
# *Exploring Immune Effects of Oral Insulin in Relatives at Risk for Type 1 Diabetes Mellitus*

## (Protocol TN-20)

## VERSION: January 15, 2016

**IND #: 76,419**

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

## PREFACE

The Type 1 Diabetes TrialNet Protocol TN20, *Exploring Immune Effects of Oral Insulin in Relatives at Risk for Type 1 Diabetes Mellitus*, describes the background, design, and organization of the study. The protocol will be maintained by the TrialNet Coordinating Center at the University of South Florida over the course of the study through new releases of the entire 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.

## Glossary of Abbreviations

| | |
|---|---|
| AE | Adverse event |
| AICD | Activation Induced Cell Death |
| APC | Antigen presenting cell |
| CBC | Complete Blood Count |
| CFR | Code of Federal Regulations |
| cGMP | Current Good Manufacturing Practice |
| CHO | Carbohydrates |
| CRF | Case report form |
| DC | Dendritic Cell |
| DPT-1 | Diabetes Prevention Trial - Type1Diabetes |
| DSMB | Data and Safety Monitoring Board |
| ELISPOT | Enzyme-Linked ImmunoSpot Assay |
| 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 |
| HbA1c | Hemoglobin A1c |
| HBsAg | Hepatitis B surface antigen |
| HIV | Human immunodeficiency virus |
| ICA | Islet cytoplasmic antibodies |
| IEC | Independent Ethics Committee |
| IGRA | Interferon-γ release assays |
| IND | Investigational New Drug |
| IRB | Institutional Review Board |
| ITN | Immune Tolerance Network |
| JDRF | Juvenile Diabetes Research Foundation |

| | |
|---|---|
| LIFT | Long Term Investigative Follow-Up |
| mIAA | Insulin Autoantibody |
| NIDDK | National Institute for Diabetes and Digestive and Kidney Diseases |
| NIH | National Institute of Health |
| NCI-CTCAE | National Cancer Institute *Common Terminology Criteria for Adverse Events* |
| NOD | Nonobese diabetic |
| OGTT | Oral Glucose Tolerance Test |
| OHRP | Office for Human Research Protections |
| PBMC | Peripheral Blood Mononuclear Cell |
| PCR | Polymerase chain reaction |
| PI | Principal Investigator |
| PO | Per Oral (by mouth) |
| QA | Quality Assurance |
| RMSE | Residual Mean Square Error |
| SAE | Serious adverse event |
| SOA | Schedule of assessments |
| SOP | Standard operating procedure |
| T1D | Type 1 diabetes mellitus |
| TCA | T Cell Assay |
| Tregs | Regulatory T cells |
| TSDR | Treg Specific Demethylation region |
| ZnT8 | Zinc Transporter 8 |

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## 1. STUDY OVERVIEW

| | |
|---|---|
| Title | *Exploring Immune Effects of Oral Insulin in Relatives at Risk for Type 1 Diabetes Mellitus* |
| IND Sponsor | National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) |
| Conducted By | Type 1 Diabetes Trial Network (TrialNet) |
| Protocol Chair | Peter Gottlieb, M.D.; Barbara Davis Center, Denver, CO |
| Accrual Objective | A minimum of 40 participants will be enrolled for this study. |
| Study Design | The study is a 2-arm, multicenter, randomized, open-labelled clinical research study. |
| Treatment Description | Each participant will receive recombinant human oral insulin capsules either through a daily regimen (67.5 mg/day) or biweekly regimen (i.e. every other week, starting at 135 mg titrating up to 500 mg as tolerated). |
| Study Duration | Participants are enrolled and followed for a total duration of 12 months. |
| Objective | The objective of this study is to assess the effects of varying doses and schedules of oral insulin on immunologic and metabolic markers in participants at risk for T1D. |
| Primary Outcome | The primary outcome is the change in immune function as assessed by level or quality of T lymphocyte or autoantibody biomarkers of β-cell specific immune response measured between 13 and 26 weeks after 1st dose versus baseline. |
| Major Inclusion Criteria | (1) Relatives of T1D proband with mIAA and at least one other diabetes-related autoantibody confirmed present |
| Major Exclusion Criteria | (1) Diagnosis of diabetes |

## 2. BACKGROUND AND SIGNIFICANCE

### 2.1. Rationale for Study

#### *2.1.1. Type 1 diabetes (T1D)*

Type 1 diabetes mellitus is an immune-mediated disease in which insulin-producing beta cells are completely or nearly completely destroyed, resulting in life-long dependence on exogenous insulin. It is a chronic and potentially disabling disease that represents a major public health and clinical concern. The number of patients being diagnosed with type 1 diabetes is increasing each year and is

approaching an epidemic level in some countries that track this information (1). Unfortunately, the increase in type 1 diabetes is the greatest in children under age five years (2).

Current management of T1D is not optimal. To avoid long-term complications, patients must attempt to maintain near normal glycemic control by frequent glucose monitoring throughout the day, by multiple daily insulin injections or use of an insulin pump, and by adjusting insulin doses for variation in diet and exercise. Such strict glycemic control is rarely achieved with current management and overly aggressive therapy results in severe hypoglycemia which can be life threatening (3). It is not possible to fully mimic the function of the beta cell, and there are no established treatments that can prevent its destruction. Thus, despite advances in diabetes care and treatment, individuals with diabetes remain at risk for early mortality and a high rate of morbidity due to complications such as retinopathy leading to blindness, neuropathy and vascular disease leading to amputations and heart disease, and nephropathy leading to renal failure. The costs of caring for diabetes and its complications are currently greater than $100 billion a year (4).

### ***2.1.2.*** *Natural History of Type 1 Diabetes Mellitus (T1D)*

Much is known about the natural history of the type 1 diabetes disease process (5). Although all people are susceptible, relatives of individuals with T1D are at much greater risk for development of the disease. In the general population, approximately 0.3% of individuals will develop T1D. In contrast, those with a close relative with T1D have a 5% incidence of disease – a 15 fold increase (6). Further risk stratification among family members depends upon genetic, immune and metabolic data (7).

Beta cell destruction generally begins in genetically susceptible individuals years before clinical onset of disease (8). The autoimmune process that causes beta cell destruction is clinically silent and can only be identified by the detection of autoantibodies in the blood such as: Islet Cell Antibodies (ICA), anti-glutamic acid decarboxylase (GAD)65ab, anti-ICA512ab/anti-IA2ab, anti-insulin autoantibodies (mIAA) (6), and the recently described antibodies to a zinc transporter (ZnT8) (9). Continued immune mediated beta cell destruction involving both B- and T-cells occurs until physiologic insulin demand cannot be met by the remaining beta cells, resulting in hyperglycemia and clinical diagnosis of T1D (10, 11).

Based on data from the Diabetes Prevention Trial-Type 1 Diabetes (DPT-1), the TrialNet Natural History/Pathway to Prevention Study, and others, the risk for developing diabetes in relatives without the disease can be defined by the presence of autoantibodies and the degree of metabolic impairment (12-14). Like the DPT-1, the ongoing TrialNet Natural History/Pathway to Prevention Study has tested more than 150,000 relatives for the presence of diabetes-associated autoantibodies during the past decade or so.

Approximately 5% of relatives tested are found to have at least one autoantibody. Further testing enables risk assessment of this population. In DPT-1, relatives with at least two autoantibodies and normal glucose tolerance have at least a 42% risk for development of T1D over 6 years. This was demonstrated in DPT-1 as well as ENDIT (European Nicotinamide Diabetes Intervention Trial), a large European trial enrolling autoantibody-positive relatives (15). This risk has been confirmed in the ongoing TrialNet Natural History/Pathway to Prevention Study (Figure 1).

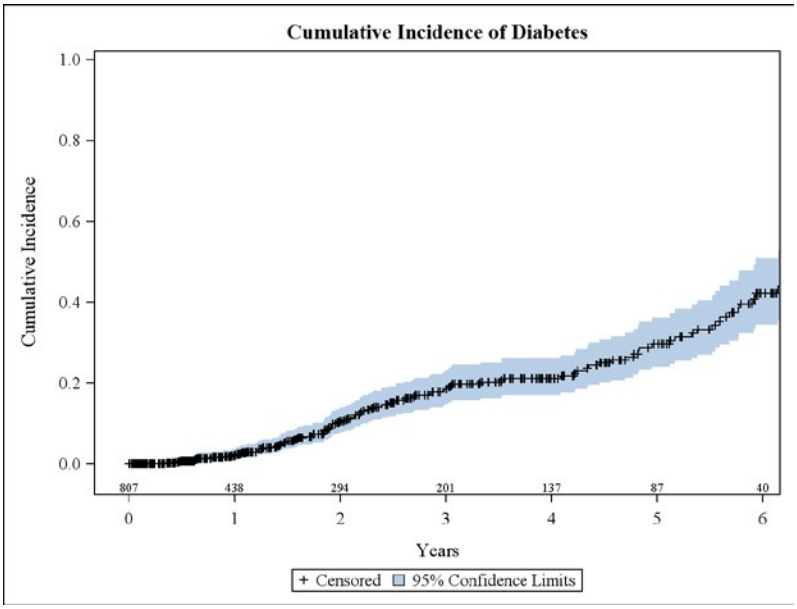



**Figure 1:** Cumulative incidence of type 1 diabetes in TN01 Natural History Pathway to Prevention Study participants with ≥ 2 autoantibodies positive and normal glucose tolerance on first OGTT Test.

Moreover, while the limited numbers of relatives followed for more than 10 years prevent precision around a 10-year risk estimate, it is noteworthy that available data from those in DPT-1 or TrialNet Natural History/Pathway to Prevention Study suggests no leveling off of this risk over time. Data from studies following individuals at risk from birth also demonstrate the continued risk for T1D over time once they develop multiple autoantibodies (Figure 2).

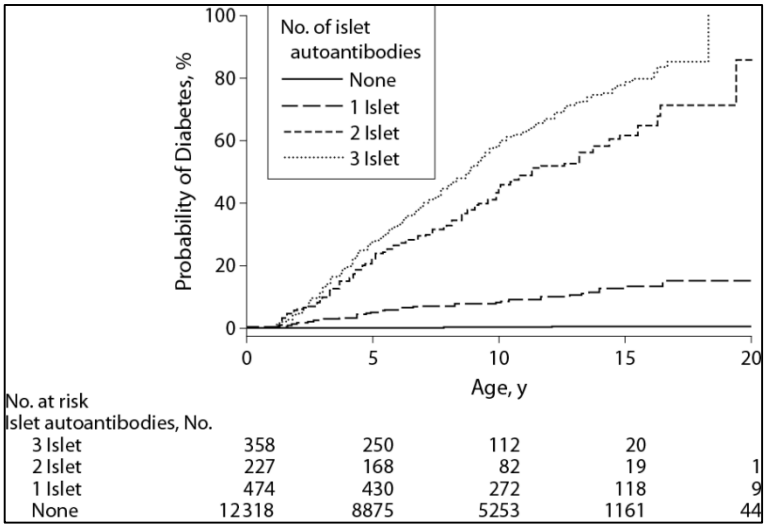



**Figure 2:** Risk for development of T1D in antibody positive children followed from birth (16).

Thus our current understanding is that *essentially all* relatives confirmed to have two or more autoantibodies will eventually develop clinical T1D.

