A Pilot, Safety and Feasibility Trial of High-Dose Omega-3 Fatty Acids and High-Dose Cholecalciferol Supplementation in Type 1 Diabetes

POSEIDON STUDY: PILOT STUDY OF OMEGA-3 AND VITAMIN D HIGH DOSES IN T1D

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PREFACE

PILOT STUDY OF OMEGA-3 AND VITAMIN D HIGH DOSES IN T1D (POSEIDON) Protocol, A Pilot, Safety and Feasibility Trial of High-Dose Omega-3 Fatty Acids and High-Dose Cholecalciferol Supplementation in Type 1 Diabetes, describes the background, design, and organization of the study. The Diabetes Research Institute will maintain the protocol over the course of the study through 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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Glossary of Abbreviations

AE	Adverse event
APC	Antigen presenting cell
ATG	Anti-Thymocyte Globulin (Thymoglobulin®)
AUC	Area under Curve
BB Rat	Bio-Breeding Rat
CBC	Complete blood count
CFR	Code of Federal Regulations
cGMP	Current Good Manufacturing Practice
CHO	Carbohydrates
CMV	Cytomegalovirus
CRF	Case report form
CRS	Cytokine Release Syndrome
DC	Dendritic Cell
DRI	Diabetes Research Institute
DSMB	Data and Safety Monitoring Board
EBV	Epstein-Barr virus
FACS	Fluorescence activated cell sorting
FDA	US Food and Drug Administration
FOXP3	Forkhead box P3
FWA	Federal-wide Assurance
GAD	Glutamate decarboxylase
GCP	Good Clinical Practice
GCSF	Granulocyte colony-stimulating factor (GCSF/Neulasta®)
HbA1c	Hemoglobin A1c
HBsAg	Hepatitis B surface antigen
HIV	Human immunodeficiency virus
ICA	Islet cytoplasmic antibodies
IGRA	Interferon-γ release assays
IL-2	Interleukin 2
IND	Investigational New Drug
IRB	Institutional Review Board
LIFT	Long Term Investigative Follow-Up
MMTT	Mixed-meal tolerance test
NCI-CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NOD	Nonobese diabetic
OHRP	Office for Human Research Protections
PBMC	Peripheral Blood Mononuclear Cell
PCR	Polymerase chain reaction
PI	Principal Investigator
QA	Quality Assurance
RMSE	Residual Mean Square Error
SAE	Serious adverse event
SC	Subcutaneous
SOE	Schedule of events
SOP	Standard operating procedure
T1D	Type 1 diabetes, previously known as insulin-dependent diabetes mellitus
Tregs	Regulatory T cells
ZnT8	Zinc Transporter 8

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1. INTRODUCTION

1.1. Study Overview

<table>
<tr><td>Title</td><td>A Pilot, Safety and Feasibility Trial of High-Dose Omega-3 Fatty Acids and High-Dose Cholecalciferol Supplementation in Type 1 Diabetes</td></tr>
<tr><td>Acronym</td><td>POSEIDON study: Pilot study of omega-3 and vitamin D high doses in T1D</td></tr>
<tr><td>Conducted By</td><td>Diabetes Research Institute</td></tr>
<tr><td>Study Phase</td><td>Phase I/IIa</td></tr>
<tr><td>Accrual Objective</td><td>56 subjects over three years</td></tr>
<tr><td>Study Design</td><td>The study is a two-arm, open label, randomized trial. All groups will receive standard intensive diabetes treatment with insulin and dietary management. There will be four groups of participants in this study

<table>
<tr><td>Group</td><td>Subject population</td></tr>
<tr><td>I</td><td>Fourteen (14) adults (18-65 years) of established T1D of more than 6 months duration (>180 days) and up to 10 years</td></tr>
<tr><td>II</td><td>Fourteen (14) adults (18-65 years) of new-onset T1D diagnosed within last 6 months (≤ 180 days)</td></tr>
<tr><td>III</td><td>Fourteen (14)children (6-17 years) of established T1D of more than 6 months duration (>180 days) and up to 10 years</td></tr>
<tr><td>IV</td><td>Fourteen (14) children (6-17 years) of new-onset T1D diagnosed within last 6 months (≤ 180 days)</td></tr>
</table>

Participants in each group will be randomly assigned in a 1:1 ratio to receive either one year of high dose Omega-3 fatty acids and Vitamin D combination (Arm A) or Vitamin D alone (Arm B). Both arms will receive Vitamin D supplementation.

Table 1. This table represents supplements used in the study, their doses, routes of administrations and description of two arms

<table>
<tr><td>Drugs with route of administration</td><td>Dose</td><td>Arm A</td><td>Arm B</td></tr>
<tr><td>Ultra-refined omega-3 EPA/DHA concentrate in Liquid or</td><td>Based on the body weight of the participant

<u>Initial Dose:</u></td><td>Three times daily orally</td><td></td></tr>
</table>
</td></tr>
</table>

	Capsule formulations (ZoneLabs® OmegaRx®2 Fish Oil – Liquid; containing 3375 mg EPA+DHA [2250 mg EPA and 1125 mg DHA] in 5 mL Of note, each 1 mL of OmegaRx®2 contains 675 mg of EPA+DHA.	150 mg of EPA+DHA per Kg body weight. *<u>Maintaince Dose:</u>* Dose will be adjusted to maintain a target AA/EPA ratio of 1.5-3.0. AA/EPA ratios will be monitored monthly until achieving target levels and as per protocol or as clinically indicated thereafter. It is 150 mg of combined EPA and DHA per kg body weight to be given either as a single or divided into smaller doses through the day.		
	Vitamin-D 25OH (Cholecalciferol)	*Based on the Vitamin D level and age of the participant* *<u>For levels 30 -39 ng/ml:</u>* *Age ≤10 years give 500 IU daily* *Age >10 years give 1000 IU daily* *<u>For levels 20 – 29 ng/ml</u>* *50,000 IU weekly for 2 months and then:* *Age ≤10 years give 1000 IU daily* *Age >10 years give 2000 IU daily* *<u>For levels < 20 ng/ml</u>*	Daily orally	Daily orally

		50,000 IU weekly for 6 months and then: *Age ≤10 years, give 1000 IU daily* *Age >10 years, give 2000 IU daily* Dose will be adjusted and monitored every four months until achieving target levels and as clinically indicated thereafter.		
Treatment Description	Subjects will be randomized to one of the two arms. The treatment arm A includes Omega-3 Fatty Acids and Vitamin D. The subjects randomized in Arm B (control group) will receive only Vitamin D supplement. The treatment assignment will be conducted in a open label fashion.				
Study Duration	All subjects will be treated and followed for one year, and will be followed for up to one additional year beyond the period of treatment. Enrollment is expected to occur over three years.				
Objective	Conduct a clinical intervention study to assess, in participants with established T1D (>6 months and up to 10 years after diagnosis) or with new onset T1D (≤6 months from diagnosis): *Primary outcome:* Stimulated (90 minute sample of a MMTT) C-peptide at the 1 year visit greater or equal to baseline level. *Secondary objectives:* Stimulated C-peptide area under the curve (AUC) during a 4-hour MMTT greater or equal to baseline level, at the 1 year visit. Reduction in HbA1c at the one year visit compared to baseline. Reduction in insulin requirement at the 1 year visit compared to baseline. Incidence of adverse events (AE) comparable to general diabetes population.				
Inclusion Criteria	Patients must meet all of the following criteria to be eligible to participate in this study: • Subject must be able to understand and provide informed consent. • Males and females, 6-17 years for children and 18-65 years of age for adult group. • For new onset T1D - from onset to 6 months (180 days) post diagnosis at the time of randomization. • For established T1D – At least 6 months and up to 10 years from the diagnosis at the time of randomization.				

Exclusion Criteria	• Affected by T1D, according to ADA standard criteria, and confirmed by positivity of at least one T1D-associated autoantibody, to GAD65, IA-2, ZnT8, or insulin autoantibody (if patient has been treated with insulin for less than 2 weeks). • T1D must be treated with insulin (except if participant is in Honeymoon period/phase). • Stimulated C-peptide peak level, at the baseline 1 visit MMTT, ≥ 0.2 ng/ml. • Female subjects of childbearing potential must have a negative pregnancy test upon study entry. • Adequate venous access to support study required blood draws. Patients *must not meet any* of the following criteria to be eligible to participate: • Inability or unwillingness of a participant to give written informed consent or comply with study protocol. • BMI>30 kg/m^2. • Contra-indications to any of the drugs used in the trial (as per package insert, e.g., knowledge of hypersensitivity to drugs or its excipients). • Uncompensated heart failure, fluid overload, myocardial infarction or liver disease or severe impairment of a vital organ within the last 6 weeks before enrollment. • Any of the following laboratory findings indicating hemoglobin <10.0 g/dL; leukocytes <3,000/μL; neutrophils <1,500/μL; lymphocytes <800/μL; platelets <100,000/μL. • Any sign or diagnosis of significant chronic active infection (e.g., hepatitis, tuberculosis, EBV, or CMV), or screening laboratory evidence consistent with a significant chronic active infection (such as positive for HIV, IGRA test for TB, or hepatitis B-C). • Ongoing acute infections, e.g., acute respiratory tract, urinary tract, or gastrointestinal tract infections. • Ongoing or anticipated use of diabetes medications other than insulin. • Current or ongoing use of non-insulin pharmaceuticals that affect glycemic control within prior 7 days of screening. • Recent recipient of any licensed or investigational live attenuated vaccine(s) within 6 weeks of randomization. • Use of investigational drugs within 3 months of participation. • Concomitant therapy with immunosuppressive drugs, immunomodulators, or cytotoxic agents, or previous therapy less than 3 months from randomization.

	• History or diagnosis of malignancy, with the exception of a history of localized basal or squamous cell carcinoma. • History of gastroparesis or other severe gastrointestinal disease.. • Presence of an allograft. • AST, ALT or Alkaline Phosphatase >2 times upper limit of normal or total bilirubin >1.5 times upper limit of normal. • History of mental illness deemed to be clinically unstable or any situation that, in the opinion of the investigator, would interfere with the participant's ability to comply with study requirements. • History of illicit drug or alcohol abuse. • Pregnancy or ongoing breastfeeding for women; unwillingness or inability of both females and males of childbearing age to use a reliable and effective form of contraception, for the entire duration of the study. • Past or current medical problems or findings from physical examination or laboratory testing that are not listed above, which, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the participant's ability to comply with study requirements or that may impact the quality or interpretation of the data obtained from the study.

2. BACKGROUND AND RATIONALE FOR THE PROPOSED TRIAL

2.1. Type 1 diabetes

Type 1 diabetes (T1D) is a chronic autoimmune disease leading to the selective destruction of insulin producing beta cells within the pancreatic islets. Autoreactive T cells and abnormalities in immune regulation, especially in regulatory T cells (Treg cells), play a key role in disease development (Pugliese, 2014). In the U.S. alone, approximately 1.3 million people, especially children and adolescents, suffer from T1D; the disease incidence is increasing in many countries (Cizza et al, 2012; Forlenza & Rewers, 2011; Pettitt et al, 2013; Vehik & Dabelea, 2011). Chronic autoimmune T cell responses against pancreatic ß-cells are considered the primary cause of T1D, leading to loss of ß-cell mass and insulin secretion and in turn life-long dependence on insulin injections (Coppieters et al, 2012; Roep & Peakman, 2012). The disease severely impacts quality of life and confers risk for both acute and chronic complications linked to significant morbidity and mortality, such as end stage renal disease, blindness, cardiovascular disease, diabetic ketoacidosis and hypoglycemia (Cengiz et al, 2013; Nathan, 2014). The economic burden caused by T1D amounts to approximately $14.4 billion in medical costs and lost income (Tao et al, 2010).

The discovery of insulin in 1922 dramatically changed the prognosis of T1D. It was the first effective therapy, which converted a fatal condition into a chronic disease. However, the prolonged life expectancy allows time for the development of long-term complications, e.g. retinopathy, neuropathy, nephropathy and vasculopathy, which are largely related to the imperfect degree of metabolic control afforded by insulin therapy. The “Diabetes Control and Complications Trial” showed those intensive insulin regimens that achieve tight blood glucose control reduced the risk of complications in T1D (Nathan, 2014). Yet this level of tight control is difficult to achieve for many patients, despite many advances in insulin therapy and blood glucose monitoring, and it bears significant risk of hypoglycemia (Steffes et al, 2003; Zinman, 1998).

T1D requires lifelong therapy with insulin injections, multiple times daily. Despite much progress in insulin preparations, insulin therapy is inadequate in most patients (Hirsch & Skyler, 2000; Pouwer & Hermanns, 2009). Insulin dosing largely remains an empiric decision and is prone to error, which in turn can lead to severe, life-threatening hyperglycemia or hypoglycemia. Blood glucose monitoring is required to adjust insulin doses. Even patients who employ frequent finger-stick blood glucose monitoring fail to maintain strict glycemic control within the ADA-specified optimal range 70% of the time (Bode et al, 2005). The use of wearable Continuous Glucose Monitoring (CGM) devices and insulin pumps represent a step forward, yet they fail to restore normal glucose metabolism and may still be hyperglycemic almost 30% of the time (Bode et al, 2005). Patients using CGM may be hypoglycemic approximately 8% of each day, with a propensity for hypoglycemic events to occur at night (Bode et al, 2005), when hypoglycemia can more likely go unnoticed and can be therefore more dangerous. In some cases, severe hypoglycemic events, i.e., those requiring the aid of another person to administer carbohydrates, glucagon, or other resuscitative assistance, can lead to seizures, unconsciousness, coma, and death. Frequent hypoglycemia lead to hypoglycemia unawareness, which increases their risk of severe hypoglycemia and serious sequelae by at least 6-fold (Seaquist et al, 2013). Moreover, severe and multiple hypoglycemic episodes are associated with increased risk of death lead to death (Weinstock et al, 2013).

Transplantation can reverse diabetes by replacing the lost beta cell mass. Both transplantation of whole pancreas and transplantation of purified pancreatic islets are possible and can lead to reversal of the diabetic state (Bruni et al, 2014; Mineo et al, 2009; Watson, 2015; White et al, 2009). Both procedures have improved over time, but many more whole pancreas transplants have been performed compared to islet transplants. Although it requires invasive surgery and has a higher risk of perioperative mortality and morbidity, pancreas transplantation is correlated with longer graft survival and function than islet transplantation. Pancreas transplantation is particularly beneficial for patients with T1D and end stage renal disease (ESRD), in whom transplantation of pancreas and kidney restores both insulin secretion and renal function for several years. However, in all forms of transplantation, patients are required to be treated with chronic immunosuppression to prevent rejection, which is associated with well-known side effects and health risks that also limit applicability of transplantation to younger patients, representing the predominant portion of newly diagnosed patients. Moreover, our own studies have shown that approximately 5-6% of pancreas transplant recipients may develop recurrence of type 1 diabetes in the transplanted pancreas, despite immunosuppression that prevents rejection (Burke et al, 2011; Vendrame et al, 2010).

There is much hope that stem cells based approaches may provide new sources of beta cells, and yet it will be critical to define immunomodulation protocols that can control islet autoimmunity and prevent rejection of transplanted cells, recurrence of disease, and as well help patients preserve insulin secretion from the residual beta cell mass at diagnosis (Orlando et al, 2014). Thus, there is a need to develop new therapies to interdict islet autoimmunity, to prevent, treat and cure T1D. Even a partial level of insulin secretion affords protection from chronic complications (Steffes et al, 2003), hypoglycemia and diabetic ketoacidosis (Ludvigsson, 2013), which can all lead to death (Realsen et al, 2012; Rosenbloom, 2010; Weinstock et al, 2013).

2.2. Omega-3 Fatty Acids

The human body can make most of the types of fats it needs from other fats or raw materials. That isn't the case for omega-3 fatty acids (also called omega-3 fats and n-3 fats). These are essential fats—the body can't make them but must get them from food. Foods high in Omega-3 include fish, vegetable oils, nuts (especially walnuts), flax seeds, flaxseed oil, and leafy vegetables.

The omega-3 fats are special for many reasons. They are an integral part of cell membranes throughout the body and affect the function of the cell receptors in these membranes. They provide the starting point for making hormones that regulate blood clotting, contraction and relaxation of artery walls, and inflammation. They also bind to receptors in cells that regulate genetic function. Likely due to these effects, omega-3 fats have been shown to help prevent heart disease and stroke, may help control lupus, eczema, and rheumatoid arthritis, and may play protective roles in cancer and other conditions.

Omega-3 fats are a key family of polyunsaturated fats. There are three main omega-3s: Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) come mainly from fish, so they are sometimes called marine omega-3s.

Alpha-linolenic acid (ALA), the most common omega-3 fatty acid in most Western diets, is found in vegetable oils and nuts (especially walnuts), flax seeds and flaxseed oil, leafy vegetables, and some animal fat, especially in grass-fed animals. The human body generally uses ALA for energy, and conversion into EPA and DHA is very limited.

Cell membranes require unsaturated fatty acids to maintain structure and function. Desaturase enzymes present in mammals add a double bond to form a monounsaturated fatty acid, oleic acid(18:1 n9), from stearate. Animals, unlike plants, can insert additional double bonds to create two classes of polyunsaturated fatty acids (PUFA), n-6 and n3. Plants convert oleic acid to linoleic acid (LA) (18:2 n-6) and desaturation of oleic produces α-linolenic (ALA) (18:3 n-3). Marine algae also produce EPA (20:5 n-3) and DHA (22:6 n-3) that are eventually transferred through the food chain to fish whose oils are high in ω-3 fatty acids. Mammals cannot synthesize LA (the predominant PUFA in Western diets) and ALA; therefore these fatty acids are "essential" and must be ingested. Mammals, unlike plants, cannot interconvert n9, n6 and n3 fatty acids, but do further convert LA to other n-6 fatty acids, (e.g., arachidonic acid (20:4 n-6)) and ALA to n-3 EPA and DHA.

