ORAL INSULIN FOR PREVENTION OF DIABETES IN RELATIVES AT RISK FOR TYPE 1 DIABETES MELLITUS

(Protocol TN-07)

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

PREFACE

The Type 1 Diabetes TrialNet Protocol TN-07, *Oral Insulin For Prevention Of Diabetes 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.

Table of Contents

1 STUDY OVERVIEW

This protocol describes the background, design, and organization of the Type 1 Diabetes TrialNet Protocol TN-07 entitled "*Oral Insulin For Prevention Of Diabetes In Relatives At Risk For Type 1 Diabetes Mellitus*." The protocol was written by Dr. Desmond Schatz, Chair of the TrialNet Oral Insulin Protocol Committee, the TrialNet Chairman's Office at Benaroya Research Institute and University of Miami, and the TrialNet Coordinating Center. Significant changes that occur to this protocol during the course of the trial require the formal approval of the TrialNet Steering Committee. The study protocol, along with the required informed consent forms, will be approved by each participating institution's Institutional Review Board (IRB) or the equivalent at international sites.

The following table presents a summary of the study design:

Title	*Oral Insulin For Prevention Of Diabetes In Relatives At Risk For Type 1 Diabetes Mellitus*
IND Sponsor	TrialNet
Conducted By	TrialNet
Protocol Chair	Desmond Schatz, M.D.; University of Florida, Gainesville FL.
Subjects	A fixed target sample size has not been specified. Rather, the study is designed as a maximum information trial in which subjects are recruited and followed until the required amount of statistical information is achieved that provides 85% power to detect a 40% risk reduction using a one-sided logrank test at the 0.05 level.
Study Design	The study is a 2-arm, multicenter, randomized, double-masked, placebo-controlled clinical trial.
Treatment Description	Subjects will receive oral insulin 7.5 mg of recombinant human insulin crystals or placebo in capsules.
Objective	The primary objective is to determine whether intervention with repeated oral administration of recombinant human insulin will prevent or delay the development of clinical Type 1 Diabetes Mellitus (T1DM) in subjects at risk for T1DM.
Primary Outcome	The primary outcome is the elapsed time from random treatment assignment to the development of diabetes among those enrolled in the primary analysis cohort consisting of subjects with insulin autoimmunity and absence of metabolic abnormalities. Criteria for diabetes onset are as defined by the American Diabetes Association (ADA) based on glucose testing, or the presence of symptoms and unequivocal hyperglycemia.
Major Inclusion Criteria	(1) Relatives of T1DM proband with mIAA and at least one other islet autoantibody present (2) Normal OGTT performed within 7 weeks prior to randomization. The primary analysis stratum and secondary analysis strata are defined based on combinations of other autoantibodies present, and presence or absence of first phase insulin response on IVGTT.

2 BACKGROUND AND SIGNIFICANCE

2.1 Rationale for Study

2.1.1 Type 1 diabetes (T1DM)

Type 1 diabetes mellitus is an immune-mediated disease in which insulin-producing beta cells are completely destroyed resulting in life-long dependence on exogenous insulin. 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 T1DM is not optimal. To avoid long-term complications, patients must 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 can rarely be achieved with current management and overly aggressive therapy results in severe hypoglycemia which can be life threatening. 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 (3).

Much is known about the natural history of the type 1 diabetes disease process. Beta cell destruction generally begins years before clinical onset as identified by the presence of circulating autoantibodies for disease relevant antigens. Though impairment in beta cell function is detected prior to clinical diagnosis, at the time of diagnosis, patients with Type 1 diabetes retain a significant amount of beta cell function as measured by C-peptide responses to a mixed meal tolerance test (MMTT) (4;5). Affected individuals often enter a honeymoon or remission phase where this insulin secretion is also seen. However, beta cell function deteriorates after diagnosis, eventually becoming undetectable and necessitating increasing reliance on exogenous insulin replacement.

2.1.2 Oral Insulin for prevention of T1DM

In the non-obese diabetic (NOD) mouse model of Type 1 Diabetes Mellitus (T1DM), it has been demonstrated that the oral administration of islet autoantigens is effective in delaying the onset of T1DM (6-10). Repeated ingestion of insulin by young, prediabetic NOD mice has been shown to inhibit their development of diabetes. It also has been shown that ingestion of glutamic acid decarboxylase (GAD), another putative β-cell antigen, by prediabetic NOD mice inhibits the development of diabetes. The results suggest that tolerance provoked by oral insulin or GAD administration can attenuate pancreatic islet autoimmunity, leading to a delay in the onset of the disease.

The hypothesis that oral insulin could delay the clinical onset of T1DM in humans was tested in the oral arm of the Diabetes Prevention Trial – type 1 diabetes (DPT-1) in which antibody positive relatives were randomized in a double-masked placebo-controlled trial (11). In the primary analysis of relatives selected and randomized in this trial on the basis of islet cell antibody (ICA) positivity with

insulin autoantibodies (IAA) ≥40 nU/ml, oral insulin did not delay or prevent development of diabetes. There was however evidence of heterogeneity of effect within the study cohort according to level of IAA. The subgroup with confirmed IAA ≥80 nU/ml not only progressed to diabetes at a faster rate than those subjects who did not have confirmed IAA ≥80 nU/ml, but also showed a potential beneficial effect of oral insulin (p=0.015). This effect was observed both in the group of subjects with IAA≥80 nU/ml as a whole, and in those recruited before a protocol change in 1997 that lowered the IAA threshold for eligibility. The presence of IAA ≥80 nU/ml was also found to be associated with other risk characteristics that suggest more rapid evolution to diabetes, including younger age, greater likelihood of presence of other autoantibodies, and greater loss of beta cell function (as suggested by lower levels of C-peptide in response to several provocative challenges).