Prior to this clinical diagnosis, autoantibody-positive relatives progress from normal glucose tolerance to impaired or indeterminate glucose tolerance (defined as any blood glucose level after ingestion of oral glucose of ≥ 200 mg/dL and/or a glucose level 2 hours after ingestion of oral glucose of 140-199 mg/dL and/or fasting glucose between 110 – 125 mg/dL during a standard oral glucose tolerance test). Participants in the TN01 Natural History/Pathway to Prevention Study with multiple autoantibody positivity and normal glucose tolerance at baseline were found to have a 38% two-year risk of progression to confirmed abnormal glucose tolerance (Figure 3). Once such abnormal glucose tolerance is present, there is a very high short-term risk of clinical diagnosis: 68% over 4 years (Figure 4). The risk is particularly high for individuals under the age of 18 (Figure 5). The extremely high risk of this group has now been demonstrated in three independent studies: DPT-1, ENDIT and the ongoing TrialNet Natural History/Pathway to Prevention Study.

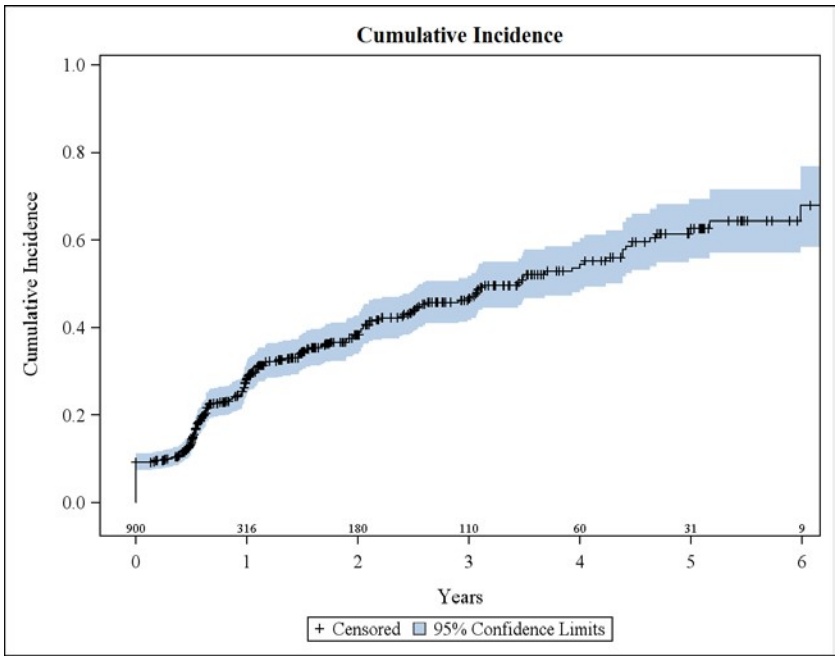



**Figure 3:** Two-year risk of confirmed abnormal glucose tolerance is 38%, and the six-year risk is 67.9% among individuals with multiple autoantibody positivity and normal glucose tolerance at baseline in the TN01 Natural History/Pathway to Prevention Study.

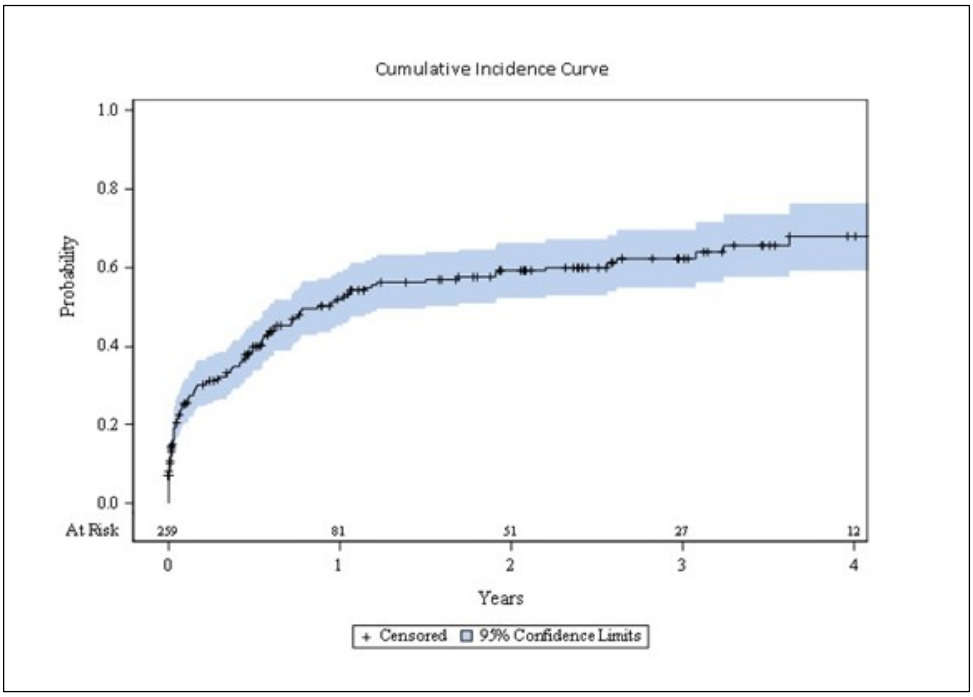



**Figure 4:** Four-year risk of diabetes onset is 68% among individuals with multiple autoantibody positivity and confirmed abnormal glucose tolerance in the TN01 Natural History/Pathway to Prevention Study.

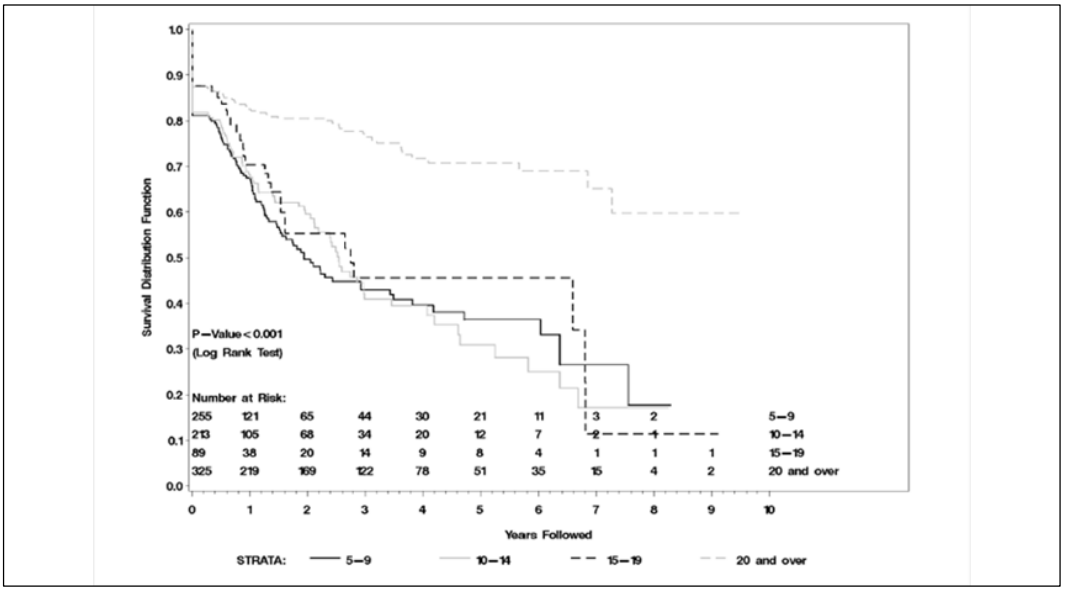



**Figure 5:** Risk of diabetes onset among multiple antibody positive relatives is highly dependent on age, with children progressing to clinical disease much faster than adults. Data from TN01 Natural History/Pathway to Prevention Study.

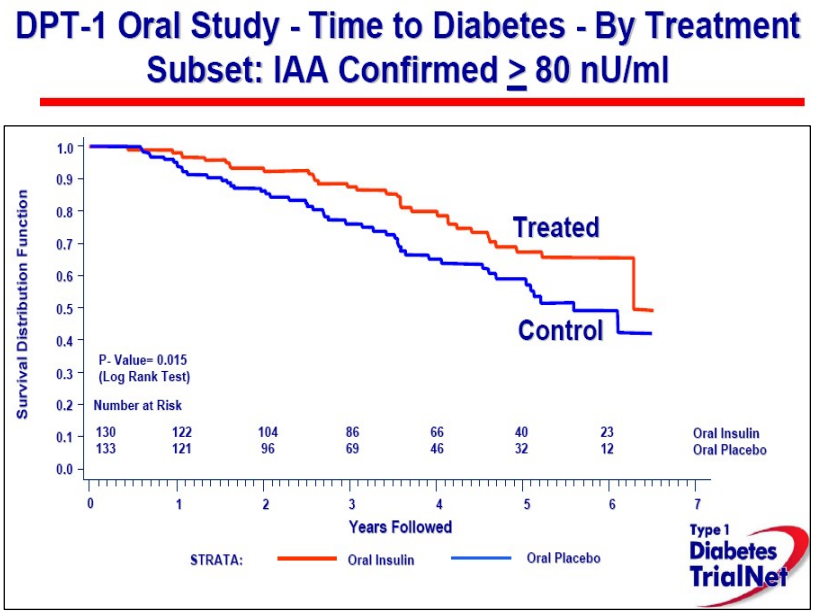



**Figure 6:** DPT-1 analysis of time to diabetes according to treatment in the subset of participants with IAA confirmed ≥80 nU/ml (1).

### ***2.1.3.*** *Oral Insulin for prevention of T1D*

The DPT-1 oral insulin study was designed to examine the hypothesis that daily administration of 7.5 mg insulin would protect participants at risk of developing type 1 diabetes from progression to overt disease. The primary results of that study, including all randomized participants, reported no beneficial effect. However, a hypothesis-generating analysis of a subgroup with high insulin autoantibody (IAA) levels (defined as ≥80 nU/ml confirmed on two occasions) showed significant benefit: annualized diabetes rate was 6.2% during treatment with oral insulin and 10.4% with placebo (P = 0.015) and median progression to diabetes was delayed by 4.5 years.

Subsequent to the DPT-1, a newer assay measuring insulin antibodies was developed which is more sensitive and specific for type 1 diabetes. This is the micro IAA assay (mIAA). Employing this new assay, Diabetes TrialNet is currently testing the hypothesis that 7.5 mg daily of oral insulin in relatives confirmed for mIAA plus another autoantibody will delay the development of clinical type 1 diabetes. As of June 2015, more than 500 individuals have randomized in this double-masked, placebo-controlled trial, which has ended enrollment as of December 2015. Results from the trial are expected in January 2017.