2.3. Omega-3 Fatty Acids and Inflammation

Omega-3 fatty acids reduce inflammatory cytokines and inflammatory prostaglandins, both of which may be related to the initial inflammation in islet cells. The mechanism of omega-3 fatty acids reducing inflammation has been shown secondary to limiting proinflammatory cytokines and the activity of the PGS2 enzyme (Litherland et al., 1999) . Therefore, it was proposed that the increased PGS2 expression and cytokine production observed in children at high-risk for developing T1D may be reduced through omega-3 fatty acid supplementation (Litherland et al., 2003). Arachidonic acid (AA), DHA, and eicosapentaenoic acid (EPA) inhibit PGS2 with similar potency, which is greater than that seen with other fatty acid analogues.
Endres et al (1989) found that the syntheses of interleukin-1 (IL-1alpha and IL-1 beta) and of tumor necrosis factor (TNF), cytokines with potent inflammatory activities, were reduced by dietary supplementation with n-3 fatty acids. The anti-inflammatory effect of these n-3 fatty acids may be mediated in part by their inhibitory effect on the production of lL-1 and TNF.

2.4. Omega-3 Polyunsaturated Fatty Acid Intake and T1D

The incidence of T1D is increasing, particularly in very young children. We hypothesize that the lack of omega-3 fatty acids in the diet has contributed to this increase. These are essential fats—the body can't make them but must get them from food. Just 100 years ago, consumption of n-6 to n-3 fatty acids in the diet was at a 1:1 ratio. In 1999, the ratio was reported to be approximately 30:1 (Simopoulos AP. 1999) . Because of the warnings to eliminate fish during pregnancy, the ratio is likely even greater during pregnancy. This results in increased production of inflammatory prostaglandins and cytokines during this critical time of fetal development. This trend towards substitution of the inflammation producing n-6 fatty acids at the expense of the anti-inflammatory n-3 fatty acids may be contributing to the increase in the number of cases of T1D diagnosed each year.

More direct evidence also suggests a relationship between omega-3 fatty acids and T1D. Decsi and colleagues demonstrated that 40 children with T1D had significantly lower DHA levels and a lower n-3: n-6 ratio than the control children, which may partially explain the altered prostanoid and inflammatory cytokine production. They conclude that the reduced availability of long-chain polyunsaturates in children with T1D suggests the need for enhanced dietary supply (Decsi et al., 2002).

Observations have been made that children who have received omega-3 fatty acid supplementation have a lower risk of T1D. Children born to women from Norwegian fishing villages had a significantly decreased risk of getting diabetes compared with the children of

women who lived in cities away from the coast (Joner G. 1992). Using a case-control design, Stene et al found that cod liver oil, either taken by the mother during pregnancy or by the child during the first year of life, was associated with a decreased risk of developing T1D (Stene et al., 2000). They concluded that either the vitamin D or the eicosapentaenoic acid (EPA) or the docosahexaenoic acid (DHA) or all three cod liver oil components have protective effects against developing T1D. In a larger and more recent study, Stene and colleagues in Norway found that supplementation with cod liver oil during the first year of life was associated with a significantly lower risk of T1D before age 15, odds ratio (OR) of 0.7 ($p<0.001$) (Stene and Joner, 2003). This is consistent with the hypothesis that omega-3 fatty acids (or vitamin D) have a protective effect. It now appears that the protective effect may occur during pregnancy or during infancy or both.

T1D is an autoimmune disease that is characterized by the destruction of insulin-producing beta cells in the pancreatic islets. Although it is not yet known what initiates the autoimmune process, it is likely that both genetic background and environmental factors contribute to the disease process. Dietary factors have been implicated in the etiology of type 1 diabetes as well as in initiating the autoimmune process that leads to clinical disease. A case-control study from Norway (Stene and Joner, 2003) reported that children with diabetes were less likely to have been given cod liver oil during infancy than children without diabetes. Given that cod liver oil contains both vitamin D and the marine omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), it was not clear whether the protective factor in cod liver oil was the vitamin D, the marine fatty acids, or both.

Although two studies reported that children with diabetes were less likely to have taken vitamin D supplements in infancy than children without diabetes (EURODIAB Substudy 2 Study Group. Vitamin D supplement in early childhood and risk for type I insulin-dependent diabetes mellitus, and Hypponen et al 2001), similar investigations focusing on the intake of marine omega-3 fatty acids have not been conducted to resolve this important question. The clinical phase of type 1 diabetes, where hyperglycemia manifests, is preceded by an asymptomatic period that varies in duration, ranging from a few months to several years, in which autoantibodies to the beta cells and their antigens are detectable in the blood. Persistent positivity of these autoantibodies confers a high risk of subsequent development of type 1 diabetes in relatives of those individuals with diabetes and in the general population. Because autoantibodies appear before clinical diabetes, examination of risk factors for the appearance of these autoantibodies would yield important clues regarding the early pathogenic events leading to autoimmunity, and perhaps the pathogenesis of type 1 diabetes itself.

Studies suggest that macrophage infiltration and inflammatory cytokine production are early events in the pathogenesis of type 1 diabetes (Chase et al., 2004; Jansen et al., 1994; Green and Flavell, 1994; Dahlen et al., 1998). Therefore, identifying factors that either promote or block the impact of these early pathogenic inflammatory events may be key to promoting or inhibiting the development of type 1 diabetes. Several studies have demonstrated a strong effect of omega-3 fatty acids on inflammatory responses in animals and humans (Endres et al., 1989; Calder, 2001; De Caterina et al., 2004). A relative deficiency of omega-3 fatty acids, a characteristic of many Western diets, may predispose to heightened inflammatory reactions and thus increase the risk for autoimmune diseases, such as type 1 diabetes. Alpha-linolenic acid (ALA) is the principal omega-3 fatty acid in Western diets and is found in the green leaves of plants, and also in selected seeds, nuts, and legumes (eg, flax, canola, walnuts, and soy). Alpha-linolenic acid may serve in a limited capacity as a precursor for EPA and DHA, 2 omega-3 fatty acids that are primarily obtained from fish. Linoleic acid is the most abundant omega-6

fatty acid in the diet and is found primarily in nut, seed, and vegetable oils. Arachidonic acid is an omega-6 fatty acid that can be derived from linoleic acid and is also found in meat and poultry. Because ALA and linoleic acid compete for key enzymes involved in fatty acid metabolism and conversion to either pro-inflammatory or anti-inflammatory eicosanoids, it is important to examine the effects of omega-3 and omega-6 fatty acid intakes together.

2.5. Vitamin D in T1D

As noted above, the observation that cod liver oil was associated with lower risk of T1D suggests the possibility of a synergistic effect of both of the components of cod liver oil (DHA and vitamin D) (Stene and Joner, 2003). A large retrospective cohort study demonstrated an association between vitamin D supplementation in the first year of life and a lower risk of T1D [24]. This association was also demonstrated in a prospective study in which infants received vitamin D supplementation (Stene et al., 2000; Stene and Joner, 2003). The mechanism may be related to the immunosuppressive properties of Vitamin D or possibly a direct effect on islet cell insulin secretion. Vitamin D receptor (VDR) gene polymorphisms have recently been associated with susceptibility to T1D in humans (Alician et al.,2009; Lowe et al., 2007). Interestingly, Stene et al (2000) found an indication for a protective effect with certain polymorphisms, but this was strongest for children who took cod liver oil during the first year of life .

Serum 25-hydroxyvitamin D (25-OHD) concentrations are largely determined by environmental factors, mainly through vitamin D intake and ultraviolet exposure. The sun is the primary source of vitamin D, which is synthesized endogenously in skin to produce cholecalciferol (vitamin D3), although a small proportion (<20%) of vitamin D comes through diet from a limited range of foods (in the form of ergocalciferol [vitamin D2] and vitamin D3) (Holick 2006). The main marker of vitamin D status is the metabolite 25-OHD, which is synthesized in the liver (Chiu et al., 2004; Norman et al., 1980).

A relationship between type 1 diabetes mellitus and vitamin D deficiency has been reported (Luong et al., 2005; Mathieu et al., 2005). The prevalence of vitamin D deficiency in patients with type 1 diabetes was 15% to 90.6% (Greer and Rogers, 2007; Svoren et al., 2009; Bener et al., 2009). There is evidence that vitamin D is important in the prevention of islet cell death and might be useful in improving the survival of islet cell grafts, and it improves the production of insulin. Low vitamin D levels were shown to have a negative effect on beta-cell function (Chiu et al., 2004; Norman et al., 1980). Regular doses of vitamin D early in life have been shown to reduce the risk of developing type 1 diabetes (Luong et al., 2005). Vitamin D treatment has also been shown to improve glycemic control and insulin sensitivity in people with type 1 and type 2 diabetes and in normal individuals. Increasing vitamin D levels from 25 to 75 nmol/L results in a 60% improvement in insulin sensitivity. (Chiu et al., 2004; Norman et al., 1980). Borissova et al., 2003; Gedik and Akalin 1986; Schwalfenberg 2008). These effects have been mainly attributed to the immunomodulatory actions of vitamin D (Luong et al., 2005).

Geographic location seems to play a part in the development of type I diabetes with risk increasing as farther from the equator. The location on Earth contributes to the amount of vitamin D thebody produces. This observation is suggestive, but not proof, that vitamin D affects the development of type 1 diabetes. Finland, a country that is at latitude far from the tropics, has the highest incidence of type 1 diabetes in the world. In the late 1960s, it was common to supplement an infant's diet with 2,000 IU

vitamin D. When researchers compared the development of type 1 diabetes in people 30 years later based on whether their mothers recalled giving them vitamin D during the first year after birth, they found an 80 percent reduction in the risk of type 1 diabetes later in life. Calcitriol makes insulin-secreting beta cells resistant to immune system attack. The active vitamin appears to reduce the production of cytokines, substances that kill beta cells.

Currently, project NCT03046927 conducted at the University of Massachusetts, Worcester is studying the role of vitamin D supplementation on the honeymoon phase of type 1 diabetes in children who are on standardized insulin treatment. The investigation is based on hypothesis that early intervention with vitamin D lead to significant changes in the approach to the early phase of type 1 diabetes with a strong emphasis on prolonging the honeymoon phase by using vitamin D and maintaining these patients on a standardized insulin regimen. The study rationale also indicates role of vitamin D in reducing the long-term complications of type 1 diabetes.

3. STUDY RATIONALE

The anti-inflammatory properties of omega-3 long-chain polyunsaturated fatty acids (LCPUFA) [eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] have been extensively reported in the literature. Derivatives from omega-3 LCPUFA have been associated with marked anti-inflammatory effects whereas arachidonic acid (AA), an omega-6 LCPUFA, is the precursor of eicosanoids associated with pro-inflammatory properties. Thus, a high AA/EPA ratio would suggest that the balance is shifted towards a pro-inflammatory state. Treatment with omega-3 fatty acids aimed at lowering the AA/EPA ratio may then prove to be beneficial particularly in diseases associated with a high inflammatory state. Bioactive metabolites such as resolvins and protectins are derived from omega-3 fatty acids and are postulated to mediate the anti-inflammatory properties of omega-3 fatty acids. These mediators act by several mechanisms including reduction of neutrophil infiltration, attenuation of TNF stimulated NF-κB activation, inhibition of interleukin-1β (IL-1β), reduction in tumor necrosis factor (TNF) and interferon-ɣ secretion, inhibition of T-cell migration and regulation of T-cell apoptosis. Notably, IL-1β and TNF have both been implicated in beta-cell death and IFN signatures as well as T cell infiltration of the pancreatic islets have been associated with type 1 diabetes (T1D).

A number of studies suggest that omega-3 fatty acids may be beneficial in T1D:

- Bellenger et al. reported that transgenic fat-1 mice, a model of endogenous omega-3 LCPUFA synthesis, were protected against streptozotocin-induced diabetes. This protection was associated with a reduction in pro-inflammatory cytokines with a concomitant increase in anti-inflammatory cytokines.
- The Diabetes Autoimmunity Study in the Young (DAISY) follows children at high risk for T1D development; in this study, dietary intake of omega-3 fatty acids was associated with a reduced risk of developing islet autoimmunity, especially of developing multiple autoantibodies, which are a strong risk factor for future disease. There was no association with the development of T1D.

- The Nutritional Intervention to Prevent (NIP) Type 1 Diabetes Study was a pilot study which evaluated the effect of DHA supplementation on inflammatory cytokine levels in infants at high genetic risk for T1D. Although DHA supplementation of infant diets was found to be safe, inflammatory cytokine production was not consistently reduced. However, the study was not designed to adjust dietary intake of omega-3 fatty acids to target specific AA/EPA ratios and it is unclear whether reaching specific ratios may translate into improved clinical outcomes.

However, in a pilot study of T1D patients with established disease (>4 years duration) omega-3 fatty acids failed to improve glycemic control after 4 weeks of therapy. This may suggest that anti-inflammatory therapies may need to be introduced early in the course of T1D in order to have an impact on prevention of beta-cell loss. In addition, considering that T1D is a chronic autoimmune and inflammatory disease, anti-inflammatory strategies may need to be administered over a prolonged period of time in order to observe a clinical benefit.

The anti-inflammatory properties of vitamin D are well established. In particular, it has been shown that Vitamin D prevents both insulitis and type 1 diabetes mellitus in mouse models of T1D and retrospective studies have shown apparent beneficial effects of vitamin D supplementation in early life on successive lifetime risk of T1DM. Vitamin D has been linked to several immunomodulatory effects. Studies in new onset T1D have shown that treatment with cholecalciferol (vitamin D3) results in both an increase in the percentage and the suppressive capacity of regulatory T-cells. A recent study in adults with vitamin D deficiency showed that daily treatment with 4,000 IU of vitamin D3 significantly reduced CD4+ T-cell activation compared to treatment with 400 units of vitamin D3. However, the role of vitamin D in preservation of beta-cell function in new onset T1D is controversial. Two studies showed no significant effect of therapy with calcitriol (1,25(OH)2D3) whereas one study using 2,000 IU of cholecalciferol daily showed a higher stimulated C-peptide at 18 months compared to placebo. Further studies evaluating the role of vitamin D supplementation in prevention of beta-cell loss at T1D onset are warranted.

Considering the safety profile and anti-inflammatory properties of high dose omega-3 fatty acids combined with Vitamin D supplementation makes this therapy a possible candidate for T1D intervention trials.

4. STUDY DESIGN

4.1. Overview

Based on the findings presented in this document, we propose to test the safety and efficacy of a regimen that combines Omega-3 Fatty Acids and Vitamin D in a design that considers timing and duration of administration in relation to their effects and predicted synergies. These agents may promote sustained immune regulation, reduce inflammation, and provide support for the residual beta cell mass. This integrated therapeutic regimen addresses major pathogenic mechanisms in T1D and thus represents a rational and well supported approach to preserve insulin secretion in T1D. This approach could halt the disease progress, preserve β-cell function and hopefully reduce dose of insulin required to manage T1D. Even partial beta cell function can facilitate disease management, reduce the likelihood of severe hypoglycemia and is associated with reduced risk of chronic complications. We hypothesize that Omega-3 Fatty Acids and Vitamin D, administered to patients with newly or

established T1D and residual stimulated C-peptide secretion will be safe and may preserve insulin secretion;

The safety will be measured by the AE profile. In addition, potential decreases in hypoglycemic events will be evaluated as this may be expected to be one of the clinical indications where combination therapy is likely to exert a clinical benefit. Other measures include the ability of combined supplement therapy to increase insulin production as assessed by serum C-peptide measurements, changes in exogenous insulin dose, and measurements of glycemic control.

The study endpoints vary between the two arms and include safety, tolerability, and efficacy. Safety endpoints include AEs over one year of treatment and an additional one year of follow-up (total two years). Efficacy endpoints include the ability of combining supplement products to produce insulin, assessed by serum C-peptide measurements following a Mixed Meal Tolerance Test (MMTT), changes in recorded exogenous insulin dose and other measures of glycemic control.

4.2. Objectives

4.2.1. Primary Objective(s)

- Stimulated (90 minute sample of a MMTT) C-peptide at the 1 year visit greater or equal to baseline level.

4.2.2. Secondary Objective(s)

- Stimulated C-peptide area under the curve (AUC) during a 4-hour MMTT greater or equal to baseline level, at the 1 year visit.
- Reduction in HbA1c at the one year visit compared to baseline.
- Reduction in insulin requirement at the 1 year visit compared to baseline.
- Incidence of adverse events (AE) comparable to general diabetes population.

4.3. Investigational Plan

This is a randomized, open-label, parallel-group, pilot phase I/IIa clinical study. Approximately 56 subjects will be randomly assigned to receive either Omega-3 Fatty Acids and Vitamin D combination or Vitamin D alone. All subjects will be given Vitamin D supplement. Patients to be included in this study are those diagnosed with new onset T1D (diagnosed within the last 180 days) or with established T1D (more than 6 months).

Participation for each subject will last approximately 2 years. Potential participants will have a screening visit to review their overall health, measure diabetes T1D-associated autoantibodies and perform a MMTT, which will also serve as the baseline MMTT. Eligible subjects will be invited to participate in the intervention trial for one year, and then followed for a second year to monitor safety and whether any potential therapeutic benefit, whether metabolic, endocrine, and/or immunological, persists after the therapy has been stopped.

We will evaluate beta cell function by examining C-peptide responses during a MMTT.

4.4. Summary of Inclusion and Exclusion Criteria

This study can fulfill its objectives only if appropriate patients are enrolled. The following eligibility criteria are designed to select patients for whom protocol treatment is considered appropriate. All relevant medical and non-medical conditions will be taken into consideration when deciding whether this protocol is suitable for a particular participant.