The *post hoc* analysis suggesting a potential beneficial effect in the subgroup with baseline confirmed IAA >80 nU/ml can be deemed only to be hypothesis generating and not as a positive confirmatory analysis. The successor group to DPT-1, the Type 1 Diabetes TrialNet clinical trials network, has therefore designed this study to explore the potential role of oral insulin in delaying or preventing Type 1 diabetes in the subgroup of IAA positive relatives in whom the apparent benefit was observed in DPT-1.

During the intervening years since the DPT-1 oral study was conducted there have been changes in methods used to assay for IAA. Improvements in technique have resulted in an assay that requires much less blood volume, now commonly referred to as the micro or mIAA assay. As well, the field has progressed to screening for other diabetes-associated autoantibodies with much less reliance on ICA. Thus, the TrialNet Natural History Study for the Development of Type 1 Diabetes, which will screen subjects for eligibility for this protocol, uses a strategy of initially testing samples for the presence of GAD65ab, ICA512 and mIAA with subsequent testing for ICA only in antibody positive subjects. This trial therefore, is different from the DPT-1 oral study in both the substitution of mIAA for the previous IAA assay (pegIAA) and in initial testing for GAD65ab, ICA512 and mIAA (with testing for ICA in antibody positive subjects) as compared to initial testing for ICA and subsequent testing for peg IAA in ICA positive subjects. However, the metabolic criteria for insulin secretion and glucose tolerance will be the same in this TrialNet study as those that were used in DPT-1. All subjects will have normal glucose tolerance determined by oral glucose tolerance testing. The primary analysis will also be restricted to subjects with a first phase insulin response (FPIR) to intravenous glucose infusion above a specified threshold. Other secondary strata will be defined based on other combinations of autoantibodies present and/or FPIR below threshold.

To partially examine the effects of these changes, 329 of 372 ICA positive samples from the DPT-1 oral study were re-analyzed using current assays, specifically, GAD65ab, ICA512, and mIAA. Importantly, among those with high titer pegIAA (>80 nU/ml), 66% (168/253) were positive for mIAA; while among those with lower titer pegIAA as identified by the original DPT-1 study 89% (68/76) were mIAA negative in the current assay.

Consistent with the DPT-1 results, the effect of oral insulin is seen among those with normal glucose tolerance who were positive for mIAA and ICA whether or not other antibodies (ICA512, GAD65ab) were present. [Figure 1]

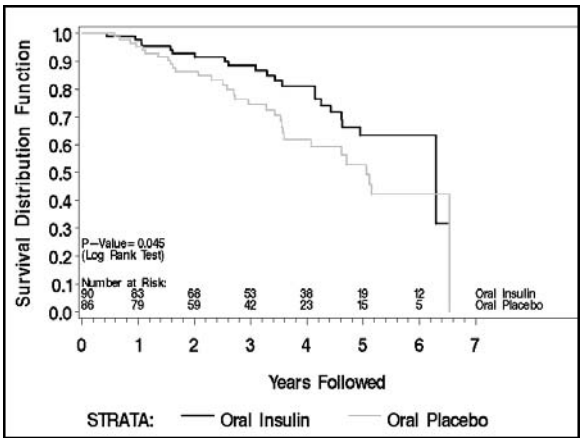

Figure 1: Effect of oral insulin on mIAA and ICA positive subjects enrolled in DPT-1Trial.

Further analysis of data from the DPT-1 Oral Study suggests that subjects with mIAA, and additionally either ICA512, or GAD65ab, also demonstrate an advantage from oral insulin treatment. [Figure 2]

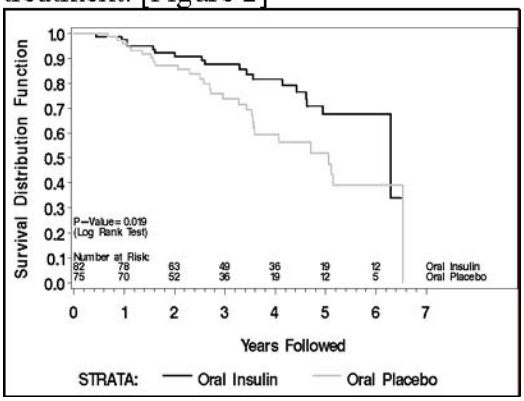

Figure 2: Effect of oral insulin on mIAA and ICA positive subjects who additionally have either ICA512 or GAD65ab enrolled in DPT-1 Trial.

Using the DPT-1 dataset, there are insufficient numbers of subjects who are ICA negative but GAD65ab and ICA512 positive to determine whether they have the same risk of progression as those ICA positive; however, emerging data from other studies suggest that this is likely true.

Therefore, the primary objective will be tested within the **Primary Analysis Stratum** consisting of those with normal glucose tolerance, above threshold FPIR, and mIAA. In addition, subjects must either have ICA, or have both GAD65ab and ICA512in the absence of ICA.

2.2 Rationale for Additional Cohorts

A secondary question is whether a beneficial effect observed in the Primary Analysis Stratum extends to other populations at different risk of diabetes. As described above, post-hoc analysis of the DPT-1 cohort indicated that the effect of oral insulin was most pronounced in those with higher pegIAA titers, now corresponding to a positive mIAA titer. This observation has led to the hypothesis that tolerance with oral insulin would be more likely in those individuals with insulin directed immune activity as indicated by the presence of mIAA. Thus, oral insulin therapy may be of benefit in

individuals with mIAA but with different combinations of ICA positivity, other autoantibody positivity and metabolic features than those in the primary cohort.
These additional secondary strata are:

Secondary 1:
mIAA confirmed, ICA confirmed with loss of FPIR

OR mIAA confirmed, ICA not confirmed, ICA512+ AND GAD65ab+ with loss of FPIR

Secondary 2:
mIAA confirmed, ICA not confirmed, ICA512+ OR GAD65ab+ with preserved FPIR

Secondary 3:
mIAA confirmed, ICA not confirmed, ICA512+ OR GAD65ab+ with loss of FPIR

The OGTT and genetic eligibility criteria would be the same as in the primary study cohort.