Others continue to investigate oral or mucosally-delivered antigen therapy in altering the course of type 1 diabetes. This work, as well as evidence from the use of antigen therapy in treating allergic disease, emphasizes the importance of the dose and dose frequency in outcome. While the DIPP study found no effect on development of type 1 diabetes in autoantibody-positive relatives treated with nasal insulin at 4-8 mg intranasal insulin per day, INIT II investigators in Australia/New Zealand continue to study nasal insulin in a similar population using a different dosing regimen (16 mg

intranasal insulin for 7 consecutive days, then once per week), which they have previously demonstrated to have a favorable immune effect (17). While it is difficult to extrapolate antigen dosing in animal models to humans, it has been noted the dose of 7.5 mg oral insulin daily chosen for the DPT-1 and TrialNet trials is about 10 fold lower than a dose shown to be effective in the NOD mouse pre-clinical model. Additionally, modelling has suggested that intermittent dosing would be more likely to induce T regulatory cells to control the immune response (18). Recently, Bonifacio et al explored different doses in a clinical setting. The Pre-POINT study evaluated different doses of oral insulin in genetically at-risk children (mean age 5 years). Using immunologic read-outs, these data indicated no untoward effects of treatment and demonstrated that the highest dose (67.5 mg daily) was most effective in favorably altering the immune response (19). As such, these investigators are pursuing the POINT study, using 67.5 mg daily in a primary prevention setting aimed at delaying or preventing the progression from genetic risk to autoantibodies.

The proposed study is planned to further address the question of dose in the context of oral insulin therapy in the prevention of type 1 diabetes in the autoantibody-positive at-risk group. We will examine the immunologic effects of 67.5 mg daily as well as 500 mg administered on alternate weeks to evaluate both dose and dose frequency in mIAA-positive relatives. Together with results from the ongoing Diabetes TrialNet Oral Insulin Trial testing 7.5 mg daily, these data will provide needed information for future studies using antigen therapy pre-diagnosis both as stand-alone and potentially in conjunction with other agents. Importantly, all participants will receive six months of active therapy and be carefully monitored for disease progression.

### 2.2. Possible Mechanisms of Action of Oral Insulin

Although numerous putative mechanisms of action of oral antigen have been mooted on the basis of nonclinical studies, there are several that emerge as rational candidates.

#### *2.2.1. Regulatory T cells (Tregs)*

Several nonclinical studies have identified the induction of T cells in response to oral antigen that are associated with, and can adoptively transfer, protection from autoimmune disease (20-21). These cells are typically autoantigen-specific, CD4+ and secrete the immune suppressive cytokines IL-10 and TGF-β. They have occasionally been termed $T_H3$ (20, 22-23) cells and are generically termed regulatory T cells or Tregs. When induced against autoantigens such as insulin, these cells and the associated cytokine secretion may also result in bystander suppression of effector T cells responding to autoantigens other than insulin, as well as “infectious” tolerance whereby regulatory T cells with specificity for non-insulin autoantigens may arise (24- 28). Oral immunotherapy has been used successfully to treat food allergy. When low doses are used and escalated every two weeks, a rise in the number of antigen-specific Tregs is detectable over time. Moreover, those individuals without clinical response had no change in antigen-specific Tregs, strongly supporting the concept that induction of these cells is an important mechanism for the action of oral tolerance. These reports also noted that the function of antigen-specific Tregs increased over time with therapy, in relation to the methylation state of FOXP3. In summary, advances in our understanding of Treg biology and the manipulation of related regulatory pathways provide a critical underpinning to this proposal.

#### *2.2.2. Deletion of effector T cells*

Another mechanism, demonstrated in animal models, by which antigen therapy may work is by way of exhaustion, anergy, or death, of antigen-specific effector T cells, through a process termed activation induced cell death (AICD). This mechanism has also been identified to be operative in humans, whereby feeding higher doses of antigen to food allergic patients induces a rapid clinical benefit

(anergy to allergens), associated with anergic and exhausted antigen-specific T cells (29). Although theoretically, high doses of antigen could lead to Treg death, this was not seen. Recent advances in the identification of antigen-specific effector T cells (e.g. using peptide-HLA multimers combined with multi-dimensional flow cytometry) has enabled the development of approaches that can explore effects of chronic antigen administration on these inflammatory mediators, including their expression of markers of chronic stimulation (30), as potential biomarkers in the context of this protocol.

#### ***2.2.3.*** *Sequestration of effector T cells*

It has been further suggested that the use of the oral route for antigen administration leads to sequestration of antigen-specific effector T cells within the extensive gut immune system (31). This is consistent with the known effect of antigen in determining T cell migration, and would theoretically lead to a loss of relevant effector cells from the site of pancreatic islet inflammation, with concomitant therapeutic effect that is sustained for the duration of the therapy.

In summary, the T cell field has matured sufficiently to allow rational hypotheses as to the mechanistic effects of oral insulin to be proposed and examined experimentally.

## 2.3. Rational for Mechanistic Assays

#### ***2.3.1.*** *Detection of autoreactive T cells and induction of T regulatory cells*

##### *2.3.1.1. Autoreactive CD4 T cells*

Assays to detect antigen-specific autoreactive T cells in T1D have been developed, replicated and validated in recent years. Modifying the standard proliferation assay to incorporate selection of CD45RO memory cells has identified disease-associated responses in T1D. Staining for intracellular cytokine production and the ELISPOT assay which measures extracellular cytokine production after antigen stimulation has been shown to detect CD4 T cell responses to insulin IA-2, GAD and ZnT8 antigens (32-33). The ELISPOT system allows for quantitative (number of spots/well = frequency) and qualitative (differential cytokine response – IFN-g, IL-10, IL-17) analysis of the response to islet cell autoantigens (34). Measuring CD4 T cells using these types of assays will be important in determining if oral insulin can lower the number of antigen-specific autoreactive T cells present in the periphery of individuals given study drug vs. those on control or modify the nature of the response based on the cytokine profile detected.

##### *2.3.1.2. Autoreactive CD8 T cells*

CD8 T cells are known to be critical to disease development in both the NOD mouse and human T1D. Although ELISPOT assays assessing their function similar to what has been described for CD4 T cells have been developed (35-36), they have not been replicated or validated in their current format. Enumeration of CD8 T cells by flow cytometry has been performed for new onset studies, clinical research studies and islet transplantation protocols and has been validated in several workshops (37-39). The quantam dot or Qdot system relies on the use of two Qdot chromophores for a particular reactivity which appears to increase the specificity and ability to detect very low frequency CD8 T cells more reliably than previously described approaches. The ability to multiplex these reagents also has reduced the amount of cells needed for these analyses and, lastly, it can be done on frozen samples. An array of class I HLA-specific islet cell peptide reagents has been designed to assess antigen reactivity to a number of islet antigen targets and will be used to measure the number of CD8 T cells present prior to and after oral insulin therapy. Again, this and other methodologies using tetramers should help us to understand the mechanism of oral antigen therapy in changing insulin-reactive CD8 T cells, as well as if it can affect bystander non-insulin-specific CD8 T cell number through bystander suppression mechanisms.

*2.3.1.3. T regulatory cells*

As discussed previously, oral insulin in NOD mice can induce T regulatory cells as one potential mechanism of its action. Detection of CD4+CD25+ Tregs can be done via flow cytometry, utilizing the combination of CD4, CD25, CD127 and FOXP3 to enumerate the number of these cells present in the periphery of participants in the trial (40). Studies in new onset T1D have allowed us to better understand the potential dysfunction that may be occurring in this regulatory cell population, which may contribute to disease onset (41-43). Bonifacio and colleagues recently reported on their work using a higher dose of oral antigen in genetically at-risk first degree relatives of T1D participants (19). They showed that higher doses of oral insulin could be associated with changes in autoantibody as well as T cell responses to oral insulin, suggestive of a mechanism for protection. The assay employed in their study took peripheral blood monocytes (PBMC's), stimulated them with insulin or proinsulin and then isolated the activated T cells and examined their transcription profiles. They found that insulin- or proinsulin-specific T cells from treated participants had increased expression of FOXP3 regulatory cells, increased expression of IL-21 which would be consistent with a vaccination effect and decreased expression of IFN-g autoreactive T cells, consistent with the intended effect of oral insulin as a vaccine inducing active immune protection in these young children. Our study will utilize these types of assays to assess the frequency of T regs and to examine their antigen-specific transcription profiles to try to confirm and extend these intriguing observations from the Pre-POINT study.

## 3. STUDY DESIGN

### 3.1. Overview

This is a 2-arm, multicenter, randomized, open-labelled clinical research study. Participants will receive varying doses and schedules of recombinant human insulin in capsules (referred to hereafter as oral insulin). In this design, each participant is randomly assigned to 1 of 2 treatment regimens with equal probability. The primary outcome is the change in immunologic parameters before and after treatment.

### 3.2. Objectives

The key questions that this mechanistic study will address are:

1. Does treatment with oral insulin affect underlying immunological mechanism(s) that are observed through changes in immune markers?
2. Are there differential changes in immunological biomarkers between different schedules of oral insulin?

In terms of a specific set of hypotheses that can be tested using samples from these component studies, we aim to examine the following:

We hypothesize that introduction of oral insulin in treatment-naïve participants:

- removes from the circulation insulin-specific pro-inflammatory T cells, detectable and recognizable by the secretion of signature cytokines such as IFN-γ and IL-17
- removes from the circulation or modifies the effector functions of insulin-specific CD8 T cells recognizable by peptide responses in ELISPOTs or using peptide-HLA multimers or other similar assays
- induces changes in gene expression of insulin-specific, proliferating T cells that indicate a change in balance of inflammation and regulation

- changes peripheral blood gene signatures (total cell populations, cell subsets, cells activated ex vivo) to indicate induction of immune regulation

All enrolled participants (relatives of probands with type 1 diabetes and with at least 2 autoantibodies) have established islet autoimmunity, a condition which generally leads to clinically overt type 1 diabetes over time. In addition to the mechanistic hypotheses described above, all participants will be closely followed for progression of disease, as oral insulin may delay or prevent such progression.

### 3.3. Summary of Inclusion/Exclusion Criteria

Participants must meet all entry criteria for the protocol as outlined below.

#### *3.3.1. Inclusion Criteria*

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

1. Participant in TrialNet Natural History/Pathway to Prevention Study (TN01) and thus, a relative of a proband with T1D and between the ages of 1-45 at the time of enrollment in TN01.

2. If most recent OGTT demonstrates Normal Glucose Tolerance, participants must be age ≥3 at time of randomization in this trial.

3. If most recent OGTT demonstrates Abnormal Glucose Tolerance, participants must be age 3-7 at time of randomization in this trial.

4. mIAA confirmed positive within the previous six months.

5. Participant must weigh ≥12 kg at the time of screening.

6. At least one other diabetes-associated autoantibody present on two separate samples, one of which was drawn within the past six months. Confirmation does not have to involve the same 2 autoantibodies.

7. Willing to provide Informed Consent or have a parent or legal guardian provide informed consent if the participant is <18 years of age.

#### *3.3.2. Exclusion Criteria*

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

1. Diagnosed with Diabetes or having their most recent OGTT with fasting glucose ≥126 mg/dl or 2 hour glucose ≥ 200 mg/dl.