We will enroll T1D patients of both genders, 6-65 years -old, to examine the Omega-3 Fatty Acids and Vitamin D supplements covering as much of the age spectrum of the disease in both established as well as new onset T1D patients. There will be no restriction on race and ethnicity. However, T1D is more prevalent in populations of European descent, and enrollment will likely reflect disease prevalence in the Miami area, where the population is diverse and enriched in ethnicities from Latin America. Subjects who fulfill the following eligibility criteria will be recruited regardless of their race or gender.

4.4.1. Inclusion Criteria

Patients **must meet all** of the following criteria to be eligible to participate in this study:

1. Subjects or their parents if under 18 years old must be able to understand and provide informed consent.
2. Males and females, 6-65 years of age.
3. For new onset T1D subjects, ≤180 days from T1D diagnosis at the time of randomization with a MMTT stimulated C-peptide peak level ≥0.2 ng/ml prior to randomization.
4. For established T1D subjects, >180 days and ≤10 years of T1D duration at the time of randomization and MMTT stimulated C-peptide peak level ≥0.2 ng/ml prior to randomization.
5. Affected by T1D, according to ADA standard criteria, and confirmed by positivity of at least one T1D-associated autoantibody, to GAD65, IA-2, ZnT8, or insulin autoantibodies (if patient has been treated with insulin for less than 2 weeks).
6. Female subjects of childbearing potential must have a negative pregnancy test upon study entry.
7. Adequate venous access to support study required blood draws.
8. T1D must be treated with insulin (except if participant is in Honeymoon period/phase).

4.4.2. Exclusion Criteria

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

1. Inability or unwillingness of a participant or their parents to give written informed consent or comply with study protocol.
2. BMI>30 Kg/m^2.

3. Contra-indications to Omega-3 Fatty Acids and/or Vitamin-D (e.g., knowledge of hypersensitivity to drugs or its excipients, allergies with fish or shellfish etc.).
4. Uncompensated heart failure, fluid overload, myocardial infarction or liver disease or severe impairment of a vital organ within the last 6 weeks before enrollment.
5. Any sign or diagnosis of significant chronic active infection (e.g., hepatitis, tuberculosis, EBV, or CMV), or screening laboratory evidence consistent with a significant chronic active infection (such as positive for HIV, IGRA test for TB, or hepatitis B-C).
6. Ongoing acute infections, e.g., acute respiratory tract urinary tract, or gastrointestinal tract infections.
7. Subjects on weight altering medications, such as Orlistat.
8. Subjects with eating disorders
9. Ongoing or anticipated use of diabetes medications other than insulin.
10. Current or ongoing use of non-insulin pharmaceuticals that affect glycemic control within prior 7 days of screening.
11. People who chronically take drugs that affect bleeding time, such as anticoagulants (“blood thinners”) or nonsteroidal anti-inflammatory drugs (NSAIDs), will not qualify to enroll in the study.
12. Recent recipient of any licensed or investigational live attenuated vaccine(s) within 6 weeks of randomization.
13. Use of investigational drugs within 4 months of participation.
14. Concomitant therapy with immunosuppressive drugs, immunomodulators, or cytotoxic agents, or previous therapy less than 3 months from randomization.
15. History or diagnosis of malignancy.
16. History of gastroparesis or other severe gastrointestinal disease.
17. History or diagnosis of malignancy with the exception of a history of localized basal or squamouos cell carcinoma. There is conflicting evidence about whether omega-3 fatty acids found in seafood and fish oil might increase the risk of prostate cancer. Until additional research on the association of omega-3 consumption and prostate cancer risk is conducted, subjects with family history of prostate cancer in a first-degree relative will be excluded from the study.
18. Presence of an allograft.
19. AST, ALT or Alkaline Phosphatase >2 times upper limit of normal or total bilirubin >1.5 times upper limit of normal.
20. History of a mental illness deemed to be clinically unstable or any situation that, in the opinion of the investigator, would interfere with the participant’s ability to comply with study requirements.

21. History of illicit drug or alcohol abuse.
22. Pregnancy or ongoing breastfeeding for women; unwillingness or inability of both females and males of childbearing age to use a reliable and effective form of contraception, for the entire duration of the study.
23. Past or current medical problems, or findings from physical examination, or laboratory testing, that are not listed above which, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the participant's ability to comply with study requirements or that may impact the quality or interpretation of the data obtained.
24. All patients who have coagulation, bleeding, or blood disorders will be excluded due to the effect of high dose of Omega 3 Fatty Acids on coagulation and bleeding process.

4.5. Informed Consent

The process of assuring that individuals are making an informed decision about participating in this study includes both verbal and written communication. The consent form will be reviewed with participants and the participant will be given time to review the written consent form and ask questions. The participant will be given a copy of their signed consent forms.

Participants will also be informed about the common adverse effects of study drugs and it will be recommended that they stop eating once they feel satiety to decrease the risk of nausea and vomiting during the treatment period. Study subjects will receive standard medical care from their endocrinologist, but the study team will closely monitor the patients to assess adverse events. At these phone or email contacts and at the study visits, the frequency of hypoglycemia will be documented. Hypoglycemia will be assessed following consensus definition (Agiostratidou, 2017). Three levels of hypoglycemia will be recorded. Level 1: glucose < 70 mg/dl (3.9 mmol/l) and glucose ≥ 54 mg/dl (3.0 mmol/l). Level 2: glucose < 54 mg/dl (3.0 mmol/l) and Level 3: a severe event characterized by altered mental and/or physical status requiring assistance.

4.6. Description of Treatment Groups

This open label protocol will enroll a total of 56 participants who will be randomly assigned (1:1) to the following groups:

- 28 participants will be assigned to receive Omega-3 Fatty Acids and Vitamin D combination following randomization, and then will be followed for an additional year.
- 28 participants will receive only Vitamin D.

4.7. Treatment Assignment

Eligible participants who have provided written informed consent will be randomized to one of the treatment groups. The participant, the clinical investigator and clinical personnel will be known to the treatment assignment.

4.8. 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 1). The participant's insulin production will be assessed by C-peptide measurements during mixed meal glucose tolerance tests (MMTT) conducted as per the study schedule. The MMTT will consist of a standard liquid meal (Boost Plus®, Nestlé HealthScience) at a dose of 6 mL/Kg to a maximum of 360 mL. The participant's diabetes control will be evaluated by measuring glycosylated hemoglobin (HbA1c) every four months.

Remaining samples will be stored in the Diabetes Research Institute for future analysis. Samples will be stored only with the participant's permission. Participants who decline consent for storage of remaining samples will still be eligible to participate in this study.

4.9. Post-treatment Follow-up

All subjects will be treated with supplement combination or vitamin D alone over a one year period. Subjects will also be asked to undergo additional follow-up post-treatment for up to one year with a visit every 4 months.

5. PATIENT MANAGEMENT

5.1. Recruitment and Screening

Information about the clinical trial will be made available to potential participants as follows: a) flyers at the Diabetes Research Institute and the Mailman Center for Child Development, University of Miami, b) on-line at www.Clinicaltrials.gov and Diabetes Research Institute Foundation webiste www.diabetesresearch.org, c) social media: Diabetes Research Institute Foundation Facebook and twitter accounts and YouTube chanel, d) by press release(s) from the Diabetes Research Institute, University of Miami.

We will also provide information about the clinical trial to physicians within the Adult and Pediatric Divisions of Endocrinology, Diabetes and Metabolism at the University of Miami. We expect this approach to result in widespread awareness of this clinical trial and lead to successful recruitment numbers.

After informed consent is obtained, 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 also be discussed during the informed consent process.

5.2. Randomization

Individuals complying with all inclusion and exclusion criteria and consenting to study participation will be randomized at the clinical site. A blinded statistician will create color and number coded envelopes using a stratified blocked randomization (new diagnosis vs established, child vs adult). Each envelope will contain a card with the corresponding treatment group assignment. Envelopes will be kept in a file cabinet in a secured room at the Diabetes Research Institute. The study PI will perform the randomization of all newly enrolled subjects in a 1:1 ratio to each of the two arms by selecting the corresponding color and number coded envelope to ensure these factors are equally distributed across the two arms of the intervention.

For each subject, the Subject Identificaton Number (SIN), treatment and participant's name will be entered into the study log.

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 target range in the absence of level 3 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 at study visits. Subjects will not be permitted to use non-insulin pharmaceuticals for glycemic control.

Glucose levels should be checked frequently using either a continuous glucose monitor (CGM) or manually via study participant's glucometer. In addition, subject will complete a 7-day Blood Sugar record and Hypoglycemia log at the screening visit (+/- 7 days) and at each study visit (+/- 7 days) starting at visit 2. Subjects using a CGM can use glucose values from the CGM to complete the 7-day blood sugar record. If subjects are using an insulin pump, pump data will be downloaded to document insulin use. Subjects on multiple daily injections will record their insulin use on the 7-day blood glucose record unless they are using a smart insulin pen with capability to record insulin dosing in which case data will be downloaded from the device and serve as the insulin dosing documentation. If patient is using a CGM and pump, downloaded data from pump and CGM will be used instead of the 7-day blood sugar log. Data obtained from participants' diabetes management devices and blood sugar log will serve as source documentation of glucose monitoring and insulin use.The study team will be evaluating the HbA1c data and provide additional guidance to the participants as needed to bring diabetes control within goals. Any episodes of level 3 hypoglycemia will prompt review by the study physician and site medical monitor with recommendations, if any, for changes in diabetes management conveyed to the participants in conjunction with the principal investigator(s).

5.4. Administration of Study Drugs

Due to current status of COVID-19 pandemic, clinical research activites have been affected and are being conducted as recommended by institutional guidelines to minimize potential exposure to SARS-CoV-2 for both research subjects and clinical research study team. Study investigational product(s)

may be mailed to participants as determined by PI. For international patients, they may be able to obtain over the counter vitamin D3 and/or omega 3 fish oil locally (in their country of residence) if the investigational product cannot be mailed overseas (at the discretion of the PI).

In addition, the study team will try to conduct as much as possible “remote” study visits (e.g telephone communication, video conferencing, obtaining study test results from Quest Diagnostics Lab) to obtain required SOE information.

5.4.1. Omega-3 Fatty Acids

Omega-3 fatty acids (omega-3s) are a group of polyunsaturated fatty acids that are important for a number of functions in the body. Some types of omega-3s are found in foods such as fatty fish and shellfish. Another type is found in some vegetable oils. Omega-3s are also available as dietary supplements. This fact sheet provides basic information about omega-3s—with a focus on dietary supplements, summarizes scientific research on effectiveness and safety, and suggests sources for additional information.

Administration of high-dose omega 3 fatty acids (18g of marine lipid concentrate) for 6 weeks to healthy volunteers reduced the AA/EPA ratio from 20.9±2.2 to 2.4±0.2 (nearly 90%) and resulted in a decrease in IL-1β, IL-1α, and TNF and was well tolerated [1]. A recent study in patients with stable coronary artery disease showed that long-term treatment (1 year) with high-dose omega 3 fatty acids (3360 mg EPA and DHA daily) was associated with an increase in specialized pro-resolving lipid mediators and enhancement of macrophage phagocytosis of blood clots [2]. These data suggest that high-doses of omega 3 fatty acids are required to mitigate cytokine responses and may be critical to generate resolvins and protectins, which have been postulated as the molecular basis for the anti-inflammatory properties of omega-3 fatty acids.

Further, high-doses of omega 3 have been associated with improved clinical outcomes. In children with ADHD, a study evaluating an 8-week treatment course with high-dose omega 3 fatty acids (250-325 mg EPA and DHA/Kg/day) aimed at achieving an AA/EPA ratio between 1.5 and 3.0 resulted in significant improvements in behavior with the AA/EPA ratio correlating with the global severity of illness scores [3, 4]. In addition, two separate case reports in children with new onset type 1 diabetes have documented treatment with high-dose of omega 3 fatty acids (55-110 mg EPA and DHA/Kg/day) for over 1 year and showed improvement in glycemic control and preservation of C-peptide [5, 6]. Notably, these high doses of omega 3 fatty acids were well tolerated.

To appropriately gauge the dose of omega 3 fatty acids we are proposing the use of the AA/EPA ratio to guide dosing. As noted above, AA/EPA ratios in the 1.5 to 3.0 range have been studied in healthy volunteers and in children with ADHD have shown an association with clinical outcomes and noted to be safe. Further, such low ratios can be achieved by diet alone as documented in the Japanese population where the average fish consumption has been reported to be one serving of 85 g (3 oz; 900 mg EPA and DHA) per day and 90% of individuals eat fish at least once a week [7].

In view of the chronic nature of the immunopathogenesis of type 1 diabetes it is then not unexpected that therapeutic interventions started at disease onset to preserve beta cell function have usually been administered for a duration of 6 month to 2 years [8-10]. It is important, however, to highlight that one of the main factors responsible for determining the duration of therapy is the safety profile of the proposed intervention.

Data from TrialNet intervention trials has allowed to study the decline in C-peptide 2 years following type 1 diabetes onset which shows a biphasic profile with a steeper decline during the first year [11] suggesting that processes involved with beta-cell loss may be more aggressive during the first year of diagnosis. Thus, therapies aimed to preserve beta-cell function in new onset disease may likely need to be administered over a prolonged period of time and for at least 1 year following disease onset.

In established disease, duration of therapy is also dependent on the mechanism of the intervention and the safety profile. Since inflammation affecting glycemic control may be a chronic state in patients with type 1 diabetes and in view of the reportedly safety profile of high-dose omega 3 fatty acids, we have proposed treating patients with established type 1 diabetes for a duration of 1 year.

5.4.1.1. Ultra-refined omega-3 EPA/DHA concentrate (ZoneLabs® OmegaRx®2 Fish Oil) Administration

Participants will receive ultra-refined omega-3 EPA/DHA concentrate (ZoneLabs® OmegaRx®2 Fish Oil – Liquid; containing 3375 mg EPA+DHA [2250 mg EPA and 1125 mg DHA] in 5 mL).
Research subjects will be started at a dose of 150 mg of EPA+DHA per Kg body weight.
For example:

- The dose for a 22.5 Kg child will be:
  150 mg x 22.5 Kg = 3375 mg of EPA+DHA fish oil corresponding to 5 mL of Omega Rx ®2 Fish Oil Liquid.
- The dose for a 30 Kg child will be:
  150 mg x 30 Kg = 4500 mg of EPA+DHA fish oil corresponding to 6.7 mL of Omega Rx ®2 Fish Oil Liquid.

Participants who are unable to tolerate the liquid formulation will be switched to the OmegaRx®2 capsule formulation at the same dose as described above.

It is 150 mg of combined EPA and DHA per kg body weight to be given either as a single or divided into smaller doses through the day.

5.4.1.2. Modification or Discontinuation of omega-3 EPA/DHA concentrate

Dose will be adjusted to maintain a target AA/EPA ratio of 1.5-3.0 as follows:

- If AA/EPA ratio is ≥ 7, double the dose and recheck ratio in one month
- If AA/EPA ratio is 4-6, increase dose by 50% and recheck ratio in one month
- If AA/EPA ratio is at target, maintain dose as prescribed s
- If AA/EPA ratio is <1.5, reduce dose by 50% and recheck in one month

AA/EPA ratios will be monitored monthly until achieving target levels and as per protocol or as clinically indicated thereafter.

5.4.1.3. Discontinuation of omega-3 EPA/DHA concentrate

Subjects would be continuously evaluated for any untoward effects of Omega-3 fatty acid supplements or negative side effects. Typically side effects consist of minor gastrointestinal symptoms, such as belching, indigestion, or diarrhea, stomach pain or discomfort, burping, heartburn, vomiting, constipation, nausea, and change in the sense of taste. Joint pain may be a side effect as well.

Patients with coagulation, bleeding, or blood disorders will be excluded. We will monitor clotting times of enrolled subjects by measuring the prothrombin time (PT/PT-INR) during all subject visits (except for visit 1), in addition to CBC with differentials (see schedule of assessment).

Symptomatic management of the side effects will be evaluated by the study physician and should the investigator believes that the study treatment is no longer in the best interest of the participant, subjects would be discontinued from the treatment. Similarly, if subject begins taking medications that affect clotting chronically, subjects would be discontinued and appropriately noted in the study record. Participants who prematurely discontinue study treatment will remain in the study and undergo all efficacy and safety assessments.

5.4.2. Vitamin D

Vitamin D is a nutrient found in some foods that is needed for health and to maintain strong bones. It does so by helping the body absorb calcium (one of bone's main building blocks) from food and supplements. People who get too little vitamin D may develop soft, thin, and brittle bones, a condition known as rickets in children and osteomalacia in adults.

Vitamin D is important to the body in many other ways as well. Muscles need it to move, for example, nerves need it to carry messages between the brain and every body part, and the immune system needs vitamin D to fight off invading bacteria and viruses. Together with calcium, vitamin D also helps protect older adults from osteoporosis. Vitamin D is found in cells throughout the body.

5.4.2.1. Vitamin D Administration

Vitamin D supplementation

1. For levels of Vitamin D 25OH of 30 -39 ng/ml:
    a. age ≤10 years give 500 IU daily
    b. age >10 years give 1000 IU daily
2. For levels of Vitamin D 25OH of 20 – 29 ng/ml <u>give 50,000 IU weekly for 2 months and then</u>:
    a. age ≤10 years give 1000 IU daily
    b. age >10 years give 2000 IU daily
3. For levels of Vitamin D 25OH of < 20 ng/ml give <u>50,000 IU weekly for 4 months and then</u>:
    a. age ≤10 years give 1000 IU daily

b. age >10 years 2000 IU daily

Repeat vitamin D 25OH levels 1 week after the last 50,000 IU dose:

- For children whose levels are not above 30 ng/ml on repeat testing, repeat treatment as above.
- For children whose levels are 30 -39 ng/ml on repeat testing:
  - if age ≤10 years increase vitamin D supplementation to 1000 IU daily
  - if age is >10 years give 2000 IU daily

Thereafter, monitor vitamin D 25OH levels every 4 months (or more frequently at the discretion of the investigator) and adjust Vitamin D treatment to maintain levels > 40 ng/ml.