The study design provides adequate power to assess the main objective within the primary stratum but does not provide power to assess this objective within the secondary strata individually or in combination. Thus, the study will provide a preliminary assessment of the effects of oral insulin therapy in subjects with different risk characteristics, and will be used to provide a preliminary assessment as to whether treatment effectiveness is similar among these different categories of subjects.

2.3 Rationale for additional outcome measures

Immune function
The underlying hypothesis of this trial is the concept of induction of immunologic tolerance to the insulin secreting beta cell through the presentation of the autoantigen (insulin) orally. Animal studies have suggested that tolerance is accompanied by characteristic changes in immune phenotypes; however, whether these changes are associated with tolerance induction in humans is unknown. A goal of this study therefore, will be to study immune function through studies of B and T cell phenotype and function including antigen specific responses. The various autoantibodies to insulin or its byproducts will also be measured longitudinally.

Genetics
While there are strong associations with particular HLA genotypes and type 1 diabetes, other genes are also thought to contribute to the disease process. These include those associated with autoimmunity per se such as PTPN22 and those associated with diabetes such as insVNTR. It is unknown whether the response to oral insulin will relate to genotype, but typing will be performed on genes related to type 1 diabetes.

Insulin secretion and insulin resistance

Type 1 diabetes is primarily thought of as a disease of islet cell destruction leading to the declining ability to secrete endogenous insulin in sufficient amounts to maintain metabolic homeostasis. While insulin secretion cannot be measured directly due to its rapid metabolism, a by-product of insulin secretion, C-peptide, can be measured in response to a meal. This measurement may be obtained in an Oral glucose tolerance test (OGTT) conducted periodically during follow-up.

Glucose homeostasis also depends on the degree of insulin resistance, or the degree of resistance of the peripheral tissues to insulin action. Recent data suggests that modeling of data obtained during the OGTT can provide a reliable estimate of the degree of insulin resistance.

These data may be correlated with fasting insulin levels as well as body weight and abdominal circumference since these are often associated with insulin resistance.

3 STUDY DESIGN

3.1 Overview

This is a double-masked, randomized, placebo controlled trial with two arms, experimental and control groups. The primary outcome is development of T1DM. There will be both primary and secondary study groups.

3.2 Objectives

The primary objective of the TrialNet Oral Insulin Trial is to determine whether intervention with repeated oral administration of recombinant human insulin, the potential autoantigen, will prevent or delay the development of clinical Type 1 Diabetes Mellitus (T1DM) in non-diabetic relatives of patients with T1DM who are positive for insulin autoantibodies but who do not have a metabolic defect (as the Primary Analysis Stratum). This intervention will be compared with placebo given in a double-masked fashion.

Secondary objectives include the description of the effects of treatment with oral insulin versus placebo in other categories of subjects defined using different combinations of autoantibodies and metabolic status (the Secondary Analysis Strata) and an assessment of the consistency of treatment effect among strata. Secondary objectives also include the assessment of the effects of treatment on immunologic and metabolic markers, and the association of these markers with the risk of diabetes onset, among other possible risk factors.

The operational objectives are to recruit, screen, randomize, and follow sufficient numbers of subjects to provide adequate statistical power to determine whether T1DM can be delayed through the administration of oral insulin.

3.3 Study Population

Recruitment and initial screening to identify subjects will be done through the TrialNet Natural History Study of the Development of Type 1 Diabetes Protocol. As part of this protocol, subjects will then undergo additional testing, and if eligible and willing, will be randomized and followed as described. Subjects determined during screening to be ineligible or unwilling to be randomized to this

protocol will be followed under the Natural History Study Protocol.

Eligible subjects are non-diabetic relatives of patients with T1DM, who have normal glucose tolerance on an OGTT, who are confirmed to be mIAA positive on two samples (collections), and who also meet the criteria for the following primary and secondary study strata based on other autoantibodies and metabolic characteristics:

Primary Analysis Stratum:
Either ICA (>= 10 JDF units) positive confirmed on two samples, or, if not confirmed for ICA, both GAD65ab and ICA512 positive on the same sample with confirmation of at least one of these autoantibodies on a separate sample.

Subjects must also have first phase insulin release (FPIR) above the threshold determined from the sum of the 1 and 3 minute insulin values from an intravenous glucose tolerance test (IVGTT). For participants age 3-7 or parents of T1DM proband the threshold is ≥ 60 μU/ml. For siblings or offspring age 8-45 or other relatives age 8-20, the threshold is ≥ 100 μU/ml.

The primary objective of the study is to assess the effects of treatment within this stratum (see Section 8).

Secondary objectives entail the assessment of treatment effects within additional strata:

Secondary Stratum 1:
Either ICA (>= 10 JDF units) positive confirmed on two samples, or, if not confirmed for ICA, both GAD65ab and ICA512 positive on the same sample with confirmation of at least one of these autoantibodies on a separate sample.

Subjects must also have first phase insulin release *below* the FPIR thresholds defined in the Primary Stratum above.

Secondary Stratum 2:
ICA, or GAD65ab or ICA512 positive. Confirmation of either GAD65 or ICA512 on a separate sample (those confirmed for ICA are in primary stratum).

Subjects must also have first phase insulin release *above* the FPIR thresholds defined in the Primary Stratum above.

Secondary Stratum 3:
ICA, or GAD65ab or ICA512 positive. Confirmation of either GAD65 or ICA512 on a separate sample (those confirmed for ICA are in secondary stratum 1).

Subjects must also have first phase insulin release *below* the FPIR thresholds defined in the Primary Stratum above.

Subjects identified through the TN Natural History Study and who satisfy all eligibility criteria and no exclusion criteria (see below) will be eligible for this protocol.

3.3.1 Inclusion Criteria

4. Have a proband* with T1DM.
2. If the proband is a sibling, parent or a child, the study participant must be 3 - 45 years of age. If the proband is a second or third degree relative (i.e. Niece, Nephew, Aunt, Uncle, Grandparent, Cousin), the study participant must be 3-20 years of age.
3 Willing to sign Informed Consent Form.
4. Has normal glucose tolerance on an OGTT performed within 7 weeks prior to randomization. If previous abnormal glucose tolerance, has had two consecutive OGTT with normal glucose tolerance.
5. mIAA confirmed positive within the previous six months.
6. At least one other antibody present on two separate samples, one of which was drawn within the past six months.