2. Prior participation in clinical research for secondary prevention of T1D.

3. History of treatment with insulin or oral hypoglycemic agent.

4. Current chronic use of medications altering stomach acid (such as H2 blockers, proton pump inhibitors and antacids).

5. History of gastric ulcer or gastric surgery.

6. History of therapy with immunosuppressive drugs or non-physiologic glucocorticoids within the past two years for a period of more than three months.

7. Has severe active disease, e.g. chronic active hepatitis, severe cardiac, pulmonary, renal, hepatic, immune deficiency and/or disease that is likely to limit life expectancy or lead to therapies such as immunosuppression during the time of the study.

8. Ongoing use of medications known to influence glucose tolerance, i.e. sulfonylureas, growth hormone, metformin, anticonvulsants, thiazide or potassium depleting diuretics, beta adrenergic blockers, niacin. Participants on such medications should be changed to a suitable alternative, if available, and will become eligible one month after medication is discontinued.

9. Pregnant, intends to become pregnant while on study, or lactating.

10. Deemed unlikely or unable to comply with the protocol or have any complicating medical issues or abnormal clinical laboratory results that interfere with study conduct or cause increased risk.

### 3.4. Study Population

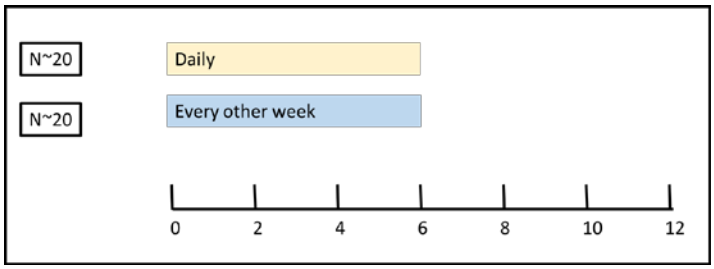
Recruitment and initial screening to identify participants will be done through the TrialNet TN01 Natural History/Pathway to Prevention Protocol. As part of this protocol and TN01, participants will then undergo additional testing, and if eligible and willing, will be randomized and followed as described. Participants determined during screening to be ineligible or unwilling to be randomized to this protocol will be offered follow-up under the Natural History/Pathway to Prevention Protocol.

### 3.5. Description of Treatment Groups

This protocol will enroll at least 40 participants who will be randomly assigned to the following groups:

- 20 participants will be assigned to receive Oral Insulin daily (67.5 mg)
- 20 participants will be assigned to receive Oral Insulin every other week (starting at 135 mg and titrating up to 500 mg every other week as tolerated)

**Figure 7**. Proposed study schema to examine changes in immunologic biomarkers in treatment-naïve and treatment –responsive participants following introduction of oral insulin as daily or every other week dosing.




### 3.6. Treatment Assignment

After participants sign the consent form, complete the screening visit(s), meet all of the inclusion criteria, and none of the exclusion criteria, and complete the baseline procedures, they will be randomized to receive one of two dose schedules of oral insulin. Participants will be randomized in equal allocations to each group. The randomization method will also be stratified by age group to ensure balance between the treatment arms for this potential confounder.

### 3.7. Study Assessments

The intervention protocol will be conducted at TrialNet Centers and Affiliates. During the course of the study, participants will frequently undergo assessments of their insulin production, immunologic status, and overall health and well-being (see Appendix A, Schedule of Assessments). All blood and serum samples for outcome determinations will be sent to the TrialNet Core Laboratories for analysis.

The primary outcome is the change in immunologic biomarkers before and after treatment. Additional outcomes include safety measures and disease progression assessed by regular glucose tolerance testing.

During the course of the study, samples will be drawn for storage at the National Institute for Diabetes and Digestive and Kidney Disease (NIDDK) Repository and at TrialNet Sites for future analysis. Participants who decline consent for these sample collections will not be eligible to participate in this study (see Section 10.4).

### 3.8. Quality Assurance

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

### 3.9. Study Timeline

Participants are enrolled and followed for a total duration of 12 months through this protocol.

### 3.10. Post-Treatment Follow up

Participants who have not developed T1D at the conclusion of the study will return to the TN01 Natural History/Pathway to Prevention Study. They may be eligible for future TrialNet prevention studies. This will be determined when future studies are developed.

Participants who develop T1D while enrolled in the TN20 Immune Effects of Oral Insulin Study will be offered follow-up in the TN16 Long Term Investigative Follow-Up in TrialNet (LIFT) Study.

## 4. PARTICIPANT MANAGEMENT

### 4.1. Screening Visit and Eligibility Assessment

Participants potentially eligible for this study will be identified through the TrialNet TN01 study. They will be notified of their eligibility by TrialNet Investigators or qualified staff. The participant will be asked to sign an informed consent document describing the purpose, risks, and benefits of this study. Assent will be obtained for children. A participant's signature indicates that he/she understands the potential risks and benefits of study participation.

The initial testing for mIAA and other autoantibodies will be done as part of TN01 screening. Those individuals who are mIAA positive will then be eligible for additional tests as part of TN01 Monitoring visit or this protocol as applicable.

### 4.2. Randomization

All eligible study participants will be randomized to one of two treatment arms, once eligibility has been confirmed. Randomization to each of the two arms will be in a 1:1 manner (i.e. equal allocation to the treatment arms). To prevent potential confounding, randomization will be stratified by age at study entry. After randomization, study medication will be dispensed to the participant per the dose and schedule of the arm to which they were randomized.

### 4.3. Drug Administration

Participants will then take study drug according to the treatment arm in which they are assigned. Those assigned to daily oral insulin will receive 67.5 mg per day. Those assigned to every other week oral insulin will receive 135 mg for the initial dose, 250 mg for the second dose, and 500 mg every other week thereafter as tolerated. Participants will be observed at the study site for 2 hours after dosing visits V0(A), V0(B), and V1. Participants will be contacted at a minimum of every other week while on treatment to facilitate compliance.

### 4.4. Treatment Modification or Discontinuation

Participants who have a screening glucose value < 50 mg/dl will not be randomized in the study. Participants who have pre-dosing glucose values <50 mg/dl will not receive oral insulin. If this occurs on visit 0 (A) such that the participant will have never taken oral insulin, the participant will be withdrawn from the study. If this occurs on subsequent dosing visits, the participant will not receive any further study medication. They will continue with study visits per protocol (Appendix A).

Participants who have post-dosing mild, prolonged, or severe hypoglycemia as defined in section 6.1.5 will not receive further study medication. They will continue with study visits per protocol (Appendix A). Participants may also be discontinued from treatment due to adverse effects of treatment that are ≥ Grade 3 and that in the judgment of the investigator are at least possibly related to the study medication. These participants will continue with study visits per protocol (Appendix A).Participants who revoke their consent to be treated will also be discontinued from treatment. These participants will also continue to be monitored through study visits per protocol unless they withdraw consent for further follow-up.

Participants with acute upper GI illness, or symptoms needing medications described in exclusion criteria 4 should withhold drug and contact study team for advice on when to resume study drug.

Participants will not be discontinued from evaluation and follow-up on this study due to non-compliance.

## 5. STUDY VISIT ASSESSMENTS

See Appendix A for detailed schedule of assessments.

### 5.1. General Assessments

General assessments for this Protocol will include:

- Informed consent
- Inclusion/exclusion criteria
- Medical history
- Physical examination including height/weight
- Concomitant medications
- Adverse events
- Pregnancy test (if female of reproductive potential)
- Treatment compliance

### 5.2. Mechanistic Outcome Assessments

Mechanistic assessments will consist of studies such as:

- DNA for testing diabetes or other immune-associated genetic markers.
- RNA for the evaluation of immune cell frequency and function by gene expression analysis
- Peripheral Blood Mononuclear Cells (PBMCs) for the evaluation of immune cell function, especially antigen-specific responses relevant to the hypothesis that oral insulin can induce a state of tolerance to islet proteins.

- Serum and plasma for the evaluation of islet autoantibody epitope, affinity, isotyping and proteomics-based assessment of immune responses.
- CBC with Differential

### 5.3. Metabolic Outcome Assessments

Metabolic assessments will consist of:

- Baseline OGTT (i.e. within 52 days of initiation of treatment), as well as OGTTs at the 6 month and 12 month visits. Glucose, insulin, C-peptide, and other analytes will be measured from OGTT samples.
- Glucose, insulin, and C-peptide will be measured before the study drug dose, and at 1 and 2 hours after dosing during study visits V0(A), V0(B), V1.
- HbA1c

### 5.4. Visit Windows

Initial treatment administration (Visit 0(A)) should begin within 7 weeks, (no more than 52 days) from the OGTT. For Visit 0(B) and Visit 1, the window is +/- 3 days of the target date. For Visit 2 through Visit 7, the window is +/- 7 days of the target date. Visit 8 should be within +/- 14 days of the targeted date.

The target date is based on the date of initial treatment administration.

## 6. ADVERSE EVENT REPORTING AND SAFETY MONITORING

### 6.1. Adverse Event Definitions

#### *6.1.1. Adverse Event*

In this clinical research study, an adverse event is any occurrence or worsening of an underlying condition of an undesirable or unintended symptom or disease.

Throughout the study, the investigator must record adverse events on source documents, regardless of the severity or perceived attribution to the study medication. The investigator should treat participants with adverse events appropriately and observe them at suitable intervals until the events resolve or stabilize.

Adverse events may be discovered through:

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

Questioning of the participant should be conducted in an objective manner.

#### *6.1.2. Adverse Reaction*

An adverse reaction means any adverse event caused by a drug. Adverse reactions are a subset of all suspected adverse events for which there is reason to conclude that the drug caused the event. Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of safety reporting, “reasonable possibility” means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than an adverse reaction, which means any adverse event caused by a drug. Examples of evidence that suggest a causal relationship (reasonable possibility) between the drug and the adverse event include:

- A single occurrence of an event that is uncommon and known to be strongly associated with drug

exposure

- One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the populations exposed to the drug
- An aggregate analysis of specific events observed in clinical research (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group.

### *6.1.3. Serious Adverse Event/Reaction*

A serious adverse event (SAE) or reaction is defined as "any adverse event occurring at any dose that suggests a significant hazard, contraindication, side effect, or precaution." An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

1. Death. A death that occurs during the study or that comes to the attention of the investigator during the protocol-defined follow-up after the completion of therapy must be reported whether it is considered to be treatment related or not.

2. A life-threatening adverse event. A life-threatening event is any adverse therapy experience that, in the view of the investigator, places the participant at immediate risk of death from the reaction as it occurred.

3. Inpatient hospitalization or prolongation of existing hospitalization, with the exception of hospitalization relating to initial diagnosis of type 1 diabetes.

4. Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions

5. Congenital anomaly or birth defect.

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

An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the participant at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

Regardless of the relationship of the adverse event to study drug, the event must be reported as a serious adverse event if it meets any of the above definitions.

### *6.1.4. Unexpected Adverse Event*

An adverse event is considered unexpected when the nature (specificity) or severity of the event is not consistent with the risks described in the protocol or informed consent document for a particular protocol required intervention. Unexpected refers to an experience that has not been previously observed. This includes events that occur more frequently than expected.