Study participants with or without a history of vitamin D deficiency who are on vitamin D replacement will continue their current dose if 25OH vitamin D levels checked at screening are in line with study targets. This may defer from the doses indicated above which apply to participants who are not on vitamin D replacement. Doses will then be adjusted at the discretion of the Principal Investigator in order to maintain 25OH vitamin D levels >40 ng/mL.

The Dietary Reference Intake Committee to Review Dietary Reference Intakes for Vitamin D and Calcium from Institutie of Medicine, recommends following upper intake levels for Vitamin D (see page 403-477)(https://www.ncbi.nlm.nih.gov/books/NBK56070/pdf/Bookshelf_NBK56070.pdf)

Table Representing Tolerable Upper Intake Levels (ULs) for Vitamin D

Age	Male	Female	Pregnancy	Lactation
0–6 months	1,000 IU (25 mcg)	1,000 IU (25 mcg)		
7–12 months	1,500 IU (38 mcg)	1,500 IU (38 mcg)		
1–3 years	2,500 IU (63 mcg)	2,500 IU (63 mcg)		
4–8 years	3,000 IU (75 mcg)	3,000 IU (75 mcg)		
≥9 years	4,000 IU (100 mcg)	4,000 IU (100 mcg)	4,000 IU (100 mcg)	4,000 IU (100 mcg)

Microcrystalline cellulose is the only excipient in Biotech Pharmacal Vitamin D3 capsule with following quantities:

- D3-5000 IU capsule contains 89 mg of microcrystalline cellulose
- D3-1000 IU capsule contains 92.8 mg of microcrystalline cellulose
- D3 50,000 IU capsule contains 115 mg of microcrystalline cellulose

Type of microcrystalline cellulose is Vivapur Type 101, it is pure microcrystalline cellulose that meets the requirements for Ph. Eur, NF, and JP monographs for Microcrystalline Cellulose and it complies with E460 (i) monograph and FCC as well.

5.4.2.2. Modification or Discontinuation of Vitamin D

Vitamin D is likely safe when taken by mouth in recommended amounts. Most people do not commonly experience side effects with vitamin D, unless too much is taken. Some side effects of excess vitamin D include weakness, fatigue, sleepiness, headache, loss of appetite, dry mouth, metallic taste, nausea, and vomiting.

Taking vitamin D for long periods of time in doses higher than 4000 units daily is possibly unsafe and may cause excessively high levels of calcium in the blood. However, much higher doses are often needed for the short-term treatment of vitamin D deficiency.

Vitamin D in very high doses can make the intestines absorb too much calcium which can cause high levels of calcium in the blood (hypercalcemia). This can cause side effects including nausea, vomiting, constipation, poor appetite, weakness, weight loss, confusion and disorientation. Hypercalcemia can also lead to kidney stones, damage to the kidneys, and cause calcium deposits in heart and lungs. We will monitor blood levels of vitamin D to minimize the risk of these side-effects.

5.4.2.3. Contraindications for Vitamin D Supplement

There are conditions where Vitamin D treatment require special precautions and these subjects will not be included in this study:

Kidney disease: Vitamin D may increase calcium levels and increase the risk of arteriosclerosis" in people with serious kidney disease. This must be balanced with the need to prevent renal osteodystrophy, a bone disease that occurs when the kidneys fail to maintain the proper levels of calcium and phosphorus in the blood.

Sarcoidosis: Vitamin D may increase calcium levels in people with sarcoidosis. This could lead to kidney stones and other problems..

Histoplasmosis: Vitamin D may increase calcium levels in people with histoplasmosis. This could lead to kidney stones and other problems..

Over-active parathyroid gland (hyperparathyroidism): Vitamin D may increase calcium levels in people with hyperparathyroidism..

Lymphoma: Vitamin D may increase calcium levels in people with lymphoma. This could lead to kidney stones and other problems..

Tuberculosis: Vitamin D might increase calcium levels in people with tuberculosis. This might result in complications such as kidney stones.

5.4.3. Assessment of Participant Compliance with Study Agent

Patients will be closely monitored to assess compliance of study drug administration. Each participant will return the empty bottles to the Coordinator to assess compliance, which in turn will be returned to the Pharmacy for appropriate on-site destruction and for documentation purposes.

5.4.4. Prior and Concomitant Treatments

The use of concomitant medications will be assessed at each study visit and recorded on an appropriate source document and CRF. Participants are allowed to use preparations of insulin as advised by the investigator or the referring physician.

The following medications are prohibited during participation in this study:

- Agents that influence insulin sensitivity or secretion (e.g. pramlintide, sulfonylureas, metformin, diphenylhydantoin, thiazide, or other potassium-depleting diuretics, beta-adrenergic blockers, niacin etc).

If participants receive, or if the investigator believes that participants must receive, any of the above medications, the case must be immediately discussed with the medical monitor. The use of prohibited medications must be documented on the source document and CRF, and a protocol deviation must be requested. A decision regarding continuation of the participant in the trial will be made by the study PI, and the medical monitor.

5.4.5. Removal of Subjects from Therapy or Subject Withdrawal or Early Termination

- **Withdrawal of consent -** Participants who withdraw consent will be asked to complete all the assessments listed for the end of treatment follow-up
- **Failure to return -** Participants who do not return for visits and who do not respond to repeated attempts by the site staff to have them return will be considered lost to follow-up.
- **Investigator judgment** - A severe or serious AE occurs, which, based on the medical judgment of the investigator, prevents completion of participation in the study.

5.4.6. Discontinuation of study drug(s) in an individual patient

The dosing and administration of investigational medication according to study specification will be discontinued for an individual participant if any of the following criteria is met:

- Subjects who have 3 severe hypoglycemic reactions on separate days (requiring assistance from another individual)
- Subjects who have nausea or vomiting that precludes adherence to diet will discontinue the use of the study drug.
- Subjects who lose more than 10 kg in weight from baseline.

- Any grade 3 or higher AE occurs that, based on the medical judgment of the investigator, the medical monitor, and the clinical trial physician, prevents completion of the course of study treatment.
- Any unexpected, treatment-related SAE resulting in permanent treatment discontinuation and not related to glycemic events.
- The investigator determines that it is in the participant's best interest to discontinue treatment.
- The participant, or participant's legal representative, requests that treatment be halted.
- The participant becomes pregnant.

Further care will be provided according to the judgment and practice of the investigator. The participant will be asked to remain in the study and participate in follow-up. If study treatment is discontinued, the medical monitor will be notified. Participants who prematurely terminate from the study will not be replaced.

5.4.7. Re-Entry into Study Treatment

In some circumstances, a participant may temporarily discontinue the study medication and/or not return to the study clinic for follow-up visits. If the participant decides to return for study treatment and/or follow-up assessments at a later date, he or she will be allowed and encouraged to do so.

The study will be conducted according to the intent-to-treat principle. This means that once randomized into the study, a participant will be expected to undergo all scheduled follow-up assessments and will remain in the assigned treatment group for purposes of statistical analysis regardless of the actual course of treatment administered. Withdrawal from treatment does not automatically entail withdrawal from the study. Withdrawal from the study will only occur if the participant dies or withdraws consent. Subjects who withdraw consent are classified as inactive but may again become active upon re-entry into the study, if they so choose.

5.4.8. Risk to the Subjects

The risks of these over the counter drugs is summarize below:

- Omega-3 Fatty Acids may cause gastrointestinal side- effects including stomach pain or discomfort, burping, heartburn, vomiting, constipation, diarrhea, nausea, and change in the sense of taste. Joint pain may be a side effect as well.
- Vitamin D in very high doses can make the intestines absorb too much calcium which can cause high levels of calcium in the blood (hypercalcemia). This can cause side effects including nausea, vomiting, constipation, poor appetite, weakness, weight loss, confusion and disorientation. Hypercalcemia can also lead to kidney stones, damage to the kidneys, and cause calcium deposits in heart and lungs. We will monitor blood levels to minimize these risks.

Allergic reaction: As with any drug, it is possible that subjects could experience an allergic reaction to OMEGA-3, or Vitamin D. Such allergic reactions include: itching, skin rash, sudden drop in blood pressure, loss of consciousness and/or associated with seizures, including the possibility of death.

Blood draw risks: Drawing blood may cause temporary pain from the needle stick, bruising or swelling at the site, and rarely, infection or fainting.

Hypoglycemia: These medications could lower blood sugar too much (hypoglycemia). This could make subjects feel tired, dizzy, sweaty, and/or nauseated. Also, it could cause heart to feel as if it is racing. There may also be other effects. Untreated hypoglycemia could cause convulsions, loss of consciousness, and can lead to death. The clinical research team will provide added training and education to subjects to better manage hypoglycemia and other diabetes related complications.

Unknown Risks: The experimental drug may have side effects that no one knows about yet. The researchers will let you know if they learn anything that might make you change your mind about participating in the study.

Females of child bearing potential must have a negative pregnancy test prior to enrolling in the study and will be required to use at least two FDA approved birth control during the study. At every study visit the sexual activity of female participants of reproductive age will be re-assessed. Pregnancy testing will be conducted in females in reproductive age at 4, 8,12 and 16 months study visit. If a subject who was previously sexually inactive becomes sexually active, she will be counseled about the need to use a reliable forms of birth control. Subjects will be requested to avoid pregnancy for 6 months following the last study administration and instructed to use birth control. If subject becomes pregnant, study intervention will be discontinued and subject withdrawn from study.

6. STUDY ASSESSMENTS

Due to current status of COVID-19 pandemic, clinical research activites have been affected and are being conducted as recommended by institutional guidelines to minimize potential exposure to SARS-CoV-2 for both research subjects and clinical research study team. Study investigational product(s) may be mailed to participants as determined by PI. For international patients, they may be able to obtain over the counter vitamin D3 and/or omega 3 fish oil locally (in their country of residence) if the investigational product cannot be mailed overseas (at the discretion of the PI).

In addition, the study team will try to conduct as much as possible “remote” study visits (e.g telephone communication, video conferencing, obtaining study test results from Quest Diagnostics Lab) to obtain required SOE information.

6.1. General Assessments

Patients visits will occur for all subjects at baseline (day 0), and every four months thereafter (month 4, 8, and 12) and consist of treatment adherence and outcome assessments. Subjects will be provided with supply of study drugs to take home and administer orally. Subjects will be educated and trained for the drug administration and study personnel will evaluate drug returns and treatment adherence. Subjects will be advised to bring the used medication back to site during their next visit. At the end of

one year, all subjects will continue with assessments up to an additional one year (months 16, 20, and 24).

The time each assessment will be performed relative to dosing is shown in the Schedule of Study Activities (Appendix 1).

General assessments include:

- Medical history: To determine if there are any clinically significant diseases or medical procedures other than the disease under study.
- Physical examination: Includes any body system with clinical signs or reported symptoms of adverse events and weight loss.
- Concomitant medications
- Adverse events

6.2. Enrollment

The research study will be explained in lay terms to each potential research participant. The potential participants will sign an informed consent before undergoing any study procedures. The consent form will be reviewed with participants and the participants will be given time to review the written consent form and ask questions. The participant will be given a copy of their signed consent form.

6.3. Screening (visit 0) and Baseline (visit 1) visits

Patients will be asked to sign consent for screening to determine eligibility. Patients will receive a physical exam, medical history will be taken, and basic physical parameters will be recorded (height, body weight, heart rate, blood pressure). Blood will be drawn for laboratory assessments (see section 6.5) for evaluation of inclusion and exclusion criteria.

A patient that meets the inclusion/exclusion entry criteria will be randomized to one of the two treatment arms. Blood will be drawn for baseline visit (visit1) assessments. The patient will receive the first treatment dose within 48 hr from the baseline visit.

6.4. Diabetes Management

During the study period, all participants will receive “intensive” management of their diabetes. The patients will continue to see their primary endocrinologist during the study period. All participants will be expected to take a sufficient number of daily insulin injections to meet the glycemic targets. In general, the expectation is that all participants will receive at least three injections of insulin daily, including short- and long-acting insulin preparations, or will utilize continuous subcutaneous insulin infusion (CSII insulin pump).

Glucose levels should be checked either via continuous glucose monitor or manually at least 4 times daily via the study participant’s glucometer. Records of glucose measurements will be kept as source documentation. Insulin use and hypoglycemia events will be recorded at each visit. Participants will be

required to record the daily amount of insulin they have used at each study visit (+/- 7 days) using the 7-day Blood Sugar and Hypoglycemia log provided. Insulin pump data or smart insulin pen data will be used for documentation of insulin use in lieu of the 7-day blood sugar log for those participants using these devices.

Upon review of these records, the investigator may make adjustments in the insulin regimen, refer a participant to a registered dietician or if necessary take other approaches that will help to maintain or improve a participant's glucose control.

6.5. Laboratory Assessments

The following laboratory assessments will be performed during the study:

- Chemistries (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, platelets, Prothrombin time [PT/PT-INR])
- Pregnancy test as appropriate
- Autoantibodies (IA2, GAD65, ZnT8, insulin autoantibodies are induced by insulin injections and thus are not useful at this stage)
- Samples for serology (i.e. Hepatitis B, Hepatitis C, EBV, CMV and Human Immunodeficiency Virus) and HbA1c
- 4-hours Mixed Meal Tolerance Test (MMTT)
- Samples for 25OH vitamin D levels and AA/EPA ratio

6.6. Mechanistic Outcome Assessments

The DRI will perform immune assays to further understand mechanisms that may be underlying the Type 1 disease process and response to therapy. For this purpose, samples will be obtained and stored for future analysis.

Mechanistic Assessments: includes samples for RNA, plasma, serum, DNA, measures of B and T cell number/function and cytokine profiles

6.7. Metabolic Outcome Assessments

Metabolic assessments will consist of:

- Glucose records and reports of hypoglycemia
- Insulin dose
- HbA1c
- Clarke Score

- Mixed meal tolerance test (MMTT)

6.8. Visit Windows

The initial study drug administration must begin within 180 days from the day of diagnosis. All subsequent treatment visits and follow up visits in Appendix 1 must occur within the time limits specified below:

The table below depicts summary of visit windows.

	Visit Windows
Visit 0	At least 2 weeks before visit 1
Visit 1	New onset group (randomization ≤180 days from T1D diagnosis) or established T1D (>180 days and <10 years from T1D diagnosis)
Visit 2-4 (Days 120, 240, 360)	± 14 days
Follow-up between year 1 and 2 Every four months	± 14 days

During the course of the study, participants who complete one year treatment will receive combination therapy of Omega-3 Fatty Acids and Vitamin D or Vitamin D alone. In addition, participants will frequently undergo assessments of their insulin production, immunological status, and overall health. The participant's insulin production will be measured by a series of MMTTs conducted regularly during the study. The participant's diabetes control will be evaluated by measuring HbA1c every four months and clinical records including insulin types, doses, administration times and blood glucose records.

6.9. Unscheduled visits

Patients will be instructed to contact the study coordinator for any health concern, side effects potentially associated with the study, or other concern that may affect their participation and require an unscheduled visit.

6.10. Sample retention

Specimens for future mechanistic studies will be obtained throughout the study. Residual specimens may be used by the investigators for studies of immunologic mechanisms involved in T1D.
Specimens collected in this trial may be used to reevaluate biologic responses as new research tools become available. These specimens will be encoded with specific study ID and stored at the Diabetes Research Institute of University of Miami.

6.11. Staggering Administration

Please note that the enrollment of patients in this trial is staggered across individuals. Initially, a total of 6 subjects will be randomized in each of the following groups:

Group	Subject population
I	Fourteen (14) adults (18-65 years) of established T1D of more than 6 months duration (>180 days) and up to 10 years
II	Fourteen (14) adults (18-65 years) of new-onset T1D diagnosed within last 6 months (≤ 180 days)
III	Fourteen (14) children (6-17 years) of established T1D of more than 6 months duration (>180 days) and up to 10 years
IV	Fourteen (14) children (6-17 years) of new-onset T1D diagnosed within last 6 months (≤ 180 days)

All 24 subjects will be monitored for acute and subacute adverse events for 90 days and a formal DSMB (Data and Safety Monitoring Board) will review the data and safety prior to treating additional subjects. This will limit the number of subjects who might be exposed to any unanticipated safety risk.

6.12. Intensive T1D 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 level 3 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 at study visits. 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 to the study team at study visits.

6.13. Safety Monitoring

The members of the Data Safety Monitoring Board (DSMB) are independent and serve in an individual capacity to provide their expertise and recommendations. They will review the 90 day data of the first 6 subjects, and then periodically review and evaluate the accumulated study data for participant safety, study conduct and progress, and, when appropriate, efficacy, and make recommendations to study investigators concerning the continuation, modification, or termination of the trial. The DSMB members will comprise of experts in the field of T1D immunotherapy and will consider study-specific data as well as relevant background knowledge about the disease, test agents, and patient population under study.

The DSMB is responsible for defining its own deliberative processes, including event triggers that would call for an unscheduled review, stopping guidelines, and voting procedures prior to initiating any data review.

6.14. *Ad-hoc* DSMB Reviews

In addition to the pre-scheduled data reviews and planned safety monitoring, the DSMB may be called upon for ad hoc reviews. The DSMB will review any event that potentially impacts safety at the request of the study investigator. In addition, the following events will trigger an *ad-hoc* comprehensive DSMB Safety Review:

- Any death that occurs in the study.
- The occurrence of a Grade 3 or higher related and unexpected SAE in three or more of the study participants who have received a study treatment.