* A proband is an individual diagnosed with diabetes before age 40 and started on insulin therapy within 1-year of diagnosis. Probands considered to have type 1 diabetes by their physician who do not meet this definition will be referred to the TrialNet Eligibility Committee.

3.3.2 Exclusion Criteria

1 Does not satisfy the above inclusion criteria.
2 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.
3 Prior participation in a clinical trial for secondary prevention of T1DM.
4 History of treatment with insulin or oral hypoglycemic agent.
5 History of therapy with immunosuppressive drugs or non-physiologic glucocorticoids within the past two years for a period of more than three months.
6 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. Subjects on such medications should be changed to a suitable alternative, if available, and will become eligible one month after medication is discontinued.
7 Pregnant or intends to become pregnant while on study or lactating.
8 Deemed unlikely or unable to comply with the protocol.
9 OGTT that reveals abnormal glucose tolerance unless two subsequent consecutive OGTT have normal glucose tolerance. Abnormal glucose tolerance is defined as:
   - fasting plasma glucose ≥ 110 mg/dL (6.1 mmol/l), AND/OR
   - 2 hour plasma glucose ≥ 140 mg/dL (7.8 mmol/l) AND/OR
   - 30, 60, or 90 minute plasma glucose ≥200 mg/dL (11.1 mmol/l)
10 Subject has HLA DQA1*0102, DQB1*0602 haplotype.

3.4 Description of Treatment Groups

Subjects will be randomized to receive either oral insulin (7.5 mg of recombinant human insulin crystals) or placebo daily.

3.5 Treatment Assignment and Double Masking

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 participants will be randomized to receive either oral insulin or placebo.

Participants will be randomized in equal allocations to each group. The randomization method will be stratified by the major TrialNet study site. This approach ensures that study site will not be a potential confounder.

This treatment assignment will be double masked. Outcome assessments will be conducted without knowledge of treatment assignment.

3.6 Study Assessments

The intervention protocol will be conducted at Centers and approved Affiliates with a General Clinical Research Center (GCRC) or equivalent facility. During the course of the study, participants will frequently undergo assessments of their insulin production, immunologic status, and overall health and well-being (see Figure 3, 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 development of clinical T1DM. Therefore, subjects will be followed until their development of T1DM or the conclusion of the study.

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. These samples will be collected only with the subject's permission. Subjects who decline consent for these sample collections will still be eligible to participate in this study (see Section 10.4).

3.7 Quality Assurance

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

3.8 Study Timeline

A fixed target sample size has not been specified and the date on which the study will terminate has not been fixed in advance. Rather, the study is designed as a *maximum information trial* in which subjects are recruited and followed until the required amount of statistical information is achieved that provides 85% power to detect a 40% risk reduction using a one-sided logrank test at the 0.05 level. The required information number is $I = D_O * D_C/(D_O + D_C) = 27.6$; where D_O, D_C are the observed numbers developing diabetes in the oral insulin and control groups, respectively. Thus, the exact total sample size and study duration are unknown.

However, projections as to the expected total sample size and end date can be conducted based on assumed values of the rate of enrollment, the control group hazard rate and the rate of losses-to-follow-up. As the study progresses, these projections can be updated based on interim estimates of these parameters.

The TrialNet Natural History Study is projected to screen 20,000 or more subjects each year to identify subjects who may be eligible for this prevention study. Preliminary estimates show that this will yield between 50 and 60 subjects annually who will meet these eligibility criteria for the primary

study stratum, and who will consent for the trial. Further, from supplemental analyses of the DPT-1, it is projected that subjects in the primary analysis stratum will have a 50% 5 year risk (cumulative incidence) of diabetes. If 50 subjects are entered each year over 6 years (N = 300 total), using the expressions in Lachin and Foulkes (15), with allowance for some losses to follow-up, it is projected that a total study duration of 7.6 years would be required to achieve I = 27.6. If 60 subjects are entered each year over 5.5 years (N = 330), a total study duration of 6.8 years would be required. A total of D_T = 115 subjects are expected to have developed diabetes by study end.

4 SUBJECT MANAGEMENT

4.1 Screening Visit and Eligibility Assessment

Subjects potentially eligible for the Oral Insulin Trial will be identified through the TrialNet Natural History Study. They will be notified of their eligibility by TrialNet Investigators at an Affiliate clinical site or the associated Clinical Center.

The initial testing for mIAA and other autoantibodies will be done as part of Natural History screening. Those individuals who are mIAA positive will then be eligible for additional tests as part of the Natural History Monitoring visit. Those with normal glucose tolerance will be eligible for enrollment into either the Primary or one of the Secondary Analysis Strata of the Oral Insulin Trial depending on their test results from both the Natural History and Oral Insulin Trial assessments.

Figure 4 summarizes the flow of subjects from the Natural History Study into the Oral Insulin initial visit and randomization.

4.2 Natural History Monitoring Visit

As described in the TrialNet Natural History Study Protocol, mIAA positive individuals will undergo a Monitoring visit consisting of oral glucose tolerance testing, confirmation of autoantibody status, and HLA evaluation. At this visit, the Oral Insulin Trial will be described to the potential participant, initiating the process of informed consent for the Oral Insulin Trial. The subject will have up to 7 weeks from the time of the OGTT until randomization.

4.3 Oral Insulin Initial Visit

The participant will be asked to sign an informed consent document describing the purpose, risks, and benefits of the Oral Insulin Trial. A participant's signature indicates that he/she understands the potential risks and benefits of study participation. During the first visit, an IVGTT will be performed.