#### *6.1.5. Grading Event Severity*

TrialNet has adopted usage of the most current version of the National Cancer Institute (NCI) Common Toxicity Criteria for Adverse Events (with the exception of hyper or hypoglycemia including the diagnosis of diabetes whether or not it requires hospitalization to initiate treatment). In the context of this trial, hypoglycemia will be defined as follows: (a) *mild hypoglycemia* is defined as signs and symptoms associated with hypoglycemia and glucose <50 mg/dl that responds to initial oral treatment and does not recur during observation period, (b) *prolonged hypoglycemia* is defined as signs and symptoms associated with hypoglycemia and glucose <50 mg/dl that responds to oral treatment but is persistent or recurs during observation period, and (c) *severe hypoglycemia* is defined as signs and symptoms associated with hypoglycemia that does not respond to oral treatment or requires assistance from others due to altered consciousness. Data regarding onset of diabetes are gathered as study measures. TrialNet Investigators will also provide an assessment of relationship of AE to study drug as not, unlikely, possibly, probably, or definitely related.

### 6.2. Adverse Event Reporting and Monitoring

Adverse events will be reported to the TrialNet Coordinating Center in accordance with the TrialNet Adverse Event Monitoring Plan (and according to study specific criteria stated in section 6.1. The investigator will grade their severity according to common toxicity criteria or study-specific criteria and will make a determination as to the relation to therapy. Events will be assessed and reported in accordance with the ICH Guidelines for Good Clinical Practice, 21 CFR 312.32 for expedited safety reporting, and per the guidance of the DHHS Office for Human Research Protections (OHRP).

The adverse event case report form for the protocol must be completed for all adverse events greater or equal to Grade 2 of the NCI CTCAE with the exception of hyper or hypoglycemia including the diagnosis of diabetes whether or not it requires hospitalization to initiate treatment. For reporting serious adverse events (SAE), the TrialNet MedWatch Form should also be completed and faxed to the TNCC *within 24 hours of when the site was notified of the event.* This will be reviewed by the TrialNet Medical Monitor, the TrialNet Safety Monitoring Committee, and the DSMB as appropriate.
Deaths must be reported immediately. Event outcome and other follow-up information regarding the treatment and resolution of the event will be obtained and reported when available, if not known at the time the event is reported. The follow-up information should contain sufficient detail to allow for a complete medical assessment of the case and an independent determination of possible causality.

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

For SAEs that are unexpected and considered possibly or probably drug related, the Medical Monitor will provide information on frequency of similar events, and generate FDA form 3500A reports (MedWatch form) for distribution to FDA, NIDDK, DSMB and site investigators. Expedited safety reports will be submitted to the IND by the NIDDK.

## 7. PARTICIPANT SAFETY

### 7.1. Risks, Benefits and Inclusion of Children

The risks of this study are presented below and in the informed consent form.

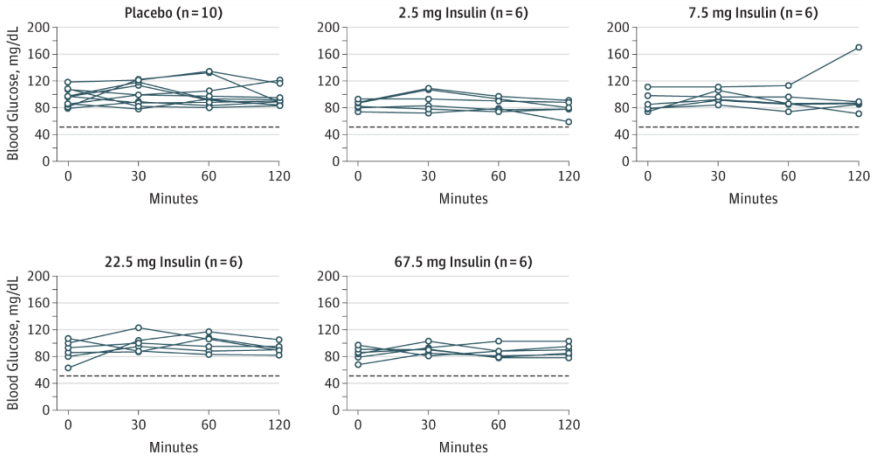
There is the prospect of direct benefit to the individual participants for their participation in the study. These potential benefits include the recognized benefits of being in a clinical study, including close monitoring offered to all participants regardless of group assignment. Further, the intervention has the prospect of direct benefit to a given participant as all participants will receive active drug, which may delay or prevent disease progression. Moreover, the study is likely to yield general knowledge about

T1D which is of importance for the understanding and amelioration of T1D in children.

The study procedures, consisting of blood draws and oral glucose tolerance testing, are minimal risk. Moreover, with over 1,349 person years of exposure, no adverse events related to oral insulin have been reported, suggesting that administration of oral insulin is also minimal risk. However, as it remains an investigational agent, taking oral insulin may be considered only slightly greater than minimal risk. Also, it offers the possibility of benefit due to the close monitoring for all participants, including children. Assent of children along with consent of the parent/legal guardian will be obtained prior to any study procedures. This research proposal in children is therefore consistent with the United States Department of Health and Human Services, Protection of Human Subjects, Subpart D, section 46.405 (research involving slightly greater than minimal risk but presenting the prospect of direct benefit to individual subjects) and with Subpart D. 50. 52 (Clinical investigations involving greater than minimal risk but presenting the prospect of direct benefit to individual subjects).

### 7.2. Expected Side Effects and Adverse Events

There were no side effects or adverse events associated with oral insulin during the DPT-1 and TN07 Oral Insulin trials, which consisted of the same study population. Participants did occasionally have mild expected adverse events associated with study procedures such as fainting and bruising with phlebotomy. While preclinical models have suggested that it is theoretically possible that oral insulin could accelerate disease, there is no evidence of disease acceleration or hypoglycemia in the ongoing TN07 trial in which >250 individuals have received oral insulin up to 8 years. There were no associated adverse effects including no hypoglycemia noted in the pilot Pre-POINT study, which used higher doses of oral insulin, similar to what will be used in this trial. As illustrated below, no change in glucose values were observed (45).




### 7.3. Protecting Against or Minimizing Potential Treatment Risks

Participants who have other active serious medical problems will not be enrolled. To assure that every other week doses of insulin are well tolerated, those in the every other week dosing group will receive an initial dose of 135 mg oral insulin and a second dose of 250 mg oral insulin, before receiving the full 500 mg dose every other week.

As noted above (section 7.2), oral insulin is not metabolically active; thus, no hypoglycemia from study medication is expected. Moreover, asymptomatic and not clinically important hypoglycemia can occur in those with beta cell dysfunction (44). Individuals with pre-treatment glucose values < 50 mg/dl at screening or at study visits V0(A), V0(B), or V1 will not receive any further study medication. Those who receive study drug at these visits will be observed for 2 hours after the dose of study medication. If signs or symptoms consistent with hypoglycemia are observed, the participant's blood glucose will be checked. If glucose is < 50 mg/dl, the participant will be treated as needed and observed at least

an additional hour before discharge when glucose ≥50 mg/dl. Any individual with mild, prolonged, or severe hypoglycemia during this period will not receive any further study medication but will continue to be monitored per protocol (Appendix A).

In the unlikely event that any individual has prolonged or severe hypoglycemia (as defined in section 6.1.5), or if 2 or more individuals have mild hypoglycemia (as defined in section 6.1.5), further enrollment will be temporarily suspended pending full review by the study group and the medical monitor.

Regular monitoring of participants and active inquiry will allow for early identification of adverse events.

### 7.4. Pregnancy

Pregnant and lactating women will not be included in this study. Females must have a negative pregnancy test prior to enrolling in the study and will be required to either use birth control or practice abstinence during the study. At every study visit, the sexual activity of female participants of reproductive age will be re-assessed. If a participant who was previously sexually inactive becomes sexually active, she will be counseled about the need to use a reliable form of birth control. All female participants of reproductive potential will undergo urine pregnancy tests at regular intervals.

## 8. STATISTICAL CONSIDERATIONS AND ANALYSIS PLAN

### 8.1. Overview

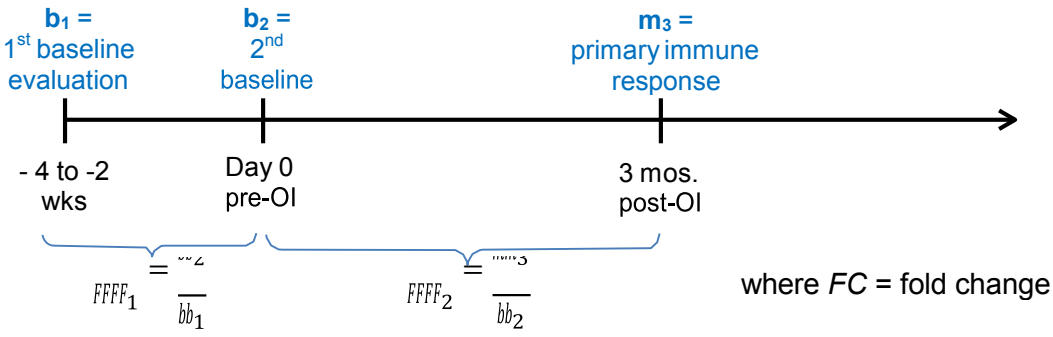
This is a randomized study designed to assess the impact of two different oral insulin regimens on immune function and markers in participants at risk for T1D. Specifically, patients will be randomized to receive either daily (67.5 mg/day) or biweekly (starting at 135 mg up to 500 mg as tolerated every other week) oral insulin dosing. The primary goals of this study are focused on assessing immune and inflammation markers before and after administration of these two different oral insulin regimens in this patient population, and how these immunological biomarker changes differ between the treatment arms.

### 8.2. Study Design, Accrual, and Study Duration

The overarching goal of this study is to evaluate how varying oral insulin regimens affect immune function and related markers. Specifically, eligible participants will be randomized to one of two oral insulin regimens defined in Section 3.5. The accrual period for this trial will be approximately 9 months; based on historical data from the ongoing TN07 study of oral insulin in this same population, we plan to enroll a total of 40 evaluable participants, where 20 participants will be randomized to each of the two treatment arms. If accrual is more rapid than expected and 40 patients are accrued and randomized to the two treatment arms prior to the end of the accrual period, additional participants may continue to be randomized to each of these treatment arms. This allows for limited over accrual to accommodate the possibility of participants dropping out prior to post-treatment sample collection, which would make them not evaluable for the primary mechanistic endpoint.

Eligible patients will be randomized to one of two oral insulin regimens in a 1:1 allocation using block randomization with variable block sizes. To ameliorate the possible confounding effects of age on the immunologic primary endpoint markers, randomization will be stratified by age at study entry (<10 vs. ≥10 years old) to ensure balance of this factor across the two arms. Given that this is a mechanistic study focusing on effects on the immune system after treatment with these varying oral insulin regimens, we will employ a treatment-received analysis approach. Any participant who receives at least one dose of oral insulin in accordance to their treatment arm will be considered evaluable and will be included in these analyses.

In lieu of a placebo arm, we will use the two baseline samples on each participant to gauge the intra- participant variability and likelihood of immunologic changes for the key biomarkers in the absence of treatment.