After review of the data, the DSMB will make recommendations regarding study conduct and/or continuation.

6.15. Temporary Suspension of Enrollment/Drug(s) Dosing or Both

A temporary halt in enrollment will be implemented if an *ad-hoc* DSMB safety review is required. Subjects already on investigational product will continue on therapy unless the *ad-hoc* DSMB review is completed and the DSMB determines that its findings warrant cessation of study drug(s). Subjects in the screening phase of the study may continue to undergo screening procedures.

6.16. Stopping rules

As stated earlier, the progress of the study will be monitored by the DSMB which will review safety data and make recommendations regarding continuation, termination, or modification of the study. Based on a 12 month enrollment period and an additional study period of 12 months, the DSMB will formally review the safety data after participant number 24 reaches day 90 of the study. The number of subjects who discontinue study treatment will also be included in the reports prepared for the DSMB.

In addition, safety data will be reviewed by the DSMB when an event occurs that is of sufficient concern to the medical monitor and/or study investigator(s) to warrant review, or when an event occurs that contributes to a stopping rule listed below.

- **Patient stopping rules**: Subjects who have 3 severe hypoglycemic reactions (requiring assistance from another individual) on separate days or who have nausea or vomiting that precludes adherence to diet will discontinue the use of the study drug. Subjects who lose more than 10 kg will also discontinue study drug. Subjects who discontinue the drug treatment will not be replaced.
- **Trial stopping rules:** Interim analysis will be conducted when recommended by the DSMB to review safety data, including metabolic, immunological assessments and adverse events. The DSMB may recommend early stopping if there is sufficient evidence to conclude that the investigational treatment is harmful based on interim analysis of safety endpoints.

6.17. Potential Benefits

The risks of this study in detailed are presented in the informed consent form. This study will examine whether combination supplement therapy intervention will preserve beta cell function, but there is no guarantee that this will occur.

This is a pilot feasibility trial with the prospect of direct benefit to the individual subjects for their participation in the study. The potential direct benefit to research subjects is that administration of specific combination supplement therapy or vitamin D alone could stabilize or improve ß-cell function, thus facilitating attainment of improved glycemic control. All subjects will benefit from being closely involved with an experienced team of diabetes experts, with the likely benefit that their glycemic control will be better than would be otherwise obtained. Thus, 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. These additional resources include frequent in-person and other contact with study associate diabetes educator throughout the duration of the study. Further, the intervention has the prospect of direct benefit to a given subject and is likely to yield general knowledge about T1D which is of importance for the understanding and amelioration of T1D. The study procedures, while greater than minimal risk, offers the possibility of benefit due to the close monitoring for all participants.

If effective in preserving or increasing ß-cell function, combination therapy could be an important new treatment to change the course of T1D, thus benefiting the individual research participant and potentially society at large. Moreover, the proposed studies will produce new information about the immune system and its regulation, in the context of T1D and in relation to therapy with these over the counter agents that would be relevant to other autoimmune diseases.

6.18. Pregnancy

Pregnant and lactating women will not be included in the study. Sexually active females must have a negative pregnancy test prior to enrolling in the study and will be required to use at least two FDA approved 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 forms of birth control. Subjects will be requested to avoid pregnancy for 6 months following the last study administration and instructed to use birth control.

6.19. 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. All participants will be required to have adequate hemoglobin to allow frequent venipuncture. The supplement drugs will be dispensed to subjects free of cost, and subjects will be closely monitored during and after the treatment.

Subjects will be counseled about the potential risk for gastrointestinal side effects and the need to report any change in health status between or at the time of visits. Directed questioning about concurrent illness will occur before each treatment.

6.20. Supplement - Drug Interaction

Vitamin D

Like most dietary supplements, vitamin D may interact or interfere with other medicines or supplements.. Here are several examples:

- Corticosteroid medicines to reduce inflammation impair how the body handles vitamin D, which leads to lower calcium absorption and loss of bone over time.
- Both the weight-loss drug orlistat (brand names Xenical® and Alli®) and the cholesterol-lowering drug cholestyramine (brand names Questran®, LoCholest®, and Prevalite®) can reduce the absorption of vitamin D and other fat-soluble vitamins (A, E, and K).
- Both phenobarbital and phenytoin (brand name Dilantin®), used to prevent and control epileptic seizures, increase the breakdown of vitamin D and reduce calcium absorption.

Omega-3 Fatty Acids

Omega-3 Fatty Acids can affect medications that influence blood clotting phenomenon, some of which includes:

- Antiplatelet Medication: Anagrelide (Agrylin®), aspirin(any brand, all doses), cilostazol (Pletal®), clopidogrel (Plavix®), dipyradamole (Persantine®), dipyridamole/aspirin (Aggrenox®), enteric-coated aspirin (Ecotrin®), ticlopidine (Ticlid®)
- Anticoagulant Medication: Anisindione (Miradon®), Arixtra, enoxaparin (Lovenox®) injection, Fragmin, heparin injection, Pradaxa, pentosan polysulfate (Elmiron®), warfarin (Coumadin®), Xerelto
- Nonsteroidal Anti-Inflammatory Drugs: Celebrex, diclofenac (Voltaren®, Cataflam®), diflunisal (Dolobid®), etodolac (Lodine®), fenoprofen (Nalfon®), flurbiprogen (Ansaid®), ibuprofen (Motrin®, Advil®, Nuprin®, Rufen®), indomethacin (Indocin®), ketoprofen (Orudis®, Actron®), ketorolac (Toradol®), meclofenamate (Meclomen®), meloxican (Mobic®), nabumeton (Relafen®), naproxen (Naprosyn®, Naprelan®, Aleve®), oxaprozin (Daypro®), piroxicam (Feldene®), salsalate (Salflex®, Disalcid®), sulindac (Clinoril®), sulfinpyrazone tolmetin (Tolectin®), trilisate (salicylate combination)
- Herbs/Vitamins: Ajoene birch bark, cayenne, Chinese black tree fungus, cumin, evening primrose oil, feverfew, garlic, ginger, ginkgo biloba, ginseng, grape seed extract, milk thistle, onion extract, St. John’s wort, tumeric, vitamins C and E.

The study team will evaluate the list of all medications and its interaction with Vitamin D or Omega-3 Fatty Acids dietary supplements patients take. Study team will educate and may alter medical treatment

if dietary supplements might interact or interfere with their prescription or over-the-counter medicines, or if the medicines might interfere with how patients body absorbs, uses, or breaks down nutrients.

7. ADVERSE EVENT REPORTING AND SAFETY MONITORING

7.1. Adverse Event Definition

7.1.1. Adverse Event (AE)

In this clinical study, 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 documents. Events not related to hypo or hyperglycemia that are Grade 2 or greater per the NCI CTCAE (see Grading Event Severity below) will be reviewed by study physician and medical monitor on the appropriate adverse event form. The investigator will 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.

7.1.1.1. Disease specific AE

For the purposes of this study, hypoglycemia events will be recorded as follows:

- Level 1: glucose < 70 mg/dl (3.9 mmol/l) and glucose ≥ 54 mg/dl (3.0 mmol/l). and
- Level 2: glucose < 54 mg/dl (3.0 mmol/l)
- Level 3: a severe event characterized by altered mental and/or physical status requiring assistance.

- . Level 2 and Level 3 hypoglycemic events must be reported as adverse events on the case report forms. *All episodes of Level 3 hypoglycemia will be reported as SAEs to the DSMB.*

Hypoglycemia grading according to CTCAE version 4.0

Adverse Event	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Hypoglycemia	<LLN-54mg/dL	<54 -40 mg/dL	<40-30 mg/dL	<30 mg/dL; life threatening consequences; seizures	Death

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Hyperglycemia will not be collected as an adverse event, unless it results in an inpatient hospitalization.

7.1.2. Serious Adverse Event

For this trial, an adverse event associated with the treatment or study procedure 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 may require medical or surgical intervention to prevent one of the outcomes listed above.

7.1.3. 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.

7.1.4. Grading Event Severity

This study will adopt the National Cancer Institute (NCI) Common Technology Criteria for Adverse Events (CTCAE) and/or study-specific criteria for classification to describe the severity of adverse events with the exception of hyper and hypoglycemia. For this study, a reportable hypoglycemic event is defined as Level 3 (a severe event characterized by altered mental and/or physical status requiring assistance) and hyperglycemic event is one resulting in Diabetes Keto Acidosis (DKA).

7.1.5. Evaluation of Adverse events

A non-serious adverse event is an AE not classified as serious. Both serious and non-serious AEs should be graded with respect to severity on the following 3 point scale and reported, in detail, on the appropriate CRF page:

Mild	Discomfort noticed, but no disruption of normal daily activities; event usually requires no intervention.
Moderate	Discomfort sufficient to reduce or affect normal daily activities; even may require intervention.
Severe	Incapacitating, with inability to perform normal daily activities; event usually requires treatment or other intervention. Subject may not be able to continue in the study.

The Investigator should evaluate the relationship of each AE to the study treatment regimen, using the following criteria:

Unrelated:	Another cause of the AE is more plausible; a clinically plausible temporal sequence is inconsistent with the onset of the AE and administration of the study treatment regimen; or a causal relationship is considered biologically impossible.
Possibly related:	There is a clinically plausible time sequence between onset of the AE and administration of the study treatment regimen, but the AE could also be attributed to concurrent or underlying disease, or the use of other drugs or procedures. “Possibly Related” should be used when the study treatment regimen is one of several biologically plausible AE causes.
Definitely Related:	The AE is clearly related to use of the study treatment regimen.

7.1.6. Adverse Event Reporting and Monitoring

Study personnel will assess adverse events and the use of concomitant medications throughout the study. 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). The adverse event case report for the protocol must be documented for all adverse events (AE) of Grade 2 or greater severity regardless of relationship to therapy. For reporting serious adverse events (SAE), the MedWatch Form should also be completed and sent to medical monitor *within 24 hours of when the site was notified of the event.* This will be reviewed by the study physicians and Medical Monitor, and the Data and Safety Monitoring Board (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 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 treatment discontinuation will be reviewed by the DSMB.

Fatal or life-threatening events thought to be caused by study drug(s) should be reported to the Medical Monitor, or CRA immediately, as follows:

Position	Name	Telephone Number	Pager Number
Medical Monitor	George W. Burke,III M.D., FACS Professor of Surgery Director, Division of Kidney and Pancreas Transplantation, 1801 NW 9th Floor, Miami, FL 33136 Email: GBurke@med.miami.edu	(305) 355-5111	(305) 355-5134
CRA	Clinical Research Operations & Regulatory Support (CRORS), Miller Office of Research, University of Miami, Miller School of Medicine, Dominion Towers 4th floor, 1400 N.W. 10th Avenue; Suite 401, Miami, FL 33136	(305) 243-0133	(305) 243-5392

7.1.7. Recording Adverse Events

To improve the quality and precision of AE data, Investigator***s should observe the following*** guidelines:

- Whenever possible, use recognized medical terms when recording AEs on the AE page of the CRF. Do not use colloquialisms, jargon, or abbreviations.
- If known, record the diagnosis (ie, disease or syndrome) rather than component signs and symptoms on ae pages of the CRF (eg, record “congestive heart failure” rather than “dyspnea”, “rales”, and “cyanosis”). However, signs and symptoms that are considered unrelated to an encountered syndrome or disease should be recorded as individual AEs on the CRF page. For example, if congestive heart failure and severe headache are observed at the same time, each event should be recorded as an individual AE.
- Adverse events occurring secondary to other events (ie, sequelae) should be identified by the primary cause. A primary ae, if clearly identifiable, generally represents the most accurate clinical term to record on ae pages of the CFR. If a primary SAE is recorded on an AE CRF page, events occurring secondary to the primary event should be described in the narrative description of the event.
- Laboratory abnormalities that are defined as clinically significant are to be considered AEs and recorded on the AE CRF page.

7.1.8. Adverse Events Requiring Expedited Reporting

Serious AEs require expedited reporting to the Sponsor or designee, regardless of the relationship of the event to the study treatment regimen. Refer to the previous section for the definition of a serious adverse event.

Investigators should report all SAEs to the Sponsor or designee within 24 hours of the observation or learning of the event. For initial SAE reports, the Investigator should record all case details that can be garnered on the SAE form and the AE CRF page.

The completed SAE form and Cover Sheet should be faxed within 24 hours to the medical monitor at (305) 355-5134 or via email GBurke@med.miami.edu. Relevant follow-up information is to be submitted to the Sponsor or its designee as soon as it becomes available.

7.1.9. Special Reporting Situations

Death: Death is an outcome of an event. The event that resulted in death should be recorded and reported on the SAE form and the AE CRF page.

Hospitalization for Surgical or Diagnostic Procedures: The illness leading to the surgical or diagnostic procedure is to be recorded as the SAE, not the procedure itself. The procedure is to be captured in the case narrative as part of the action taken in response to the illness.

7.1.10. Laboratory Test Result Abnormalities

The following laboratory test result abnormalities should be captured on the non-serious AE CRF page or SAE Report Form (paper or electronic) as appropriate:

- Any laboratory test result that is clinically significant or meets the definition of an SAE
- Any laboratory test result abnormality that required the subject to have study drug discontinued or interrupted
- Any laboratory test result abnormality that required the subject to receive specific corrective therapy.

It is expected that wherever possible, the clinical rather than laboratory term would be used by the reporting investigator (e.g. anemia versus low hemoglobin value).

7.1.11. Pregnancy

If, following initiation of the study product(s), it is subsequently discovered that a study subject is pregnant or may have been pregnant at the time of investigational product exposure, including up to 12 weeks after last product administration, the study product(s) will be permanently discontinued in an appropriate manner (e.g. dose tapering if necessary for subject safety).

They must be reported to the medical monitor within 24 hours by fax to (305) 355-5134 with a confirmatory telephone call to (305) 355-5111 and in accordance with SAE reporting procedures described above.

Any pregnancy that occurs in a female partner of a male study participant should be reported to the medical monitor.

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7.1.12. Overdose

All occurrences of overdose must be reported as SAEs (see SAE reporting section for details).

An overdose is defined as the accidental or intentional administration of any dose of a product that is considered both excessive and medically important. All occurrences of overdose must be reported as an SAE.

7.1.13. Other Safety Considerations

Any significant worsening noted during interim or final physical examinations, electrocardiogram, x-ray filming, any other potential safety assessment required or not required by protocol should also be recorded as a non-serious or serious AE, as appropriate, and reported accordingly.

8. DATA QUALITY ASSURANCE

Accurate, consistent, and reliable data will be ensured through the use of standard practices and procedures. These are described in the following sections.

8.1. Data Collection, Monitoring, and Transfer

Adverse events will be collected from the initiation of study drug and be followed to resolution or until 30 days after the participant completes participation, whichever comes first.

Adverse events may be collected as follows:

- Observing the participant.
- Questioning the participant in an objective manner.
- Receiving an unsolicited complaint from the participant.

An abnormal value or result from a clinical or laboratory can also indicate an adverse event. If this is the case, then the evaluation that produced the value or result should be repeated until the value or result returns to normal or can be explained and the participant's safety is not at risk. If an abnormal value or result is determined by the investigator to be clinically significant, it must be indicated as such on the appropriate laboratory evaluation form(s), and must also be reported as an adverse event on the adverse event form. Abnormal vital sign measurements or laboratory finding deemed not clinically significant by the site investigator must be documented as such and are not required to be listed in the

adverse event source documents or adverse event case report form. The DRI will perform data collection, monitoring, and transfer tasks. A Clinical Research Monitor designated by Sponsor will be responsible for monitoring the clinical study in accordance with current federal and relevant foreign regulations. The Monitor will periodically collect the CRFs and brought them to Sponsor. Data on CRFs will be double-entered into the database system and verified.

8.2. Quality Control and Quality Assurance

The database will be verified against the CRFs as outlined in the monitoring plan.

9. STATISTICAL CONSIDERATIONS AND ANALYSIS PLAN

9.1. Overview

This study will test the hypothesis that combination therapy, administered for one year to patients with either new onset or established T1D with residual stimulated C-peptide, will retain C-peptide secretion compared to therapy with vitamin D alone.

9.2. Endpoints/Outcomes

Primary endpoint:

- Stimulated (90 minute sample of a MMTT) C-peptide at the 1 year visit greater or equal to baseline level.

Secondary Objective(s)

- Stimulated C-peptide area under the curve during a 4-hour MMTT greater or equal to baseline level, at the 1 year visit.
- Reduction in HbA1c at the one year visit compared to baseline.
- Reduction in insulin requirement at the 1 year visit compared to baseline.
- Incidence of adverse events (AE) comparable to general diabetes population.

9.3. Measures to Minimize Bias

Participants who completed the screening visit and are determine to be eligible for study participation will undergo randomization. Participants will be randomized by the study PI at the clinical site. The participants will be informed regarding the intervention assignment.

9.4. Analysis Plan

The details of the analyses will be provided in the statistical analysis plan (SAP) below.

9.4.1. Analysis Populations

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. 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.

9.4.2. Primary Analysis of Primary Endpoint(s)/Outcome(s)

The metabolic primary outcome is the 90-minute stimulated C-peptide following MMTT, conducted at the 1 year visit. The primary statistical hypothesis to be assessed is that the proportion of patients with maintained or increased level of 90-minute at 12 months as compared to baseline will be greater in subjects receiving combination therapy than for subjects receiving vitamin D alone.

The analysis of the primary outcome will initially involve a two sample test of proportions comparing the proportion of patients in the combination therapy group maintaining or increasing 90-C-peptide with respect to baseline with the proportation of vitamin D alone treated subjects with 90-minute C-peptide that is maintained or increased from baseline value. A covariate-adjusted analysis will additionally be performed by way of a logistic regression model, with a categorical index of treatment group adjusted for baseline age and time since diagnosis; this can provide us with adjustment for potential confounding of these factors and/or provide increased efficiency and precision by including these potentially significant explanatory variables.