Assignment to study stratum will depend upon both antibody results and results from their IVGTT. Classification as "above threshold FPIR" will require only one IVGTT with this result. Subjects whose initial FPIR is below threshold will undergo repeat IVGTT no later than the day of randomization. If the second IVGTT is "above threshold", this classifies the subject in the "above threshold" category for stratum determination. Thus, classification as "below threshold" requires two "below threshold" IVGTT results. This process is to address the first-test effect observed in the DPT-1 whereby a "below threshold" FPIR on the initial visit was often not-confirmed.

Any participant either not eligible or not willing to be randomized into the Oral Insulin Trial is eligible for continued follow-up as part of the TrialNet Natural History Study.

4.4 Randomization

The participant will be eligible for randomization in one of the study strata if they satisfy all of the following:

1. mIAA positive on two separate samples
2. Positive for at least one other autoantibody on two samples
3. Does not meet the HLA exclusion criteria
4. Has normal glucose tolerance by OGTT, and if previous abnormal glucose tolerance, has two consecutive OGTTs with normal glucose tolerance.
5. Age ≥ 3 years.

Randomization must occur within 7 weeks of an OGTT in order to ensure that participants have normal glucose tolerance at time of randomization.

Prior to randomization, all entry criteria will be reviewed to ensure subject eligibility.

After randomization, study medication will be dispensed to the participant.

4.5 Administration of Study Medication

All subjects will take one capsule of study medication (7.5 mg of recombinant insulin or placebo) daily for the duration of the study. Study medication will be dispensed at each 6-month visit. Subjects will remain on the same dose of insulin/placebo throughout the trial.

4.6 Treatment Discontinuation

Subjects may be discontinued from treatment due to adverse effects of treatment that in the judgment of the investigator are related to the study medication. Subjects will also be discontinued from treatment who revoke consent to be treated.

Subjects will not be discontinued from treatment due to non-compliance or the apparent lack of preliminary beneficial effect, so-called treatment failure.

4.7 Intent-to-Treat Design

This study is designed and will be implemented under the intention-to-treat principle in which all subjects randomized will be included in all analyses. To minimize bias under this principle, this requires adoption of an intent-to-treat design. To the extent possible, all subjects randomized into the study should continue all scheduled follow-up assessments until the time of onset of diabetes, death, or the declared end of the study. All subjects discontinued from treatment by the investigator, or who refuse to continue treatment, or who fail to comply with treatment, should continue to be followed under this policy.

A subject whose follow-up lapses during the study will be termed *inactive*. No subject will be

designated as a study dropout. Inactive subjects will not be permanently withdrawn from the study and will be allowed to return to follow-up, and continue with randomized treatment, at any time provided that the subject has not developed diabetes. Those still inactive at the end of the study will be classified as lost to follow-up, and the date so lost will be that at which the subject was last known to be free of diabetes.

5 STUDY VISIT ASSESSMENTS

Participants will be seen at a study site and contacted by phone during follow-up to collect study data, perform required blood testing, determine changes in diabetes status and monitor study related adverse events and treatment compliance.

The schedule of evaluations and laboratory studies is presented in Figure 3, Schedule of Assessments (refer to Section 3.6). A summary of assessments for the protocol is given below.

All assessments will be performed on samples or data obtained from either the Natural History Study or this trial.

All participants randomized into this study will be seen at a study site for a follow-up evaluation three and six months after randomization and every six months thereafter. Participants will be contacted by phone between 6-monthly clinic visits to assess changes in diabetes status, medication compliance and adverse events. These phone contacts will occur approximately 3 months from the date of the participants previous clinic visit.

5.1 General Assessments

General assessments for this Protocol will include:

- Informed consent
- Inclusion/exclusion criteria
- Medical history including lifestyle assessment
- Physical examination including height/weight, abdominal circumference
- Concomitant medications
- Adverse events
- Treatment compliance

5.2 Laboratory Assessments

The following laboratory assessments will be performed during the study:

- Islet Autoantibodies

5.3 Mechanistic Outcome Assessments

Mechanistic assessments may include:

- DNA for testing other diabetes or 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 number and function.
- Serum and plasma for assays such as the evaluation of islet autoantibody epitope, affinity,

isotyping and proteomics based assessment of immune responses.

5.4 Metabolic Outcome Assessments

Metabolic assessments may include:

- HbA1c l
- OGTT
- Insulin secretion
- Insulin sensitivity
- IVGTT

5.5 Visit Windows

Randomization and initial treatment administration should begin within 7 weeks (no more than 52 days) from the OGTT. The subsequent treatment visits should be conducted within 6 weeks before or after the scheduled date based on each subject's date of randomization.

6 ADVERSE EVENT REPORTING AND SAFETY MONITORING

6.1 Adverse Event Definitions

6.1.1 Adverse Event

In this clinical trial, an adverse event is any occurrence or worsening of an undesirable or unintended sign, symptom or disease.

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

Adverse events may be discovered through:

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

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

6.1.2 Serious Adverse Event

For this trial, an adverse event associated with the treatment or study procedures that suggest a significant hazard, contraindication, side effect or precaution (as described below) is to be reported as a serious adverse event (SAE).

A serious adverse event (experience) or reaction is any untoward medical occurrence that:

- results in death,
- is life-threatening,
- requires inpatient hospitalization or prolongation of existing hospitalization,

- results in persistent or significant disability/incapacity, or
- is a congenital anomaly/birth defect.

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

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

6.1.4 Grading Event Severity
TrialNet has adopted usage of the National Cancer Institute (NCI) Common Toxicity Criteria for Adverse Events (CTCAE) and/or study-specific criteria for classification to describe the severity of adverse events.

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 (see Manual of Operations). They will be graded as to severity according to common toxicity criteria or study-specific criteria and the investigator will make a determination as to the relation to therapy. Events will be assessed and reported in accordance with the ICH Guidelines for Good Clinical Practice 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. 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 three to six months (and, as needed) of adverse events by treatment group assignment. Serious adverse events as well as adverse events leading to study discontinuation will be reviewed by the DSMB.