### 8.3. Analysis Plan

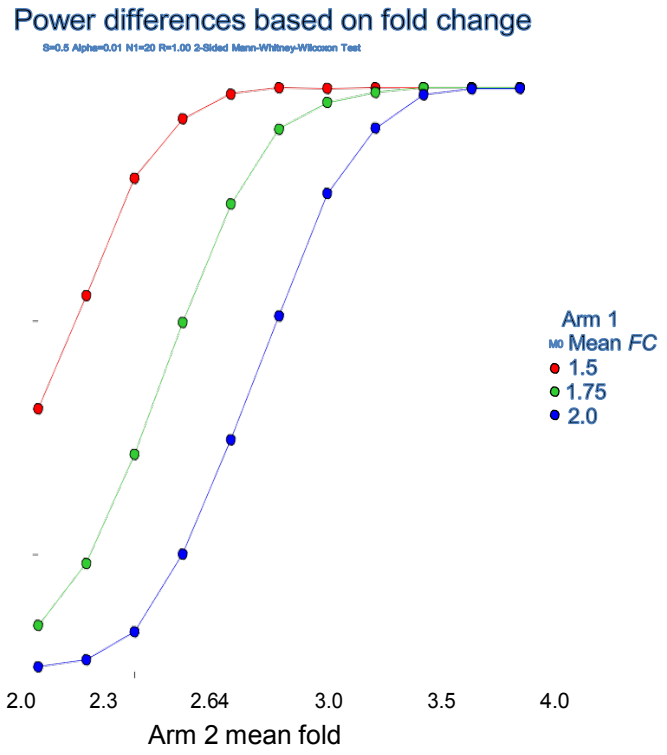
Immune function can be assessed through multiple (often correlated) markers as well as additional cytokine/inflammation/genetic markers; however, our primary outcome is the change in immune function as assessed by level or quality of T lymphocyte or autoantibody biomarkers of β-cell specific immune response measured between 13 and 26 weeks after 1st dose versus baseline. As described in Section 3.2, specific outcome measures may include CD4 auto-antigen specific ELISPOTs for IFN-γ and IL-17, and CD8 peptide-HLA multimers (Q-dots) and other assays from just before treatment to 3-months post-treatment. To identify the most promising regimen of oral insulin in terms of immunologic change, we will compare these changes in the selected immunobiomarkers between the treatment arms. Specifically, we will calculate the fold change from baseline pre-treatment to 3 months after oral insulin treatment is started. In order to adjust for potential intra-participant variability that may exist in these measures in the absence of treatment, we will "normalize" these fold change measures such that you have the 3-month post-treatment sample marker levels divided by the day 0 marker levels as the fold change of interest, and then divide that by the fold change observed in the "control phase", or the no-treatment phase, so to speak, with the ratio of the day 0 baseline to the sample marker levels 2-4 weeks prior to treatment (i.e. $FC_2 \div FC_1$ from above figure).

We recognize that some of these measures may not have detectable levels at baseline or even after treatment. Since it is important to capture not only a maintenance of undetectable counts of these markers but also to capture if there is an increase in these markers (for better or worse), we will impute the midpoint between 0 and the lower limit of detection (LLOD) specific to the assay and plate/run for participants identified as having measures below the LLOD for that marker. In the setting where levels below the LLOD can be estimated from a standard curve with the assay, we will still consider these unreliable estimates below the LLOD and will impute the midpoint as well. If levels for a participant remain undetectable before and after treatment, then this imputation allows them to be captured as having a fold change equal to one for that marker, indicating no change. This will, however, allow capture of an increase in these levels for that marker in that participant if pre-treatment levels are below the LLOD but increase above that threshold post-treatment. This is particularly important for measures such as the ELISPOT measures of IFN-γ and IL-17, where an increase in these markers is considered a negative outcome.

In this setting, a fold change or ratio equal to 1 reflects no change in the marker(s) after treatment with oral insulin. For each of the markers (e.g. ELISPOT IFN-γ, ELISPOT IL-17, peptide-HLA multimers – naïve, effector, stem-like) we will use nonparametric two-sided Mann-Whitney-Wilcoxon (rank sum) tests to compare the fold changes in these markers (via ranks) between the arms. Since we are comparing fold change measures for each participant, and how this responsiveness to oral insulin therapy differs based on dose and schedule, we expect that each arm will have some level of responsiveness and thus our comparison will be focused on which induces a greater change to the markers. With 20 participants per treatment arm, we will have at least 80% power to detect a difference in the fold changes between the arms for each of the markers (vs. the null hypothesis that there is no difference between the arms). This is based on the assumption that the true mean fold change for one

arm is 1.5 (a 50% increase) and that the true mean fold change in the other treatment arm is at least 2.3 (i.e. a 2.3 fold increase in marker level). This conservatively constrains the Type I error to 0.01 (based on a Bonferroni correction to accommodate the simultaneous evaluation of up to five different immunologic markers in these comparisons). These calculations assume an underlying normal distribution of the measures after a $\log_2$ transformation of these ratios, and assume standard deviations of 0.5 for these underlying distributions. Even if the less effective treatment schedule is more effective than the above scenario assumption and has a true mean fold change of 1.75, we will still have at least 80% power to detect a difference in marker fold change between the arms if the true mean fold change for the other arm is 2.64. The same power statements hold for the inverse of these; i.e. to detect significant differences for these magnitudes of differences in the reduction of these markers (e.g. mean fold change of 0.67 vs. 0.435). Even in the unlikely case that the true underlying distribution of these fold changes for the markers is actually a uniform distribution, we will still have at least 80% power to detect a difference between the arms if the average fold changes are 2.8 vs. 1.5 or 4 vs. 1.75.

In addition to looking at differences between the treatment arms, we will also evaluate each of the arms individually to test if there are significant changes in the fold changes in each of the markers after oral insulin treatment. With 20 participants in an arm, we will have at least 90% power to detect a significant change in a marker if the true mean fold change is at least 1.4 (i.e. a fold increase by 1.4 or greater) or a decrease of the same magnitude (fold change of 0.72) versus the null hypothesis in this single-arm evaluation that there is no change in these markers before and after treatment (with the same other assumptions as above: significance level of 0.01, underlying normal distribution for the $\log_2$ transformed values, standard deviation of 0.5 on that scale). Even if there is greater variability in the measures for the underlying distribution (e.g. sd=0.75), we will still have at least 80% power to detect a significant change in these markers after oral insulin treatment if the true mean fold change is 1.6 (or conversely, 0.625) versus the null hypothesis that the true mean fold change is 1.0.




In an ancillary manner, we will use each participant as his or her own control, and assess changes in each of the primary biomarkers using a paired test (Wilcoxon signed rank test) to evaluate the differences in the fold change between the two baseline evaluations ($FC_1$) vs. the fold change between the pre-treatment ($b_2$) and post-treatment ($m_3$) evaluations ($FC_2$). With 20 participants in a group, we will have at least 90% power to detect a significant difference in the means, if the true mean fold change after treatment is 1.52 or greater vs. no change (fold change of 1) in the two baseline assessments, again constraining the two-sided significance level to 0.01, assuming an underlying normal distribution of the $\log_2$ transformed values and sd=0.5. We will also evaluate the overall effect of oral insulin regardless of dose/schedule versus the biomarker changes without treatment. With 40 participants, we will have at least 90% power to

detect a significant difference in fold change if the true mean fold change after treatment is 1.35 or greater vs. no change (fold change of 1) between the two baseline assessments.

To visually assess patterns of change, we will graphically analyze the fold change for each of the immunologic markers at each of the timepoints for each treatment arm. Since the kinetics of immune change and response at certain timepoints are currently unknown, we will evaluate this first using descriptive statistics to characterize the fold change measures at each of the timepoints after treatment is started: 1, 2, 3, and 6, as well as after treatment is completed: 7, 8, 9, and 12 months. Repeated measures ANOVA models will also be used to compare the responsiveness to oral insulin regimens in terms of fold changes for each of the immunologic biomarkers. Multivariate ANOVA (MANOVA) models will also be used to compare more broadly the impact of these regimens on immune function, where the different cell type markers will be assessed through this model for CD4+ T cells and for CD8+ T cells. To assess longer-term impact of an oral insulin regimen on immune cell mobilization, we will evaluate fold changes in these markers after completion of therapy (e.g. at 7 and 9 months after initiation of treatment) to assess whether or not any immune stimulation is maintained. Pairwise comparisons of the fold change in markers will be made between arms for the various timepoints using two-sided nonparametric Wilcoxon rank sum tests, as well as graphically using side-by-side boxplots.

In addition, we will evaluate the impact of treatment arm and other demographic and clinical factors such as age, gender, and autoantibody profile in the univariate setting on these biomarkers, as well as assess the influence of treatment arm when adjusting for age and other factors in the multivariable setting. Here, we can also assess the influence of other immunologic markers of interest on these changes in the primary immune biomarkers being evaluated above. Since timing of immune response is not well understood in this setting, we will also evaluate changes in these markers over time, particularly after treatment is completed.

In a secondary manner, we will also evaluate multiple additional immunologic markers, such as phenotypic markers: naïve, effector memory, and central memory CD4+ T cells and CD8+ T cells as well as regulatory T cells. These markers will be measured as absolute numbers per mL, and will be assessed at baseline (i.e. prior to oral insulin treatment) as well as at multiple timepoints during and after treatment (Appendix A). Given that our focus is on how these oral insulin regimens affect immune cell mobilization and thus a response by the immune system, we will assess the fold change at the various timepoints (i.e. the ratio of the post-treatment level to the baseline marker levels).

Secondary endpoints will also include clinical responses to treatment as measured by metabolic markers before and after treatment. C-peptide levels along with insulin and glucose levels before and after treatment will be captured and evaluated to better characterize these participants, particularly in relation to changes in immune markers.

### 8.4. Toxicity and Tolerability

Analysis of this trial will include summarization of the toxicity and tolerability by arm using NCI's CTCAE with the exception of hypoglycemia, which will be summarized by arm according to definitions in section 6.1.5. Frequency and severity of adverse events and tolerability of the regimen in each of the treatment arms will be collected and summarized using descriptive statistics. As per NCI CTCAE, the term toxicity is defined as adverse events that are classified as either possibly, probably, or definitely related to study treatment. The maximum grade for each type of toxicity will be recorded for each patient, and frequency tables will be reviewed to determine toxicity patterns. In addition, we will review all adverse event data that is graded as 3, 4, or 5 and classified as either "unrelated" or "unlikely to be related" to study treatment in the event of an actual relationship developing. The

incidence of severe (grade 3+) adverse events or toxicities will be described.

In addition, we will also capture the proportion of participants who go off treatment due to adverse reactions or those who refuse further treatment for lesser toxicities that inhibit their willingness to continue participation on the trial. These tolerability measures will be assessed within each of the treatment arms and we will evaluate differences in these measures between the arms. All participants who have received at least one dose in a treatment arm will be evaluable for toxicity and tolerability.