9.4.3. Supportive Analyses of the Primary Endpoint(s)/Outcome(s)

90-min C-peptide measured at screening and , 12 months and 24 months in the different treatment groups will additionally be fit to a mixed linear regression model, allowing estimation and testing of means and differences between groups at each time point, while simultaneously accommodating the correlation arising from the longitudinal study design in estimates of variances used in tests of significance. We will also estimate and test the differential rate of change in mean 90-min C-peptide over time between these groups, by incorporating an interaction term between time point and treatment group into the models. Although this investigation is not specifically powered to test this interaction, this might provide us with useful insight as to the differences in the effect of combination therapy over time on 90-min C-peptide.

9.4.4. Descriptive Analyses

In addition to the detailed statistical analysis described above, descriptive analysis will include reporting and comparison baseline characteristics of the study subjects to evaluate whether any substantial differences exist between the vitamin D alone and combination treatment groups for key variables such as age, gender, BMI, HbA1c, baseline C-peptide, insulin requirements and other variables such as concomitant use of other medications to determine any potential bias. These will involve standard two sample comparisons such as T-tests for continuous variables (possibly transformed when necessary to preserve distributional assumptions) and Chi-square or Fisher’s exact tests for categorical variables. As described earlier, potentially confounding factors arising from imbalance will be included into more formal statistical models of the investigation.

9.5. Interim Analyses

The DSMB will be convened to review safety and efficacy data. Descriptive interim analyses will include distributions of endpoints, and adverse events. Interim analyses will be conducted periodically during the study and will be reviewed by the DSMB for assessment of effectiveness and safety. Group-sequential spending function with an O'Brien-Fleming boundaries may be used to protect the type I error probability from early and multiple testing and to assess the significance of the interim results that emerge during the trial.

9.5.1. Interim Analysis of Efficacy Data

Interim analysis for efficacy will be planned when half the subjects have reached their endpoint. However we note that this is a small trial and if we were to consider a priori planning for interim analysis the limited number of observations could lead to unstable estimates of efficacy. This can be further discussed with the DSMB. The inflation factor needed for augmenting sample size to preserve power if we were to plan for interim analysis at one look with group sequential methods using standard Obrien-Fleming boundaries is 1.02, which does not significantly affect the parameters of our design (sample size needs to be inflated by 1.02).

9.5.2. Interim Analysis of Safety Data

Interim analysis may be conducted as recommended by the DSMB, to review safety data, including metabolic, immunological assessments and adverse events. The DSMB may recommend early stopping if there is sufficient evidence to conclude that the investigational treatment is harmful based on interim analysis of safety endpoints.

At any time, if there are significantly more adverse events in the experimental arm based on Fisher's exact tests for comparison, early termination should be considered. For example, a scenario where 9 patients have enrolled with 6 in the combination treatment arm and 3 in the vitamin D alone arm with 5 adverse events in the combination treatment arm and 0 in the vitamin D treatment arm would be considered significantly increased likelihood of adverse events in the combination treatment arm and would potentially result in trial termination. The same holds in the scenario where 15 patients have been enrolled with 10 in the combination treatment arm and 5 in the vitamin D alone treatment arm, where the combination treatment group experiences 9 adverse events while the vitamin D group experiences only 1. Similar scenarios exist throughout the trial and at any time if the exact test comparing groups under the null that the likelihood of adverse events is equal between groups exceeds a significance threshold of 0.05, this would warrant early termination.

9.5.3. Futility Analysis

Futility is unlikely to be shown during a one year treatment period, but the DSMB can decide to terminate the study should futility be shown. We do not intend to stop the study for futility as we intend to obtain an estimate and 95% confidence interval for the effect of treatment between groups at the 1 year mark

using the full information offered by our design. Additionally, not stopping for futility incorporates the consideration that the C-peptide AUC curves of combination therapy and vitamin D alone groups may separate later on follow-up, as AUC values decline further in the vitamin D alone group. Essentially all type 1 diabetes trials have been allowed to reach their one-year endpoint, even those that showed no efficacy.

9.6. Statistical Hypotheses

Primary hypothesis:

- Null: Patients receiving combination supplement therapy and patients in the Vitamin-D alone group have comparable proportions with maintained or increased C-peptide production, assessed by the measurement of the stimulated 90-minute C-peptide at one year of follow up.
- Alternative: Patients receiving combination therapy have higher proportions with maintained or increased C-peptide production, assessed by the measurement of the stimulated 90-minute C-peptide as compared to the proportion of patients with maintained or increased C-Peptide in the vitamin D alone group at one year of follow up.

9.7. Sample Size Considerations

Metabolic primary outcome

Sample size considerations correspond to the primary hypothesis which is that the proportion of patients with maintained or increased 90-min C-pepetide at 12 months compared to baseline value is greater in the combination therapy group than in the vitamin D alone group. Standard power calculations for two sample tests of proportions indicate that 12 subjects in each group are needed to achieve 80% power to detect differences between less than 5% in the vitamin D alone group and at least 50% in the combination therapy group. Allowing for 10% dropout/attrition, sample size considerations include an allowance for 10% of the subjects to have missing data (one-year MMTT was not done or subject withdrew prior to the one-year assessment).

Primary endpoints should be available for all enrolled subjects. An exception will be if a death occurs or if the subject withdraws consent to be followed, although we expect this to be very limited. We also have included consideration for attrition in our design. Should there be any missing data, missing data will be assessed for mechanism of missingness and to see if missing at random assumptions are valid. If necessary, appropriate imputation strategies can be applied for sensitivity analysis. We will explore the data to assess if missingness at a given time point is related to observed data at the previous time point. If we find that, conditional on treatment group, observed values strongly predict subsequent values where observed, but are not related to missingness at subsequent time points, we may regard the missing data as a random sample of previously observed data.

10. ETHICAL CONSIDERATIONS & COMPLIANCE WITH GOOD CLINICAL PRACTICE

10.1. Statement of Compliance

The study will be conducted in accordance with the protocol, Good Clinical Practices, the relevant ICH guidelines, the applicable regulatory requirements, and the ethical principles that have their origins in the Declaration of Helsinki. As required by United States Food and Drug Administration (FDA) Code of Federal Regulations (CFR) (21 CFR 56) and the Declaration of Helsinki, the study protocol, amendments, and Informed Consent form will be reviewed and approved, according to 21 CFR §50 and §56, respectively, by University of Miami's IRB. Any amendments to the protocol or consent materials must also be approved before they are implemented.

10.2. Informed Consent

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

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

10.3. Study Subject Confidentiality

The investigational site participating in this study will maintain the highest degree of confidentiality permitted for the clinical and research information obtained from subjects. As a part of the quality assurance and legal responsibilities of an investigation, the investigational site must permit authorized representatives of the Quality Assurance division of University of Miami 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. Study records with the study subject's information for internal use at the clinical site 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 Diabetes Research Institute. Case report forms will identify participants by the unique Identification Number. At the end of the study, all study databases will be archived at the Center, and the data collection forms will be saved for long-term storage.

Stored samples including genetic 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, and any mechanistic studies will not be made known to the participant.

11. STUDY ADMINISTRATION

This study is investigator initiated study sponsored by the investigators from Diabetes Research Institute. Funding will cover the costs of administration and laboratory tests associated with this study during the participant's period of follow-up and procedures related to drug administration. Study drugs will be free of charge for the participant's entire length of treatment.

Since dosage of study drugs is dependent on blood levels (i.e., 25OH vitamin D and AA/EPA ratio) and results may take up to 1 week to be ready following blood collection during the participants' visits, participants who live far from the study site and logistically cannot come to the site to pick-up their study drug outside of their scheduled study visit windows, may have the study drugs shipped to them. Both study drugs, vitamin D and omega 3 fatty acids, are stable at room temperature and do not require additional special handling during shipment except for adequate packing of the omega 3 bottles to ensure they do not break during transportation.

11.1. Groups and Committees

11.1.1. Protocol Development Committee

As acknowledged on the preface of this study protocol, protocol development committee met periodically over four months to finalize this protocol. Members of this committee bring interdisciplinary and translational expertise in pediatrics, immunology, transplantation and endocrinology of T1D, research nurse, research pharmacist and regulatory experts. Committee consists of Subject Matter Experts with unique and proven expertise in different immune pathways involved in T1D.

11.1.2. Clinical Site

The study will be conducted at Diabetes Research Institute and Mailman Center for Child Development. Principal Investigators of the participating clinical site will oversee all operations. The clinical sites will conduct the trial and collect all laboratory and data collection form information for analysis. Clinical research team meets every Friday of the week to discuss development and issues related to all clinical research studies at the institute and facilitate evaluation of the trial management.

11.1.3. Medical Monitor for the Clinical Protocol

This trial includes the medical monitor independent of the study (George Burke, M.D., Professor of Surgery at the University of Miami Miller School of Medicine).

11.1.4. Clinical Site Monitoring

In order to conduct this study consistent with established research principles and ICH-GCP guidelines, there will be site visits conducted during the study to evaluate study conduct. Clinical Research Operation and Regulatory Support (CRORS) is part of the Research, Research Education & Innovative Medicine (RIM) Department, within the Miller School of Medicine at the University of Miami. The CRORS is independent of the Diabetes Research Institute and will provide source data verification and periodic monitoring 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). The site will be monitored by the Clinical Research Associates from the CRORS.

11.1.5. Data and Safety Monitoring Board (DSMB)

The members of the DSMB are independent and serve in an individual capacity to provide their expertise and recommendations. They will review the 90 day data of the first 24 subjects, and then periodically review as per the charter and evaluate the accumulated study data for participant safety, study conduct and progress, and, when appropriate, efficacy, and make recommendations to study investigators concerning the continuation, modification, or termination of the trial. The DSMB members are experts in the field of T1D immunotherapy and will consider study-specific data as well as relevant background knowledge about the disease, test agents, and patient population under study. All adverse events will be recorded on the adverse event forms, which will be provided to the local IRBs, per their reporting requirements. The DSMB will independently evaluate whether the adverse events constitute grounds to discontinue the study.

11.1.6. DRI Office of Regulatory Support

This office of Quality Assurance and Regulatory Affairs provides standardized auditing functions, educational training, and regulatory support for clinical research at the Diabetes Research Institute.

11.1.7. Human Subjects Research Office & Institutional Review Boards (HSRO & IRB)

The HSRO provides administrative support for the University of Miami IRBs, which review proposed research involving human subjects to ensure the protection of those subjects and regulatory compliance.

11.1.8. Clinical Research Centers

Pediatric endocrinology clinics at the Mailman Center for Child Development and adult endocrinology clinics at Diabetes Research Institute and Clinical Translational Research Site at the University of Miami Miller School of Medicine provide comfortable, safe and effective environment to conduct professional, high-quality research involving human subjects.

11.1.9. Investigational Drug Services (Research Pharmacy)

The Investigational Drug Service (IDS) was established to provide pharmacy support to investigators in managing randomized, clinical trials at the University of Miami. The IDS also has the capability to dispensing complex investigational therapies and as drug safety experts therein can provide customized value that is increasingly recognized as a mechanism for assuring safety of study patients, ensuring adherence to protocol guidelines, and minimizing the liability associated in conducting drugs or supplement trials.

11.1.10. Core Laboratories

Core laboratories participating in the study include:

- Clinical research laboratories routinely providing services to the University of Miami.
- The Laboratory of Dr. Alberto Pugliese at the Diabetes Research Institute, University of Miami, Miami, Florida, where samples from patients will be processed, divided in aliquots, frozen as needed for future analysis.

11.2. Preservation of the Integrity of the Study

All presentations and publications using study data must protect the main objectives of the trial. Data integrity will be maintained and the investigators will publish study results and will apply accepted academic policies and regulations governing the publication.

11.3. Participant Reimbursement and Compensation

Participants will not be compensated for visits attended in the study. The supplement(s) during the course of the study will be provided at no charge to the subjects.

12. STUDY TIMELINE

It is anticipated that patient enrollment will occur during the first three years of the trial. All subjects will be followed until one year after initial treatment, with up to one year further follow-up after the treatment period has ended. Study visits will have a window of ±14 days starting from visit 2.

APPENDIX A: Schedule of Events (SOE)

Visit number	0	1	2	3	4	5 F1	6 F2	7 F3
Days of the trial[1]	-1	1	120	240	360	480	600	720
Months	1	1	4	8	12	16	20	24
Informed Consent	X							
Medical History	X		X	X	X	X	X	X
Physical Exam[2]	X		X	X	X	X	X	X
CBC with differential	X	X	X	X	X			
Prothrombin time (PT/PT-INR)	X		X	X	X	X	X	X
Chemistries	X		X	X	X			
Pregnancy test (blood sample)[5]	X		X	X	X	X		
Serum for islet autoantibodies	X		X	X	X	X	X	X
Serology	X							
Mechanistic assessments[3]		X	X	X	X	X	X	X
Hemoglobin A1c	X		X	X	X	X	X	X
Stimulated C-peptide at 90 min	X				X			X
MMTT 4 hour	X				X			X
Randomization		X						
Diabetes Assessments (insulin adjustment and Hypoglycemia awareness)	X	X	X	X	X	X	X	X
Clarke Score	X				X			X
Dispensing or Administration of Study Drug • Omega-3 Fatty Acids + Vitamin-D OR • Vitamin-D		X[6]	X	X				
Vitamin D levels [4]	X		X	X	X	X	X	X
AA/EPA Ratio[7]	X		X	X	X	X	X	X
Counting of Returned Study Drug			X	X	X			
Subject 7-day blood sugar record and hypoglycemia log	X		X	X	X	X	X	X
Concomitant Medication Review	X	X	X	X	X			
Adverse Events Assessment			X	X	X	X	X	X

1. Screening Visit: Screening MMTT must be at least 3 weeks after diagnosis and preferably within one month (37 days) of randomization for recent onset T1D. For established T1D, screening must take place after 180 days of diagnosis.
2. Physical Exam: Routine exam at screening and quarterly.

3. Mechanistic Assessments: includes samples for RNA, plasma, serum, DNA, measures of B and T cell number/function and cytokine profiles. The schedule for these assessments may vary as appropriate. At no time will the blood draw volume exceed what is allowable according to the subject's body weight.
4. Vitamin D levels will be monitored every 4 months or more frequently at the discretion of the investigator in order to adjust dose to achieve target levels.
5. Pregnancy testing will be conducted in females in reproductive age at 4, 8,12 and 16 months study visit.
6. Study drug(s) will be administered within 48hr from baseline visit (visit 1).
7. Dose will be adjusted to maintain a target AA/EPA ratio of 1.5-3.0. AA/EPA ratios will be monitored monthly until achieving target levels and as per protocol or as clinically indicated thereafter.

F1-F3. Follow-Up after 12 Months: Visits will be conducted approximately every 4 months (16, 20, and 24 months).

APPENDIX B: Blood Sample Collection Amounts by Age Group and Weight

- **Ages ≥18 y/o (Adults)**

Maximum Draw Allowed Per 8 Week Period: 10.5 mL/kg or 550 mL

Visit number	0	1	2	3	4	5 F1	6 F2	7 F3
Days of the trial	-1	1	120	240	360	480	600	720
Months	1	1	4	8	12	16	20	24
CBC with differential	2 mL	2 mL	2 mL	2 mL	2 mL			
Prothrombin time (PT/PT-INR)	1.8 mL		1.8 mL	1.8 mL	1.8 mL	1.8 mL	1.8 mL	1.8 mL
Chemistries	3.5 mL		3.5 mL	3.5 mL	3.5 mL			
Pregnancy test ¥	¥		¥	¥	¥	3.5 mL		
Serum for islet autoantibodies	3.5 mL		3.5 mL	3.5 mL	3.5 mL	3.5 mL	3.5 mL	3.5 mL
Serology	3.5 mL							
Mechanistic assessments§		21 mL	21 mL	21 mL	21 mL	21 mL	21 mL	21 mL
Hemoglobin A1c	2 mL		2 mL	2 mL	2 mL	2 mL	2 mL	2 mL
Simulated C-peptide at 90 min*	X				X			X
MMTT 4 hour	38.5 mL				38.5 mL			
Vitamin D levels	3.5 mL		3.5 mL	3.5 mL	3.5 mL	3.5 mL	3.5 mL	3.5 mL
AA/EPA Ratio	2 mL		2 mL	2 mL	2 mL	2 mL	2 mL	2 mL
Total Blood collection per visit	60.3 mL	23 mL	39.3 mL	39.3 mL	77.8 mL	37.3 mL	33.8 mL	33.8 mL

¥ Pregnancy test for visits -1; 120; 240, and 360 will be performed using same serum sample for chemistry**.** For visit 480 a 3.5 mL SST tube (serum sample) will be collected to perform the test in the absence of a chemistry sample requirement

§ Mechanistic Assessments: includes samples for RNA, plasma, serum, DNA, measures of B and T cell number/function and cytokine profiles. The schedule for these assessments may vary as appropriate. At no time will the blood draw volume exceed what is allowable according to the subject's body weight.