6.3 Protecting Against or Minimizing Potential Treatment Risks

Subjects will not be enrolled who have other active serious medical problems. Regular monitoring of subjects and active inquiry will allow for early identification of adverse events.

7 PARTICIPANT SAFETY

7.1 Expected Side Effects and Adverse Events

There were no side effects or adverse events associated with oral insulin during the DPT-1 which consisted of the same study population and same dose of drug. Subjects did occasionally have mild expected adverse events associated with study procedures such as fainting and bruising with phlebotomy. Although unlikely, it is possible that oral insulin could accelerate disease.

7.2 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 use birth control during the study. At every study visit the sexual activity of female participants of reproductive age will be re-assessed. If a subject who was previously sexually inactive becomes sexually active, she will be counseled about the need to use a reliable form of birth control. Female subjects will undergo urine pregnancy tests at regular intervals.

8 STATISTICAL CONSIDERATIONS AND ANALYSIS PLAN

Analyses of study data will be conducted to address the primary and secondary objectives of the trial, other stated objectives, and other interrelationships among elements of study data of interest to the investigators and of relevance to the objectives of the study. Such analyses may also entail the use of data from other studies in combination with data from this study. Likewise, data from this study may be used in combination with data from another study to address objectives of that study. Analyses by gender and race/ethnicity, as appropriate, are also planned.

All analyses will be conducted under the intention-to-treat principle whereby all outcome data in all randomized subjects will be included in all analyses as appropriate.

8.1 Primary Outcome

The primary outcome is the elapsed time from random treatment assignment to the development of diabetes among those enrolled in the primary analysis cohort consisting of subjects with insulin autoimmunity and absence of metabolic abnormalities.

Criteria for diabetes onset are, as defined by the American Diabetes Association (ADA), based on glucose testing, or the presence of unequivocal hyperglycemia with acute metabolic decompensation (diabetic ketoacidosis). One of the following criteria must be met on two occasions as soon as possible but no less than one day apart for diabetes to be defined:

1. Symptoms of diabetes plus casual plasma glucose concentration ≥ 200 mg/dL (11.1 mmol/l). Casual is defined as any time of day without regard to time since last meal. The classic symptoms of diabetes include polyuria, polydipsia, and unexplained weight loss.

OR

2. Fasting plasma glucose ≥ 126 mg/dL (7 mmol/l). Fasting is defined as no caloric intake for at least 8 hours.

OR

3. 2 hour plasma glucose ≥ 200 mg/dL (11.1 mmol/l). The test should be performed using a glucose load containing the equivalent of 1.75g/kg body weight to a maximum of 75 g anhydrous glucose dissolved in water.

It is preferred that at least one of the two testing occasions involve an OGTT.

Cases identified will be confirmed as having diabetes if the glucose values to make these determinations were obtained in a TrialNet laboratory and documentation of symptoms was performed by TrialNet study personnel.

A **TrialNet Diabetes Onset Adjudication Committee** will review all relevant information for each subject otherwise diagnosed as having developed diabetes who does not meet the above criteria. The Committee will determine whether the diagnosis of diabetes in each such subject is sufficiently sound so as to include that subject among the cases who have reached the primary outcome in the statistical analysis. The Committee will review each case masked to treatment assignment.

8.2 Primary Analysis

The primary objective of the study is to assess the effect of oral insulin therapy versus placebo on the risk of diabetes onset in the population of subjects in the Primary Analysis Stratum as defined on the basis of the eligibility criteria elaborated in Section 3.3.

The cumulative incidence of diabetes onset over time since randomization within each group will be estimated from a modified Kaplan-Meier estimate of the "diabetes-free" survival function. The difference between groups in the cumulative incidence functions, and the associated hazard functions, will be tested at the 0.05 level, one-sided, using the Mantel-logrank test. The estimates of cumulative incidence and the test will adjust for periodic outcome assessment visits (12) to assess diabetes status. A one-sided test is employed since the objective is to confirm a preliminary finding from the prior DPT-1 Oral Insulin Trial. The relative risk of diabetes onset between groups will be estimated from the discrete Cox Proportional Hazards (PH) model (12). The critical value for the test statistic, and confidence intervals, in this primary analysis will be determined by the group-sequential procedure (Section 8.5 below).

8.3 Secondary Outcomes and Analyses

A variety of secondary analyses are planned that include the following.

1 The effects of treatment with oral insulin versus placebo will be described in the Secondary Analysis Strata defined in Section 3.3, each consisting of other categories of subjects defined using different combinations of autoantibodies and metabolic status. This will entail the same analyses as above for the Primary Analysis within each of the three secondary strata. An additional analysis will assess the effect of treatment within the Secondary strata combined using a Cox PH model stratified by the three secondary cohort strata, with separate estimates of the hazard ratio for oral insulin versus placebo within each stratum, and with a likelihood ratio test of the oral insulin treatment effect among all Secondary strata combined. Such analyses will be largely descriptive because the study is not designed to provide adequate power to detect an effect of oral insulin treatment within these

secondary cohorts individually or combined.

2 The overall effect of oral insulin versus placebo treatment in the combined strata will be assessed by an analysis as described above using all subjects in a Cox PH model analyses stratified by the four analysis strata (Primary plus three Secondary), with a likelihood ratio test of the oral insulin treatment effect among all strata combined. If the magnitude of the oral insulin treatment effect within the secondary analysis cohorts approaches that in the primary cohort, then the power of the study to detect an overall effect in all combined cohorts will be increased.

3 The consistency of the oral insulin versus placebo treatment effect will be assessed among strata. This will entail an analysis stratified by each of the four analysis strata (Primary plus three Secondary) and with a test of homogeneity of the treatment effect among strata, i.e. a group by stratum interaction, in a Cox PH Model.