In order to closely monitor for hypoglycemia, all participants will have pre- and post-dose glucose, insulin, and C-peptide levels for each treatment arm evaluated. We will closely monitor and summarize glucose levels before and after treatment as specified. Incidence of mild, prolonged, and severe hypoglycemia events will be summarized based on the definitions in Section 6.1.5.

Although unlikely and not expected, we will closely monitor for hypoglycemia events as defined in Section 6.1.5. If at any time any patient is reported to have prolonged or severe hypoglycemia or if at any time two or more patients are reported as having mild hypoglycemia based on these definitions, we will temporarily suspend accrual to the trial pending full review by the study team, Medical Monitor.

### 8.5. Primary Outcome

The primary outcome is the change in immune function as assessed by level or quality of T lymphocyte or autoantibody biomarkers of β-cell specific immune response measured between 13 and 26 weeks after 1$^{st}$ dose versus baseline.

## 9. ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE

### 9.1. Statement of Compliance

This study will be conducted in compliance with the protocol and consistent with current Good Clinical Practices (GCP), adopting the principles of the Declaration of Helsinki, and all applicable regulatory requirements (*ICH E6, 45CFR46, and FDA 21CFR sections 11, 50, 56, 312)*.

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

### 9.2. Participating Centers

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

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

### 9.3. Informed Consent

The process of assuring that individuals (and parent/guardian if less than 18 years of age) are making an informed decision about participating in this study includes both verbal and written communication.

Written material includes a Participant Handbook, Volunteer Understanding Assessment, and written consent forms. The consent form describes the procedures, risks, and benefits for the study. The consent form will be reviewed with participants (and their guardian in the case of participants under 18 years of age) and the participant will be given time to review the written consent form and ask questions. An assent form has also been developed for participants less than 18 years of age (unless local IRB requirements differ in procedure).

As part of the informed consent process, the participant and/or parent or guardian (if the participant is less than 18 years of age) will also complete a short, written Volunteer Understanding Assessment that is designed to ensure that the participant understands the study, as well as what is being asked of him/her. The participant will be given a copy of their signed consent/assent forms.

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

The informed consent form must be updated or revised whenever 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 participant's participation in the study.

Participants will be re-consented if they reach the age of 18 years while enrolled in the study.

### 9.4. Study Participant Confidentiality

Study participant data, which is for reporting purposes, will be stored at the TrialNet Coordinating Center. Data sent to the Coordinating Center will identify participants by the unique TrialNet Identification Number. The data entry system at the Coordinating Center is a secured, password-protected computer system. At the end of the study, all study databases will be archived at the Coordinating Center for long-term storage.

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

# 10. STUDY ADMINISTRATION

## 10.1. Organizational Structure

This study is part of Type 1 Diabetes TrialNet, which is funded by the National Institutes of Health, principally the National Institute of Diabetes, Digestive and Kidney Diseases. Funding will cover the costs of administration and laboratory tests associated with this study during the participant's period of follow-up. Eli Lilly and Company will provide oral insulin crystals free of charge.

The proposed study medication, oral insulin, is not commercially available. Eli Lilly and Company is providing the oral insulin crystals for this study.

## 10.2. Groups and Committees

### *10.2.1. Oral Insulin Trial Protocol Committee*

The Study Chair and TrialNet Executive Committee will receive periodic reports from the TNCC on the progress of the study. These will include accrual rates and baseline demographic characteristics. Interim data summaries provided to others (except those that could lead to unmasking of study outcome) will first be supplied to the Study Chair for review. Criteria and results of ongoing monitoring of the TrialNet labs in terms of reproducibility will also be provided on a routine basis and reported on during the Joint Prevention Study Chair Committee meetings, as scheduled. As appropriate, abstracts and manuscripts dealing with the progress of the trial shall be directed by the TN20 Immune Effects of Oral Insulin Trial Protocol Committee.

### *10.2.2. TrialNet Chairman's Office, TrialNet Coordinating Center, and the TrialNet Hub*

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

### *10.2.3. Clinical Sites*

Principal Investigator at each participating TrialNet clinical site will oversee all operations at that site. The clinical sites will forward all laboratory and data collection form information to the TNCC for analysis. Direct communication and site visits, as needed, will facilitate evaluation of the trial management.

### *10.2.4. Safety Monitoring Committee and Medical Monitor*

The Type 1 Diabetes TrialNet Safety Monitoring Subcommittee (SMS) is responsible for establishing policies and procedures for assurance of safety monitoring of TrialNet protocols and of TrialNet participants. The Safety committee will review all serious AEs and receive summary reports of all AEs.

All adverse events will be recorded on the adverse event forms, which will be sent to the local IRBs/REBs, per their reporting requirements, and to the TNCC.

An independent physician will be designated to serve as the medical monitor for this study who will maintain regular contact with the study and the Study Chair. (S)he will review all adverse event reports and will file event reports with regulatory authorities as appropriate

#### *10.2.5. Clinical Site Monitoring*

In order to conduct this study with established research principles and ICH-GCP guidelines, site visits will be conducted during the study to evaluate study conduct and ensure participant safety. All sites will be monitored by the TNCC and appropriate TrialNet committees for participant enrollment, compliance with protocol procedures, completeness and accuracy of data entered on the case report forms (CRFs), the occurrence and reporting of adverse events (AEs) and serious adverse events (SAEs), site pharmacy accountability/operations, and to confirm the presence of appropriate IRB/REB regulatory approvals/documents.

#### *10.2.6. Data and Safety Monitoring Board (DSMB)*

The DSMB will meet approximately every 6 months to review the potential toxicity and tolerability of the study treatments as defined in Sections 8.4.1 and 7.3 based on interim summaries and reports prepared by the TNCC. The DSMB will independently evaluate whether there are grounds to modify or discontinue the study.

### 10.3. Sample and Data Storage

Stored samples, including genetic material, 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.

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

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

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

Genetic and other biological material will be stored for future use with the permission of the study participant as described above. The results of these future analyses will not be made known to the participant.

### 10.4. Preservation of the Integrity of the Study

The scientific integrity of the trial dictates that results be reported on a study-wide basis; thus, an individual clinical site will not report the data collected from its site alone. All presentations and publications using TrialNet data must protect the main objectives of the trial. Data that could be perceived as threatening these objectives will not be presented prior to release of the primary study outcomes. The TrialNet Publications and Presentations Committee will approve the timing of

presentations of data and the meetings at which they might be presented, and the publication of results and the selection of the journal to which each paper will be submitted for publication. Study results should be discussed with the news media only upon authorization of the Executive Committee, but never before the results are presented. Any written statements about this study that are shared with national media should be approved by the Executive Committee and the National Institute of Diabetes, Digestive and Kidney Diseases before release.

### 10.5. Participant Reimbursement and Compensation

Participants may be compensated for each visit attended in the study. In compliance with ICH Guidance E6, the amount and method of payments to participants shall be designed to avoid coercion or undue influence on the study participants. Payments to participants will be prorated and not wholly contingent on completion of the trial by the participant.

## Appendix A: Schedule of Assessments

| Month of Trial | | 0 | 2 weeks | 1 | 2 | 3 | 6 | 7 | 8 | 9 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Visit number | -1 | 0 ( Visit A) | 0 (Visit B)[1] | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Informed Consent | X | | | | | | | | | | |
| Inclusion/exclusion Criteria | X | | | | | | | | | | |
| Medical History | X | | | | | | | | | | |
| Physical Exam | (X[2]) | (X[2]) | | | | | | | | | X |
| Concomitant Medications | X | X | X | X | X | X | X | X | X | X | X |
| Adverse Events[3] | X | X | X | X | X | X | X | X | X | X | X |
| Pregnancy test (if female of reproductive potential) | X | X | X | X | X | X | X | X | X | X | X |
| Drug Dispensation | | X[4] | X[4] | X[4] | X | X | | | | | |
| Assess Treatment Compliance | | | | X | X | X | X | | | | |
| Oral Glucose Tolerance Test | X[5] | | | | | | X | | | | X |
| HbA1c | (X[6]) | (X[6]) | | | | X | X | | | X | X |
| Diabetes Associated Autoantibodies | X[6] | | | | | | | | | | |
| Samples for Mechanistic Assays[7] | X | X | | X | X | X | X | X | X | X | X |

[1] Visit V0 (B) only applies to participants in the 500mg every other week treatment group .

[2] Physical Exam may be performed at the -1 or 0 visit, and is not required at both.

[3] Complete directed Physical Exam, if appropriate, for evaluation of AE

[4] Participants will be observed for 2 hours after dosing with samples obtained for glucose, insulin, C-peptide prior to and 1 and 2 hours after dosing.

[5] TN20 Screening OGTT and/or autoantibody confirmation can be performed as either part of the TN01 Natural History/Pathway to Prevention Study visit, or the TN20 screening visit. Participants who are not confirmed mIAA and other autoantibody positive prior to screening may use results from the screening visit or tie-breaker PRN visit for TN20 randomization eligibility.

[6] HbA1c may be collected at either the -1 or 0 visit, and is not required at both.

[7] May include serum, plasma, whole blood, PBMC, RNA, DNA, and CBC with differential. Schedule of assessments may vary as appropriate. Total blood draw volume in adults will not exceed 10.5 ml/kg or 550 ml, whichever is smaller, over an 8 week period. For children, no more than 5 ml/kg will be drawn at any single visit and no more than 9.5 ml/kg over an 8 week period.

## References


1 Silink M. Childhood diabetes: a global perspective. Horm Res 2002; 57 Suppl 1:1-5.

2 Karvonen M, Pitkaniemi J, Tuomilehto J. The onset age of type 1 diabetes in Finnish children has become younger. The Finnish Childhood Diabetes Registry Group. Diabetes Care 1999; 22(7):1066-1070.

3 Miller KM, Foster NC, Beck RW, et al.; T1D Exchange Clinic Network. Current state of type 1 diabetes treatment in the U.S.: updated data from the T1D Exchange clinic registry. Diabetes Care 2015;38:971– 978

4 Yach D, Stuckler D, Brownell KD. Epidemiologic and economic consequences of the global epidemics of obesity and diabetes. Nat Med 2006; 12(1):62-66.

5 Atkinson MA: ADA Outstanding Scientific Achievement Lecture 2004. Thirty years of investigating the autoimmune basis for type 1 diabetes: why can't we prevent or reverse this disease? Diabetes 54:1253-1263, 2005.

6 Riley WJ, Maclaren NK, Krischer J, Spillar RP, Silverstein JH, Schatz DA, Schwartz S, Malone J, Shah S, Vadheim C, et al.: A prospective study of the development of diabetes in relatives of patients with insulin-dependent diabetes. N Engl J Med 323:1167-1172., 1990.

7 Sherr J, Sosenko J, Skyler JS, Herold KC: Prevention of type 1 diabetes: the time has come. Nat Clin Pract Endocrinol Metab 4:334-343, 2008.

8 Eisenbarth GS: Type I diabetes mellitus. A chronic autoimmune disease. N Engl J Med 314:1360-1368, 1986.

9 Wenzlau JM, Juhl K, Yu L, Moua O, Sarkar SA, Gottlieb P, Rewers M, Eisenbarth GS, Jensen J, Davidson HW, Hutton JC: The cation efflux transporter ZnT8 (Slc30A8) is a major autoantigen in human type 1 diabetes. Proc Natl Acad Sci U S A 104:17040-17045, 2007.