Mechanistic Samples (total volume: 21 ml) as follows:

Pro-inflammatory cytokines: 3.5 ml
Phenotyping: 2 mL
RNA: 2.5 mL
DNA: 2.5 mL
Resolvins: 2 mL
LPS activation T-cell stimulation assay: 3 mL
Plasma: 2 mL
Serum: 3.5 mL

*****Stimulated c-peptide at 90 min is a time point of the MMTT. No extra sample collection needed**.**

- **Ages 6 to <18 y/o**
- **Weight at screening visit (Kg): 27 and greater**

Maximum Draw Allowed Per Day (5mL/Kg): 135 mL/kg
Maximum Draw Allowed Per 8 Week Period (9.5 mL/Kg): 257 mL

Visit number	**0**	**1**	**2**	**3**	**4**	**5 F1**	**6 F2**	**7 F3**
Days of the trial	**-1**	**1**	**120**	**240**	**360**	**480**	**600**	**720**
Months	**1**	**1**	**4**	**8**	**12**	**16**	**20**	**24**
CBC with differential	**2 mL**	**2 mL**	**2 mL**	**2 mL**	**2 mL**			
Prothrombin time (PT/PT-INR)	**1.8 mL**		**1.8 mL**	**1.8 mL**	**1.8 mL**	**1.8 mL**	**1.8 mL**	**1.8 mL**
Chemistries	**3.5 mL**		**3.5 mL**	**3.5 mL**	**3.5 mL**			
Pregnancy test **¥**	**¥**		**¥**	**¥**	**¥**	**3.5 mL**		
Serum for islet autoantibodies	**3.5 mL**		**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**
Serology	**3.5 mL**							
Mechanistic assessments**§**		**21 mL**	**21 mL**	**21 mL**	**21 mL**	**21 mL**	**21 mL**	**21 mL**
Hemoglobin A1c	**2 mL**		**2 mL**	**2 mL**	**2 mL**	**2 mL**	**2 mL**	**2 mL**
Simulated C-peptide at 90 min*****	**X**				**X**			**X**
MMTT 4 hour	**38.5 mL**				**38.5 mL**			
Vitamin D levels	**3.5 mL**		**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**
AA/EPA Ratio	**2 mL**		**2 mL**	**2 mL**	**2 mL**	**2 mL**	**2 mL**	**2 mL**
Total Blood collection per visit	**60.3 mL**	**23 mL**	**39.3 mL**	**39.3 mL**	**77.8 mL**	**37.3 mL**	**33.8 mL**	**33.8 mL**

¥ Pregnancy test for visits -1; 120; 240, and 360 will be performed using same serum sample for chemistry**.** For visit 480 a 3.5 mL SST tube (serum sample) will be collected to perform the test in the absence of a chemistry sample requirement

§ Mechanistic Assessments: includes samples for RNA, plasma, serum, DNA, measures of B and T cell number/function and cytokine profiles. The schedule for these assessments may vary as appropriate. At no time will the blood draw volume exceed what is allowable according to the subject's body weight.

Mechanistic Samples (total volume: 21 ml) as follows:

Pro-inflammatory cytokines: 3.5 ml
Phenotyping: 2 mL
RNA: 2.5 mL
DNA: 2.5 mL
Resolvins: 2 mL
LPS activation T-cell stimulation assay: 3 mL
Plasma: 2 mL
Serum: 3.5 mL

*****Stimulated c-peptide at 90 min is a time point of the MMTT. No extra sample collection needed**.**

- **Ages 6 to <18 y/o**
- **Weight at screening visit (Kg): 25 to < 27**

Maximum Draw Allowed Per Day (5mL/Kg): 125 mL/kg
Maximum Draw Allowed Per 8 Week Period (9.5 mL/Kg): 238 mL

Visit number	**0**	**1**	**2**	**3**	**4**	**5 F1**	**6 F2**	**7 F3**
Days of the trial	**-1**	**1**	**120**	**240**	**360**	**480**	**600**	**720**
Months	**1**	**1**	**4**	**8**	**12**	**16**	**20**	**24**
CBC with differential	**2 mL**	**2 mL**	**2 mL**	**2 mL**	**2 mL**			
Prothrombin time (PT/PT-INR)	**1.8 mL**		**1.8 mL**	**1.8 mL**	**1.8 mL**	**1.8 mL**	**1.8 mL**	**1.8 mL**
Chemistries	**3.5 mL**		**3.5 mL**	**3.5 mL**	**3.5 mL**			
Pregnancy test **¥**	**¥**		**¥**	**¥**	**¥**	**3.5 mL**		
Serum for islet autoantibodies	**3.5 mL**		**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**
Serology	**3.5 mL**							
Mechanistic assessments**§**		**21 mL**	**21 mL**	**21 mL**	**21 mL**	**21 mL**	**21 mL**	**21 mL**
Hemoglobin A1c	**2 mL**		**2 mL**	**2 mL**	**2 mL**	**2 mL**	**2 mL**	**2 mL**
Simulated C-peptide at 90 min*****	**X**				**X**			**X**
MMTT 4 hour	**38.5 mL**				**38.5 mL**			
Vitamin D levels	**3.5 mL**		**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**
AA/EPA Ratio	**2 mL**		**2 mL**	**2 mL**	**2 mL**	**2 mL**	**2 mL**	**2 mL**
Total Blood collection per visit	**60.3 mL**	**23 mL**	**39.3 mL**	**39.3 mL**	**77.8 mL**	**37.3 mL**	**33.8 mL**	**33.8 mL**

¥ Pregnancy test for visits -1; 120; 240, and 360 will be performed using same serum sample for chemistry**.** For visit 480 a 3.5 mL SST tube (serum sample) will be collected to perform the test in the absence of a chemistry sample requirement

§ Mechanistic Assessments: includes samples for RNA, plasma, serum, DNA, measures of B and T cell number/function and cytokine profiles. The schedule for these assessments may vary as appropriate. At no time will the blood draw volume exceed what is allowable according to the subject's body weight.

Mechanistic Samples (total volume: 21 ml) as follows:

Pro-inflammatory cytokines: 3.5 ml
Phenotyping: 2 mL
RNA: 2.5 mL
DNA: 2.5 mL
Resolvins: 2 mL
LPS activation T-cell stimulation assay: 3 mL

Plasma: 2 mL
Serum: 3.5 mL

*****Stimulated c-peptide at 90 min is a time point of the MMTT. No extra sample collection needed**.**

- **Ages 6 to <18 y/o**
- **Weight at screening visit (Kg): 23 to < 25**

Maximum Draw Allowed Per Day (5mL/Kg): 115 mL/kg
Maximum Draw Allowed Per 8 Week Period (9.5 mL/Kg): 219 mL

Visit number	**0**	**1**	**2**	**3**	**4**	**5 F1**	**6 F2**	**7 F3**
Days of the trial	**-1**	**1**	**120**	**240**	**360**	**480**	**600**	**720**
Months	**1**	**1**	**4**	**8**	**12**	**16**	**20**	**24**
CBC with differential	**2 mL**	**2 mL**	**2 mL**	**2 mL**	**2 mL**			
Prothrombin time (PT/PT-INR)	**1.8 mL**		**1.8 mL**	**1.8 mL**	**1.8 mL**	**1.8 mL**	**1.8 mL**	**1.8 mL**
Chemistries	**3.5 mL**		**3.5 mL**	**3.5 mL**	**3.5 mL**			
Pregnancy test **¥**	**¥**		**¥**	**¥**	**¥**	**3.5 mL**		
Serum for islet autoantibodies	**3.5 mL**		**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**
Serology	**3.5 mL**							
Mechanistic assessments**§**		**22 21 mL**	**21 mL**	**21 mL**	**21 mL**	**21 mL**	**21 mL**	**21 mL**
Hemoglobin A1c	**2 mL**		**2 mL**	**2 mL**	**2 mL**	**2 mL**	**2 mL**	**2 mL**
Simulated C-peptide at 90 min*****	**X**				**X**			**X**
MMTT 4 hour	**38.5 mL**				**38.5 mL**			
Vitamin D levels	**3.5 mL**		**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**
AA/EPA Ratio	**2 mL**		**2 mL**	**2 mL**	**2 mL**	**2 mL**	**2 mL**	**2 mL**
Total Blood collection per visit	**60.3 mL**	**23 mL**	**39.3 mL**	**39.3 mL**	**77.8 mL**	**37.3 mL**	**33.8 mL**	**33.8 mL**

¥ Pregnancy test for visits -1; 120; 240, and 360 will be performed using same serum sample for chemistry**.** For visit 480 a 3.5 mL SST tube (serum sample) will be collected to perform the test in the absence of a chemistry sample requirement
§ Mechanistic Assessments: includes samples for RNA, plasma, serum, DNA, measures of B and T cell number/function and cytokine profiles. The schedule for these assessments may vary as appropriate. At no time will the blood draw volume exceed what is allowable according to the subject's body weight.

Mechanistic Samples (total volume: 21 ml) as follows:

Pro-inflammatory cytokines: 3.5 ml
Phenotyping: 2 mL
RNA: 2.5 mL
DNA: 2.5 mL

Resolvins: 2 mL
LPS activation T-cell stimulation assay: 3 mL
Plasma: 2 mL
Serum: 3.5 mL

*****Stimulated c-peptide at 90 min is a time point of the MMTT. No extra sample collection needed**.**

- **Ages 6 to <18 y/o**
- **Weight at screening visit (Kg): 21 to < 23**

Maximum Draw Allowed Per Day (5mL/Kg): 105 mL/kg
Maximum Draw Allowed Per 8 Week Period (9.5 mL/Kg): 200 mL

Visit number	**0**	**1**	**2**	**3**	**4**	**5 F1**	**6 F2**	**7 F3**
Days of the trial	**-1**	**1**	**120**	**240**	**360**	**480**	**600**	**720**
Months	**1**	**1**	**4**	**8**	**12**	**16**	**20**	**24**
CBC with differential	**2 mL**	**2 mL**	**2 mL**	**2 mL**	**2 mL**			
Prothrombin time (PT/PT-INR)	**1.8 mL**		**1.8 mL**	**1.8 mL**	**1.8 mL**	**1.8 mL**	**1.8 mL**	**1.8 mL**
Chemistries	**3.5 mL**		**3.5 mL**	**3.5 mL**	**3.5 mL**			
Pregnancy test **¥**	**¥**		**¥**	**¥**	**¥**	**3.5 mL**		
Serum for islet autoantibodies	**3.5 mL**		**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**
Serology	**3.5 mL**							
Mechanistic assessments**§**		**21 mL**	**21 mL**	**21 mL**	**21 mL**	**21 mL**	**21 mL**	**21 mL**
Hemoglobin A1c	**2 mL**		**2 mL**	**2 mL**	**2 mL**	**2 mL**	**2 mL**	**2 mL**
Simulated C-peptide at 90 min*****	**X**				**X**			**X**
MMTT 4 hour	**38.5 mL**				**38.5 mL**			
Vitamin D levels	**3.5 mL**		**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**
AA/EPA Ratio	**2 mL**		**2 mL**	**2 mL**	**2 mL**	**2 mL**	**2 mL**	**2 mL**
Total Blood collection per visit	**60.3 mL**	**23 mL**	**39.3 mL**	**39.3 mL**	**77.8 mL**	**37.3 mL**	**33.8 mL**	**33.8 mL**

¥ Pregnancy test for visits -1; 120; 240, and 360 will be performed using same serum sample for chemistry**.** For visit 480 a 3.5 mL SST tube (serum sample) will be collected to perform the test in the absence of a chemistry sample requirement
§ Mechanistic Assessments: includes samples for RNA, plasma, serum, DNA, measures of B and T cell number/function and cytokine profiles. The schedule for these assessments may vary as appropriate. At no time will the blood draw volume exceed what is allowable according to the subject's body weight.

Mechanistic Samples (total volume: 21 ml) as follows:

Pro-inflammatory cytokines: 3.5 ml

Phenotyping: 2 mL
RNA: 2.5 mL
DNA: 2.5 mL
Resolvins: 2 mL
LPS activation T-cell stimulation assay: 3 mL
Plasma: 2 mL
Serum: 3.5 mL

*****Stimulated c-peptide at 90 min is a time point of the MMTT. No extra sample collection needed**.**

- **Ages 6 to <18 y/o**
- **Weight at screening visit (Kg): 19 to < 21**

Maximum Draw Allowed Per Day (5mL/Kg): 95 mL/kg
Maximum Draw Allowed Per 8 Week Period (9.5 mL/Kg): 181 mL

Visit number	**0**	**1**	**2**	**3**	**4**	**5 F1**	**6 F2**	**7 F3**
Days of the trial	**-1**	**1**	**120**	**240**	**360**	**480**	**600**	**720**
Months	**1**	**1**	**4**	**8**	**12**	**16**	**20**	**24**
CBC with differential	**2 mL**	**2 mL**	**2 mL**	**2 mL**	**2 mL**			
Prothrombin time (PT/PT-INR)	**1.8 mL**		**1.8 mL**	**1.8 mL**	**1.8 mL**	**1.8 mL**	**1.8 mL**	**1.8 mL**
Chemistries	**3.5 mL**		**3.5 mL**	**3.5 mL**	**3.5 mL**			
Pregnancy test **¥**	**¥**		**¥**	**¥**	**¥**	**3.5 mL**		
Serum for islet autoantibodies	**3.5 mL**		**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**
Serology	**3.5 mL**							
Mechanistic assessments[§]		**21 mL**	**21 mL**	**21 mL**	**21 mL**	**21 mL**	**21 mL**	**21 mL**
Hemoglobin A1c	**2 mL**		**2 mL**	**2 mL**	**2 mL**	**2 mL**	**2 mL**	**2 mL**
Simulated C-peptide at 90 min*****	**X**				**X**			**X**
MMTT 4 hour	**38.5 mL**				**38.5 mL**			
Vitamin D levels	**3.5 mL**		**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**
AA/EPA Ratio	**2 mL**		**2 mL**	**2 mL**	**2 mL**	**2 mL**	**2 mL**	**2 mL**
Total Blood collection per visit	**60.3 mL**	**23 mL**	**39.3 mL**	**39.3 mL**	**77.8 mL**	**37.3 mL**	**33.8 mL**	**33.8 mL**

¥ Pregnancy test for visits -1; 120; 240, and 360 will be performed using same serum sample for chemistry**.** For visit 480 a 3.5 mL SST tube (serum sample) will be collected to perform the test in the absence of a chemistry sample requirement
§ Mechanistic Assessments: includes samples for RNA, plasma, serum, DNA, measures of B and T cell number/function and cytokine profiles. The schedule for these assessments may vary as appropriate. At no time will the blood draw volume exceed what is allowable according to the subject's body weight.

Mechanistic Samples (total volume: 21 ml) as follows:

Pro-inflammatory cytokines: 3.5 ml
Phenotyping: 2 mL
RNA: 2.5 mL
DNA: 2.5 mL
Resolvins: 2 mL
LPS activation T-cell stimulation assay: 3 mL
Plasma: 2 mL
Serum: 3.5 mL

*****Stimulated c-peptide at 90 min is a time point of the MMTT. No extra sample collection needed**.**

- **Ages 6 to <18 y/o**
- **Weight at screening visit (Kg): 17 to < 19**

Maximum Draw Allowed Per Day (5mL/Kg): 85 mL/kg
Maximum Draw Allowed Per 8 Week Period (9.5 mL/Kg): 162 mL

Visit number	**0**	**1**	**2**	**3**	**4**	**5 F1**	**6 F2**	**7 F3**
Days of the trial	**-1**	**1**	**120**	**240**	**360**	**480**	**600**	**720**
Months	**1**	**1**	**4**	**8**	**12**	**16**	**20**	**24**
CBC with differential	**2 mL**	**2 mL**	**2 mL**	**2 mL**	**2 mL**			
Prothrombin time (PT/PT-INR)	**1.8 mL**		**1.8 mL**	**1.8 mL**	**1.8 mL**	**1.8 mL**	**1.8 mL**	**1.8 mL**
Chemistries	**3.5 mL**		**3.5 mL**	**3.5 mL**	**3.5 mL**			
Pregnancy test **¥**	**¥**		**¥**	**¥**	**¥**	**3.5 mL**		
Serum for islet autoantibodies	**3.5 mL**		**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**
Serology	**3.5 mL**							
Mechanistic assessments[§]		**21 mL**	**21 mL**	**21 mL**	**21 mL**	**21 mL**	**21 mL**	**21 mL**
Hemoglobin A1c	**2 mL**		**2 mL**	**2 mL**	**2 mL**	**2 mL**	**2 mL**	**2 mL**
Simulated C-peptide at 90 min*****	**X**				**X**			**X**
MMTT 4 hour	**38.5 mL**				**38.5 mL**			
Vitamin D levels	**3.5 mL**		**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**
AA/EPA Ratio	**2 mL**		**2 mL**	**2 mL**	**2 mL**	**2 mL**	**2 mL**	**2 mL**
Total Blood collection per visit	**60.3 mL**	**23 mL**	**39.3 mL**	**39.3 mL**	**77.8 mL**	**37.3 mL**	**33.8 mL**	**33.8 mL**

¥ Pregnancy test for visits -1; 120; 240, and 360 will be performed using same serum sample for chemistry**.** For visit 480 a 3.5 mL SST tube (serum sample) will be collected to perform the test in the absence of a chemistry sample requirement

§ Mechanistic Assessments: includes samples for RNA, plasma, serum, DNA, measures of B and T cell number/function and cytokine profiles. The schedule for these assessments may vary as appropriate. At no time will the blood draw volume exceed what is allowable according to the subject's body weight.