4 Subgroup analyses will be conducted comparing the effects of oral insulin versus placebo on the risk of diabetes within subsets of the study cohort, such as among men versus among women. Such analyses will be conducted separately within the primary analysis stratum alone, and within the combined cohort stratified by analysis strata (Primary and three Secondary), with a test of the group by subgroup factor interaction in a Cox PH Model. Subgroups of the population will be classified by age (children <= 12 years of age, adolescents 13-17 years and adults >= 18 years), gender, race/ethnicity, specific antibody status at baseline, and above versus below threshold FPIR at baseline. Differences in the treatment effect between subgroups will be tested using a covariate by group effect in a Cox PH model (12).

Similar analyses will be conducted using the values of quantitative baseline factors including weight, BMI, and the immunologic and metabolic factors described in Section 2.3 that include the autoantibody titers, basal C-peptide, stimulated C-peptide (peak and AUC mean), measures of insulin resistance modeled from the OGTT, and the FPIR. The dependence of the treatment effect on the quantitative levels of a covariate will also be assessed by a covariate by treatment group interaction in a PH model. Such an analysis will also be conducted to assess the effects of age and FPIR as quantitative covariates.

Additional factors may be defined before unmasking of the study data to the investigators. The analyses will distinguish between factors specified prior to unmasking, and those identified post-hoc during analysis.

5. Longitudinal analyses will assess the effects of oral insulin versus placebo treatment on immunologic and metabolic markers over time up to the onset of diabetes. Differences between groups in the mean levels of quantitative factors over time will be assessed using a normal errors linear model for repeated measures (13). Differences between groups in the prevalence of qualitative factors over time will be assessed using generalized estimating equations for categorical measures (13). Generalized estimating equations may also be employed for the analysis of quantitative factors when the normal errors assumptions are violated.

Once a subject develops diabetes, that subject will no longer be followed for longitudinal assessment of these factors. Thus, these analyses will describe the differences between groups in

factors among those who remain free of diabetes.

6. The association of demographic, genetic, immunologic, metabolic, and lifestyle factors, among others, both at baseline and over time, with the risk of diabetes onset will be assessed in Cox PH Models over time. If the proportional hazards assumption does not apply, an appropriate parametric regression model may be employed. The effects of changes in longitudinal factors on diabetes risk will be assessed using time-dependent covariates for these factors. Analyses will be conducted separately within the oral insulin and placebo groups, and differences between groups in covariate effects (group by covariate interactions) will be assessed. Models will then be assessed within the two groups combined, taking account of any group by covariate interactions.

8.4 Study Power and Maximum Information Design

The prior DPT-1 Oral Insulin Trial was designed to detect a 50% reduction in risk of diabetes overall. However, among those in the "high IAA intermediate risk" stratum, the observed relative risk was 0.566, corresponding to a 43.4% risk reduction. Thus, this study has been designed to provide 85% power to detect a 40% risk reduction using a one-sided test at the -0.05 level.

The study is designed as a maximum information trial in which subjects are recruited and followed until the required amount of statistical information is achieved.

At any point in time during the study, the information in the data for a logrank test is provided by $I = (D_O D_C)/D_T$. where D_O and D_C refer to the number of subjects who have developed diabetes in the oral insulin and control groups, respectively, and D_T refers to the total number of such subjects (14). The information required to provide 85% power to detect a 40% risk reduction with a one-sided logrank test at the 0.05 significance level is $I = 27.551$. Under this design the study will be terminated when the observed numbers of events (D_O, D_C, D_T) provides $I \geq 27.551$.

The final test of significance will employ the group sequential critical value to protect against inflation in the type I error probability due to interim assessments of the emerging data for review by the DSMB. However, there is only a minimal loss in power with this approach so that the fixed sample size power based on the above information calculation is virtually identical to the group sequential power. Thus, there is no need to adjust for the group sequential critical values in these computations.

Under a maximum information design, the exact total sample size and study duration are unknown, although projections of each can be generated a priori, and then monitored as the study progresses (14). At this time we project that the study will be able to enroll 50-60 subjects per year into the primary analysis stratum who will have a 50% 5 year risk of diabetes (see Section 3.8). During the DPT-1 oral insulin trial, 3 of 186 subjects were lost to follow-up of diabetes status over 4 years. With this information it is possible to use the expressions in Lachin and Foulkes (15) to make a projection as to the total study duration needed to accrue the information $I = 27.6$ needed to provide 85% power to detect a 40% risk reduction. If 50 subjects are entered each year over 6 years ($N = 300$ total), it is projected that a total study duration of 7.6 years would be required. If 60 subjects are entered each year over 5.5 years ($N = 330$), a total study duration of 6.8 years would be required. In each case, a total of $D_T = 115$ subjects are expected to have developed diabetes by study end.

However, the exact rate of recruitment, control hazard rate (or 5 year risk) and rate of losses to follow-

up are unknown. Thus, no specific time has been specified at which the study will end. Rather, the study will end when the accrued numbers of subjects developing diabetes provide I = 27.551. As the study progresses, projections of the study end will be computed based on the observed rate of enrollment, the observed hazard rate and the observed rate of loss-to-follow-up.

8.5 Interim Monitoring Plan

Interim analyses will be conducted periodically during the study and will be reviewed by the TrialNet Data and Safety Monitoring Board (DSMB) for assessment of effectiveness and safety. The Lan-DeMets (16) spending function with an O'Brien-Fleming boundary will be used to protect the type I error probability for the primary outcome analyses, and to assess the significance of the interim results that emerge during the trial. Kim, Boucher and Tsiatis (17) describe the application of such group-sequential procedures in a maximum information design.
The DSMB may terminate the trial prematurely if a statistically significant effect is observed and it is considered that all major trial objectives have been met.

The DSMB will also consider early termination due to absence of a treatment effect (i.e. futility) based on computations of conditional power conducted both under the initial study design and under the current trend of the data (18). Stopping for futility may inflate the type II error probability. The methods described in Lachin (19) will be employed to ensure that the type II error probability under the original design assumptions is not inflated beyond 0.20, or power reduced below 0.80, by a decision to terminate for futility.

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.