10 Sosenko JM, Palmer JP, Greenbaum CJ, Mahon J, Cowie C, Krischer JP, Chase HP, White NH, Buckingham B, Herold KC, Cuthbertson D, Skyler JS: Patterns of metabolic progression to type 1 diabetes in the Diabetes Prevention Trial-Type 1. Diabetes Care 29:643-649, 2006.

11 Tsai EB, Sherry NA, Palmer JP, Herold KC: The rise and fall of insulin secretion in type 1 diabetes mellitus. Diabetologia 49:261-270, 2006.

12 Effects of insulin in relatives of patients with type 1 diabetes mellitus. N Engl J Med 346:1685-1691, 2002.

13 Effect of intensive therapy on residual beta-cell function in patients with type 1 diabetes in the diabetes control and complications trial. A randomized, controlled trial. The Diabetes Control and Complications Trial Research Group. Ann Intern Med 128:517-523, 1998.

14 Skyler JS, Krischer JP, Wolfsdorf J, Cowie C, Palmer JP, Greenbaum C, Cuthbertson D, Rafkin-Mervis LE, Chase HP, Leschek E: Effects of oral insulin in relatives of patients with type 1 diabetes: The Diabetes Prevention Trial--Type 1. Diabetes Care 28:1068-1076, 2005.

15 Gale EA, Bingley PJ, Emmett CL, Collier T: European Nicotinamide Diabetes Intervention Trial (ENDIT): a randomised controlled trial of intervention before the onset of type 1 diabetes. Lancet 363:925-931, 2004

16 Ziegler AG, Rewers M, Simell O, Simell T, Lempainen J, Steck A, Winkler C, Ilonen J, Veijola R, Knip M, Bonifacio E, Eisenbarth GS: Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. JAMA 309(23):2473-247, 2013.

17 Fourlanos S1, Perry C, Gellert SA, Martinuzzi E, Mallone R, Butler J, Colman PG, Harrison LC: Evidence that nasal insulin induces immune tolerance to insulin in adults with autoimmune diabetes. Diabetes 4:1237-45, 2011.

18 Fousteri G, Chan JR, Zheng Y, Whiting C, Dave A, Bresson D, Croft M, von Herrath M: Virtual Optimization of Nasal Insulin Therapy Predicts Immunization Frequency to Be Crucial for Diabetes Protection. Diabetes 59 (12): 3148, 2010.

19 Bonifacio E, Ziegler AG, Klingensmith G, Schober E, Bingley PJ, Rottenkolber M, Theil A, Eugster A, Puff R, Peplow C, Buettner F, Lange K, Hasford J, Achenbach P; Pre-POINT Study Group: Effects of high-dose oral insulin on immune responses in children at high risk for type 1 diabetes: the Pre-POINT randomized clinical trial. JAMA 313(15):1541-9, 2015.

20 Bergerot I, Fabien N, Maguer V, Thivolet C: Oral administration of human insulin to NOD mice generates CD4+ T cells that suppress adoptive transfer of diabetes. J Autoimmun 7:655–63, 1994.

21 Zhang ZH, Davidson L, Eisenbarth G, Weiner HL: Suppression of diabetes in nonobese diabetic mice by oral administration of porcine insulin. Proc Natl Acad Sci USA 88:10252–6, 1991.

22 Polanski M, Melican NS, Zhang J, Weiner HL: Oral administration of the immunodominant B-chain of insulin reduces diabetes in a co-transfer model of diabetes in the NOD mouse and is associated with a switch from Th1 to Th2 cytokines. J Autoimmun 10(4):339-46, 1997.

23 Hancock WW, Polanski M, Zhang J, Blogg N, Weiner HL: Suppression of insulitis in non-obese diabetic (NOD) mice by oral insulin administration is associated with selective expression of interleukin-4 and -10, transforming growth factor-beta, and prostaglandin-E. Am J Pathol 147(5):1193-9, 1995.

24 Chen Y, Kuchroo VK, Inobe J, Hafler DA, Weiner HL: Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. Science 265(5176):1237-40, 1994.

25 al-Sabbagh A, Miller A, Santos LM, Weiner HL: Antigen-driven tissue-specific suppression following oral tolerance: orally administered myelin basic protein suppresses proteolipid protein-induced experimental autoimmune encephalomyelitis in the SJL mouse. Eur J Immunol 24(9):2104-9, 1994.

26 Maron R, Guerau-de-Arellano M, Zhang X, Weiner HL: Oral administration of insulin to neonates suppresses spontaneous and cyclophosphamide induced diabetes in the NOD mouse. J Autoimmun 16(1):21-8, 2001.

27 Sadeghi H, Bregenholt S, Wegmann D, Petersen JS, Holmgren J, Lebens M: Genetic fusion of human insulin B-chain to the B-subunit of cholera toxin enhances in vitro antigen presentation and induction of bystander suppression in vivo. Immunology 106(2):237-45, 2002.

28 Bergerot I, Arreaza GA, Cameron MJ, Burdick MD, Strieter RM, Chensue SW, Chakrabarti S, Delovitch TL: Insulin B-chain reactive CD4+ regulatory T-cells induced by oral insulin treatment protect from type 1 diabetes by blocking the cytokine secretion and pancreatic infiltration of diabetogenic effector T-cells. Diabetes 48(9):1720-9, 1999.

29 Bedoret D, Singh AK, Shaw V, Hoyte EG, Hamilton R, DeKruyff RH, Schneider LC, Nadeau KC, Umetsu DT: Changes in antigen-specific T cell number and function during oral desensitization in cow's milk allergy enabled with omalizumab. Mucosal Immunol 5(3): 267–276, 2012.

30 Skowera A, Ladell K, McLaren JE, Dolton G, Matthews KK, Gostick E, Kronenberg-Versteeg D, Eichmann M, Knight RR, Heck S, Powrie J, Bingley PJ, Dayan CM, Miles JJ, Sewell AK, Price DA, Peakman M: β-cell-specific CD8 T cell phenotype in type 1 diabetes reflects chronic autoantigen exposure. Diabetes 64(3):916-25, 2015.

30 Laouar, A et all. CD70+ antigen-presenting cell controls the expansion and differentiation of T cells in the intestinal mucosa. Nat Immuno. (6)7: 698-706, 2005.

32 Arif S, Tree TI, Astill TP, Tremble JM, Bishop AJ, Dayan CM, Roep BO, Peakman M: Autoreactive T cell responses show proinflammatory polarization in diabetes but a regulatory phenotype in health. J Clin Invest 113(3):451-63, 2014.

33 Dang M, Rockell J, Wagner R, Wenzlau JM, Yu L, Hutton JC, Gottlieb PA, Davidson HW: Human type 1 diabetes is associated with T cell autoimmunity to zinc transporter 8. J Immunol 15;186(10):6056-63, 2011.

34 Arif S, Moore F, Marks K, Bouckenooghe T, Dayan CM, Planas R, Vives-Pi M, Powrie J, Tree T, Marchetti P, Huang GC, Gurzov EN, Pujol-Borrell R, Eizirik DL, Peakman M: Peripheral and islet interleukin-17 pathway activation characterizes human autoimmune diabetes and promotes cytokine-mediated β-cell death. Diabetes 60(8):2112-9, 2011.

35 Martinuzzi E, Novelli G, Scotto M, Blancou P, Bach JM, Chaillous L, Bruno G, Chatenoud L, van Endert P, Mallone R: The frequency and immunodominance of islet-specific CD8+ T-cell responses change after type 1 diabetes diagnosis and treatment. Diabetes 57(5):1312-20, 2008.

36 Pinkse GG, Boitard C, Tree TI, Peakman M, Roep BO: HLA class I epitope discovery in type 1 diabetes: independent and reproducible identification of proinsulin epitopes of CD8 T cells--report of the IDS T Cell Workshop Committee. Ann N Y Acad Sci. 1079:19-23, 2006.

37 Roep BO, Solvason N, Gottlieb PA, Abreu JR, Harrison LC, Eisenbarth GS, Yu L, Leviten M, Hagopian WA, Buse JB, von Herrath M, Quan J, King RS, Robinson WH, Utz PJ, Garren H; BHT-3021 Investigators, Steinman L: Plasmid-encoded proinsulin preserves C-peptide while specifically reducing proinsulin-specific CD8 $^{+}$ T cells in type 1

38 Velthuis JH, Unger WW, Abreu JR, Duinkerken G, Franken K, Peakman M, Bakker AH, Reker-Hadrup S, Keymeulen B, Drijfhout JW, Schumacher TN, Roep BO: Simultaneous detection of circulating autoreactive CD8+ T-cells specific for different islet cell-associated epitopes using combinatorial MHC multimers. Diabetes 59(7):1721-30, 2010.

39 Mallone R, Scotto M, Janicki CN, James EA, Fitzgerald-Miller L, Wagner R, Gottlieb P, Thorpe J, Jospe N, Durinovic-Bellò I, Boitard C, Lou O, Dayan CM, Wong FS; T-Cell Workshop Committee: Immunology of Diabetes Society T-Cell Workshop: HLA class I tetramer-directed epitope validation initiative T-Cell Workshop Report-HLA Class I Tetramer Validation Initiative. Diabetes Metab Res Rev 27(8):720-6, 2011. Presented at IDS 2015

40 Liu W, Putnam AL, Xu-Yu Z, Szot GL, Lee MR, Zhu S, Gottlieb PA, Kapranov P, Gingeras TR, Fazekas de St Groth B, Clayberger C, Soper DM, Ziegler SF, Bluestone JA: CD127 expression inversely correlates with FoxP3 and suppressive function of human CD4+ T reg cells. J Exp Med 10;203(7):1701-11, 2006.

41 Cerosaletti K, Schneider A, Schwedhelm K, Frank I, Tatum M, Wei S, Whalen E, Greenbaum C, Kita M, Buckner J, Long SA: Multiple autoimmune-associated variants confer decreased IL-2R signaling in CD4+ CD25(hi) T cells of type 1 diabetic and multiple sclerosis patients. PLoS One 8(12):e83811, 2013.

42 Long SA, Rieck M, Sanda S, Bollyky JB, Samuels PL, Goland R, Ahmann A, Rabinovitch A, Aggarwal S, Phippard D, Turka LA, Ehlers MR, Bianchine PJ, Boyle KD, Adah SA, Bluestone JA, Buckner JH, Greenbaum CJ; Diabetes TrialNet and the Immune Tolerance Network: Rapamycin/IL-2 combination therapy in patients with type 1 diabetes augments Tregs yet transiently impairs β-cell function. Diabetes 61(9):2340-8, 2012.

43 Long SA, Buckner JH: Combination of rapamycin and IL-2 increases de novo induction of human CD4(+)CD25(+)FOXP3(+) T cells. J Autoimmun 30(4):293-302, 2008.

44 Group, Diabetes Prevention Trial – Type 1 Diabetes Study. The New England Journal of Medicine. 1685-1691, 2002.

45 JAMA 2015; 313(15:1541-1549).