Mechanistic Samples (total volume: 21 ml) as follows:

Pro-inflammatory cytokines: 3.5 ml
Phenotyping: 2 mL
RNA: 2.5 mL
DNA: 2.5 mL
Resolvins: 2 mL
LPS activation T-cell stimulation assay: 3 mL
Plasma: 2 mL
Serum: 3.5 mL

*****Stimulated c-peptide at 90 min is a time point of the MMTT. No extra sample collection needed**.**

- **Ages 6 to <18 y/o**
- **Weight at screening visit (Kg): 16 to < 17**

Maximum Draw Allowed Per Day (5mL/Kg): 80 mL/kg
Maximum Draw Allowed Per 8 Week Period (9.5 mL/Kg): 152 mL

Visit number	**0**	**1**	**2**	**3**	**4**	**5 F1**	**6 F2**	**7 F3**
Days of the trial	**-1**	**1**	**120**	**240**	**360**	**480**	**600**	**720**
Months	**1**	**1**	**4**	**8**	**12**	**16**	**20**	**24**
CBC with differential	**2 mL**	**2 mL**	**2 mL**	**2 mL**	**2 mL**			
Prothrombin time (PT/PT-INR)	**1.8 mL**		**1.8 mL**	**1.8 mL**	**1.8 mL**	**1.8 mL**	**1.8 mL**	**1.8 mL**
Chemistries	**3.5 mL**		**3.5 mL**	**3.5 mL**	**3.5 mL**			
Pregnancy test **¥**	**¥**		**¥**	**¥**	**¥**	**3.5 mL**		
Serum for islet autoantibodies	**3.5 mL**		**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**
Serology	**3.5 mL**							
Mechanistic assessments**§**		**21 mL**	**21 mL**	**21 mL**	**21 mL**	**21 mL**	**21 mL**	**21 mL**
Hemoglobin A1c	**2 mL**		**2 mL**	**2 mL**	**2 mL**	**2 mL**	**2 mL**	**2 mL**
Simulated C-peptide at 90 min*****	**X**				**X**			**X**
MMTT 4 hour	**38.5 mL**				**38.5 mL**			
Vitamin D levels	**3.5 mL**		**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**	**3.5 mL**
AA/EPA Ratio	**2 mL**		**2 mL**	**2 mL**	**2 mL**	**2 mL**	**2 mL**	**2 mL**
Total Blood collection per visit	**60.3 mL**	**23 mL**	**39.3 mL**	**39.3 mL**	**77.8 mL**	**37.3 mL**	**33.8 mL**	**33.8 mL**

¥ Pregnancy test for visits -1; 120; 240, and 360 will be performed using same serum sample for chemistry**.** For visit 480 a 3.5 mL SST tube (serum sample) will be collected to perform the test in the absence of a chemistry sample requirement

§ Mechanistic Assessments: includes samples for RNA, plasma, serum, DNA, measures of B and T cell number/function and cytokine profiles. The schedule for these assessments may vary as appropriate. At no time will the blood draw volume exceed what is allowable according to the subject's body weight.

Mechanistic Samples (total volume: 21 ml) as follows:

Pro-inflammatory cytokines: 3.5 ml
Phenotyping: 2 mL
RNA: 2.5 mL
DNA: 2.5 mL
Resolvins: 2 mL
LPS activation T-cell stimulation assay: 3 mL
Plasma: 2 mL
Serum: 3.5 mL

*****Stimulated c-peptide at 90 min is a time point of the MMTT. No extra sample collection needed**.**

REFERENCES

- Alcina A, Fedetz M, Ndagire D, Fernandez O, Leyva L, Guerrero M, bad-Grau MM, Arnal C, Delgado C, Lucas M, Izquierdo G, Matesanz F (2009) IL2RA/CD25 gene polymorphisms: uneven association with multiple sclerosis (MS) and type 1 diabetes (T1D). PLoSONE 4: e4137
- Bener A, Alsaied A, Al-Ali M, Al-Kubaisi A, Basha B, Abraham A, et al. High prevalence of vitamin D deficiency in type 1 diabetes mellitus and healthy children. Acta Diabetol. 2009;46:183–9. [PubMed]
- Bode BW, Schwartz S, Stubbs HA, Block JE (2005) Glycemic characteristics in continuously monitored patients with type 1 and type 2 diabetes: normative values. Diabetes care 28: 2361-2366
- Borissova AM, Tankova T, Kirilov G, Dakovska L, Kovacheva R. The effect of vitamin D3 on insulin secretion and peripheral insulin sensitivity in type 2 diabetic patients. Int J Clin Pract. 2003;57:258–61.
- Burke GW, III, Vendrame F, Pileggi A, Ciancio G, Reijonen H, Pugliese A (2011) Recurrence of Autoimmunity Following Pancreas Transplantation. CurrDiabRep
- Calder PC. Dietary fatty acids and the immune system. Nutr Rev. 1998;56(1 pt 2):S70-S83. 12. Calder PC. Polyunsaturated fatty acids, inflammation, and immunity. Lipids. 2001;36(9):1007- 1024.
- Cengiz E, Xing D, Wong JC, Wolfsdorf JI, Haymond MW, Rewers A, Shanmugham S, Tamborlane WV, Willi SM, Seiple DL, Miller KM, Dubose SN, Beck RW (2013) Severe hypoglycemia and diabetic ketoacidosis among youth with type 1 diabetes in the T1D Exchange clinic registry. PediatrDiabetes: 10
- Chase HP, Cooper S, Osberg I, et al. Elevated Creactive protein levels in the development of type 1 diabetes. Diabetes. 2004;53(10):2569-2573.
- Chiu KC, Chu A, Go VL, Saad MF. Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. Am J Clin Nutr. 2004;79:820–5.
- Coppieters KT, Dotta F, Amirian N, Campbell PD, Kay TW, Atkinson MA, Roep BO, von Herrath MG (2012) Demonstration of islet-autoreactive CD8 T cells in insulitic lesions from recent onset and long-term type 1 diabetes patients. JExpMed 209: 51-60
- Dahle´ n E, Dawe K, Ohlsson L, Hedlund C. Dendritic cells and macrophages are the first and major producers of TNF-alpha in pancreatic islets in the nonobese diabetic mouse.J Immunol. 1998;160(7):3585- 3593
- De Caterina R, Madonna R, Massaro M. Effects of omega-3 fatty acids on cytokines and adhesion molecules. Curr Atheroscler Rep. 2004;6(6):485- 491.
- Decsi T, Minda H, Hermann R, Kozári A, Erhardt E, Burus I, Molnár S, Soltész G. 2002. Polyunsaturated fatty acids in plasma and erythrocyte membrane lipids of diabetic children. Prostaglandins Leukot Essent Fatty Acids. 67:203-210
- Endres S, Ghorbani R, Kelley VE, Georgilis K, Lonnemann G, van der eer JW, Cannon JG, Rogers TS, Klempner MS, Weber PC, et al. 1989. The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. N Engl J Med 320:265-71.

- Endres S, Ghorbani R, Kelley VE, et al. The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. N Engl J Med. 1989;320(5):265-271.
- Ferrannini E, Mari A, Nofrate V, Sosenko JM, Skyler JS (2010) Progression to diabetes in relatives of type 1 diabetic patients: mechanisms and mode of onset. Diabetes 59: 679-685
- Forlenza GP, Rewers M (2011) The epidemic of type 1 diabetes: what is it telling us? CurrOpinEndocrinolDiabetes Obes 18: 248-251
- Gedik O, Akalin S. Effects of vitamin D deficiency and repletion on insulin and glucagon secretion in man. Diabetologia. 1986;29:142–5.
- Green EA, Flavell RA. Tumor necrosis factoralpha and the progression of diabetes in non-obese diabetic mice. Immunol Rev. 1999;169:11-22.
- Greer RM, Rogers MA, Bowling FG, Buntain HM, Harris M, Leong GM, et al. Australian children and adolescents with type 1 diabetes have low vitamin D levels. Med J Aust. 2007;187:59–60.
- Hirsch IB, Skyler JS (2000) The Management of Type 1 Diabetes. In Endotext, De Groot LJ, Beck-Peccoz P, Chrousos G, Dungan K, Grossman A, Hershman JM, Koch C, McLachlan R, New M, Rebar R, Singer F, Vinik A, Weickert MO (eds). South Dartmouth (MA): MDText.com, Inc.
- Holick MF. High prevalence of vitamin D inadequacy and implications for health. Mayo Clin Proc. 2006;81:353–73
- Hypponen E, Laara E, Reunanen A, Ja¨ rvelin MR, Virtanen SM. Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. Lancet. 2001;358 (9292):1500-1503.
- Jansen A, Homo-Delarche F, Hooijkaas H, Leenen PJ, Dardenne M, Drexhage HA. Immunohistochemical characterization of monocytes-macrophages and dendritic cells involved in the initiation of the insulitis and beta-cell destruction in NOD mice. Diabetes. 1994; 43(5):667-675.
- Joner G. 1992. The epidemiology of insulin-dependent diabetes mellitus in Norway: incidence, prevalence, microvascular complications and mortality (Thesis). Haukeland University Hospital and Aker University Hospital, Oslo.
- Litherland SA, She JX, Schatz D, Fuller K, Hutson AD, Peng RH, Li Y, Grebe KM, Whittaker DS, Bahjat K, Hopkins D, Fang Q, Spies PD, North K, Wasserfall C, Cook R, Dennis MA, Crockett S, Sleasman J, Kocher J, Muir A, Silverstein J, Atkinson M, Clare-Salzler MJ. 2003. Aberrant monocyte prostaglandin synthase 2 (PGS2) expression in type 1 diabetes before and after disease onset. Pediatr Diabetes 4(1):10-8.
- Litherland, S.A. S.A. Xie, Hutson, Wasserfall C, Whittaker D, She, Hofig, Dennis, Fuller, Cook, Schatz, Moldawer and Clare-Salzler M. 1999. Aberrant prostaglandin synthase 2 expression defines an antigen-presenting cell defect for insulin-dependent diabetes mellitus. J. Clin. Invest 104:515-52.
- Ludvigsson J (2013) C-peptide in diabetes diagnosis and therapy. Frontiers in bioscience (Elite edition) 5: 214-223
- Luong K, Nguyen LT, Nguyen DN. The role of vitamin D in protecting type 1 diabetes mellitus. Diabetes Metab Res Rev. 2005;21:338–46.
- Mari A, Ferrannini E (2008) Beta-cell function assessment from modelling of oral tests: an effective approach. Diabetes ObesMetab 10 Suppl 4:77-87. doi: 10.1111/j.1463-1326.2008.00946.x.: 77-87

- Mathieu C, Gysemans C, Giulietti A, Bouillon R. Vitamin D and diabetes. Diabetologia. 2005;48:1247–57.
- Mineo D, Pileggi A, Alejandro R, Ricordi C (2009) Point: steady progress and current challenges in clinical islet transplantation. Diabetes Care 32: 1563-1569
- Nathan DM (2014) The diabetes control and complications trial/epidemiology of diabetes interventions and complications study at 30 years: overview. Diabetes Care 37: 9-16
- Norman AW, Frankel JB, Heldt AM, Grodsky GM. Vitamin D deficiency inhibits pancreatic secretion of insulin. Science. 1980;209:823–5
- Orlando G, Gianello P, Salvatori M, Stratta RJ, Soker S, Ricordi C, Dominguez-Bendala J (2014) Cell replacement strategies aimed at reconstitution of the beta-cell compartment in type 1 diabetes. Diabetes 63: 1433-1444
- Pettitt DJ, Talton J, Dabelea D, Divers J, Imperatore G, Lawrence JM, Liese AD, Linder B, Mayer-Davis EJ, Pihoker C, Saydah SH, Standiford DA, Hamman RF (2013) Prevalence of Diabetes Mellitus in U.S. Youth in 2009: The SEARCH for Diabetes in Youth Study. Diabetes Care
- Pouwer F, Hermanns N (2009) Insulin therapy and quality of life. A review. Diabetes Metab ResRev 25 Suppl 1:S4-S10.: S4-S10
- Pugliese A (2014) Advances in the etiology and mechanisms of type 1 diabetes. Discovery medicine 18: 141-150
- Realsen J, Goettle H, Chase HP (2012) Morbidity and mortality of diabetic ketoacidosis with and without insulin pump care. Diabetes technology & therapeutics 14: 1149-1154
- Roep BO, Peakman M (2012) Antigen targets of type 1 diabetes autoimmunity. Cold Spring HarbPerspectMed 2: a007781
- Rosenbloom AL (2010) The management of diabetic ketoacidosis in children. Diabetes Ther 1: 103-120
- Schwalfenberg G. Vitamin D and diabetes: Improvement of glycemic control with vitamin D3 repletion. Can Fam Physician. 2008;54:864–6.
- Seaquist ER, Anderson J, Childs B, Cryer P, Dagogo-Jack S, Fish L, Heller SR, Rodriguez H, Rosenzweig J, Vigersky R (2013) Hypoglycemia and diabetes: a report of a workgroup of the American Diabetes Association and the Endocrine Society. Diabetes care 36: 1384-1395
- Simopoulos AP. 1999. Essential fatty acids in health and chronic disease. Am J Clin Nutr 70:560S-569S.
- Steffes MW, Sibley S, Jackson M, Thomas W (2003) Beta-cell function and the development of diabetes-related complications in the diabetes control and complications trial. Diabetes care 26: 832-836
- Stene LC, Joner G, the Norwegian Childhood Diabetes Study Group. Use of cod liver oil in the first year of life associated with lower risk of childhood onset type 1 diabetes: a large population based case-control study. 2003. Am Journ Clin Nutr 78:1128-34.
- Stene LC, Ulriksen J, Magnus P, Joner G. 2000. Use of cod liver oil during pregnancy associated with lower risk of Type I diabetes in the offspring. Diabetologia 43:1093-8.
- Stene LC, Joner G; The Norwegian Childhood Diabetes Study Group. Use of cod liver oil during the first year of life is associated with lower risk of childhoodonset type 1 diabetes: a large, population-based, casecontrol study. Am J Clin Nutr. 2003;78(6):1128- 1134.

- Svoren BM, Volkening LK, Wood JR, Laffel LM. Significant vitamin D deficiency in youth with type 1 diabetes Mellitus. J Pediatr. 2009;154:132–4. [PMC free article]
- Tao B, Pietropaolo M, Atkinson M, Schatz D, Taylor D (2010) Estimating the cost of type 1 diabetes in the U.S.: a propensity score matching method. PLoSONE 5: e11501
- Vehik K, Dabelea D (2011) The changing epidemiology of type 1 diabetes: why is it going through the roof? Diabetes Metab ResRev 27: 3-13
- Vendrame F, Pileggi A, Laughlin E, Allende G, Martin-Pagola A, Molano RD, Diamantopoulos S, Standifer N, Geubtner K, Falk BA, Ichii H, Takahashi H, Snowhite I, Chen Z, Mendez A, Chen L, Sageshima J, Ruiz P, Ciancio G, Ricordi C, Reijonen H, Nepom GT, Burke GW, III, Pugliese A (2010) Recurrence of type 1 diabetes after simultaneous pancreas-kidney transplantation, despite immunosuppression, is associated with autoantibodies and pathogenic autoreactive CD4 T-cells. Diabetes 59: 947-957
- Watson CJ (2015) The current challenges for pancreas transplantation for diabetes mellitus. Pharmacological research : the official journal of the Italian Pharmacological Society 98: 45-51
- Weinstock RS, Xing D, Maahs DM, Michels A, Rickels MR, Peters AL, Bergenstal RM, Harris B, Dubose SN, Miller KM, Beck RW (2013) Severe Hypoglycemia and Diabetic Ketoacidosis in Adults with Type 1 Diabetes: Results from the T1D Exchange Clinic Registry. JClinEndocrinolMetab
- White SA, Shaw JA, Sutherland DE (2009) Pancreas transplantation. Lancet 373: 1808-1817
- Zinman B (1998) Glucose control in type 1 diabetes: from conventional to intensive therapy. ClinCornerstone 1: 29-38
- Endres, S., et al., The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. N Engl J Med, 1989. 320(5): p. 265-71.
- Elajami, T.K., et al., Specialized proresolving lipid mediators in patients with coronary artery disease and their potential for clot remodeling. FASEB J, 2016. 30(8): p. 2792-801.
- Germano, M., et al., Plasma, red blood cells phospholipids and clinical evaluation after long chain omega-3 supplementation in children with attention deficit hyperactivity disorder (ADHD). Nutr Neurosci, 2007. 10(1-2): p. 1-9.
- Sorgi, P.J., et al., Effects of an open-label pilot study with high-dose EPA/DHA concentrates on plasma phospholipids and behavior in children with attention deficit hyperactivity disorder. Nutr J, 2007. 6: p. 16.
- Baidal, D.A., et al., Combination high-dose omega-3 fatty acids and high-dose cholecalciferol in new onset type 1 diabetes: a potential role in preservation of beta-cell mass. Eur Rev Med Pharmacol Sci, 2016. 20(15): p. 3313-8.
- Cadario, F., et al., Can Type 1 diabetes progression be halted? Possible role of high dose vitamin D and omega 3 fatty acids. Eur Rev Med Pharmacol Sci, 2017. 21(7): p. 1604-1609.
- Mozaffarian, D., JELIS, fish oil, and cardiac events. Lancet, 2007. 369(9567): p. 1062-3.
- Skyler, J.S., Primary and secondary prevention of Type 1 diabetes. Diabet Med, 2013. 30(2): p. 161-9.
- Moran, A., et al., Interleukin-1 antagonism in type 1 diabetes of recent onset: two multicentre, randomised, double-blind, placebo-controlled trials. Lancet, 2013. 381(9881): p. 1905-15.
- Mastrandrea, L., et al., Etanercept treatment in children with new-onset type 1 diabetes: pilot randomized, placebo-controlled, double-blind study. Diabetes Care, 2009. 32(7): p. 1244-9.

- Greenbaum, C.J., et al., Fall in C-peptide during first 2 years from diagnosis: evidence of at least two distinct phases from composite Type 1 Diabetes TrialNet data. Diabetes, 2012. 61(8): p. 2066-73.

Agiostratidou G, et al, Standardizing Clinically Meaningful Outcome Measures Beyond HbA_{1c} for Type 1 Diabetes: A Consensus Report of the American Association of Clinical Endocrinologists, the American Association of Diabetes Educators, the American Diabetes Association, the Endocrine Society, JDRF International, The Leona M. and Harry B. Helmsley Charitable Trust, the Pediatric Endocrine Society, and the T1D Exchange. Diabetes Care. 2017 Dec;40(12):1622-1630