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

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 at each of the participating clinical sites. HIPAA regulations will be followed by each participating institution in accordance with each institution's requirements. The participating international sites will obtain approval from their corresponding review boards in accordance with their local procedures and institutional requirements.

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

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

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 Patient Handbook 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 Quiz that is designed to ensure that the subject understands the study, as well as what is being asked of him/her. The participant will be given a copy of their consent/assent forms. The ongoing consent of participants should be assessed on a continual basis as part of the informed consent process per Good Clinical Practice.

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

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

9.4 Study Subject Confidentiality

Study records with the study subject's information for internal use at the clinical sites will be secured at the study site during the study. At the end of the study, all records will continue to be kept in a

secure location. There are no plans to destroy the records.

Study subject data, which is for reporting purposes, will be stored at the University of South Florida Coordinating Center. Case report forms 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, and the data collection forms will be electronically scanned and saved in electronic format for long-term storage. All paper copies of the forms will ultimately be destroyed after the data is transferred.

Genetic and other biological material will be stored for future use with the permission of the study subject as described in Section 10.4. The results of these future analyses will not be made known to the participant.

9.5 Risks, Benefits, and Inclusion of Children

The risks of this study are presented in the informed consent form and are described in Chapter 7. While there is no guaranteed benefit, there is the prospect of direct benefit to the individual subjects for their participation in the study. These potential benefits include that the diagnosis of diabetes will likely be made earlier via close monitoring, decreasing the risk of life threatening diabetic keto-acidosis.

The study procedures are minor increase over minimal risk and offer the possibility of benefit in the close monitoring and thus early detection of disease for all children. Further, the intervention may have direct benefit to a given subject and is likely to yield general knowledge about T1DM which is of importance for the understanding and amelioration of T1DM in children. Assent of children along with consent of the parents will be obtained prior to any study procedures. This research proposal in children is therefore consistent with United States Department of Health and Human Services, Protection of Human Subjects, Subpart D, Section 46.405 (Research involving greater than minimal risk but presenting the prospect of direct benefit to the 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).

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.

10.2 Groups and Committees

10.2.1 Oral Insulin Trial Protocol Committee

The Oral Insulin Trial Committee, the TrialNet Clinical Monitoring Group, Laboratory Monitoring Group, Steering Committee and Data and Safety Monitoring Board will receive periodic reports from the TrialNet Coordinating Center on the progress of the study. These will include accrual rates and

baseline demographic characteristics. Throughout the study, the Oral Insulin Trial Protocol Committee and the Laboratory Monitoring Group will review various indices of the performance of the TrialNet central laboratories including the reproducibility of results, within assay coefficients of variation, autoantibody rates of positivity and confirmation.

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

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

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

10.2.4 TrialNet Laboratories
TrialNet core laboratories will be utilized to perform tests and assays for this trial. All laboratory results will be forwarded to the TrialNet Coordinating Center for analysis.

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

10.2.6 Data and Safety Monitoring Board (DSMB)
The DSMB will meet approximately every 6 months to review the interim effectiveness and potential toxicity of the study treatments based on interim analyses of indicators of effectiveness and safety prepared by the Coordinating Center. The DSMB will independently evaluate whether there are grounds to discontinue the study.

10.3 Partnering with Industry

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

10.4 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 for notification to them about future studies. Approval from the TrialNet Steering Committee and the NIDDK would be required before such linkage could occur. Researchers from outside of TrialNet will not be permitted to identify samples.

Data collected for this study will be sent to the TrialNet Coordinating Center 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. Thus, whereas a sample can be destroyed upon a participant's request during the existence of the TrialNet, it can no longer be destroyed once TrialNet is completed. However, there will still be the potential to link data derived from the samples with data that had been derived from TrialNet studies. Once TrialNet is completed, researchers will only obtain access to samples through grant proposals approved by the NIDDK. The NIDDK will convene an external panel of experts to review requests for access to samples.

10.5 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 the masking 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.

10.6 Participant Reimbursement and Compensation

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

Oral Insulin Study: Schedule of Assessments

	TN NH	Oral Trial Initial Visit	Baseline			1YR		2YR		3YR		4YR		5YR		6YR[6] …	
	Monitoring	I	0	3	6	12	18	24	30	36	42	48	54	60	66	72…	END
METABOLIC STUDIES																	
OGTT	X[1]				X	X	X	X	X	X	X	X	X	X	X	X	X
IVGTT		X	(X)[2]														
HbA1C	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X
IMMUNOLOGIC STUDIES																	
Islet autoantibodies	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
DNA (Including HLA)	X																
Samples for Mechanistic/future studies[3]		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
CLINICAL MEASURES																	
Medical History/AE assessment		X[4]		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Family history		X[4]															
Urine pregnancy test if applicable		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical exam including lifestyle assessments		X[4]				X		X		X		X		X		X	
Limited Physicalexam[5]				X	X		X		X		X		X		X		X
Dispense medication			X		X	X	X	X	X	X	X	X	X	X	X	X	
Assess medication compliance				X	X	X	X	X	X	X	X	X	X	X	X	X	X

[1]OGTT must be within 7 weeks of randomization and result must be NGT. If previous abnormal OGTT, subject must have two consecutive OGTT with NGT
[2]Second IVGTT required only if first FPIR is below threshold.
[3]Samples may include serum, plasma, whole blood, and PBMC. Total blood draw volume in adults ≤150 ml at each visit. For children, no more than 5 ml/kg will be drawn at any single visit and no more than 9.5 ml/kg over a 8 week period. Thus mechanistic samples and/or islet autoantibodies scheduled at initial visit may be omitted due to blood volume limitations.
[4]May be performed either at initial or baseline visit.
[5]Height, weight, BP, abdominal circumference (abdominal circumference not done at 3 month visit).
[6]Participants continue every 6 month visits until development of diabetes or study end (see Section 8.4 of OIT Protocol: Study Power and Maximum Information Design)

Figure 4: Oral Insulin Trial Flow Chart

Study Stage

Natural History Study Screening Stage

First, second, or third degree relative

Autoantibody determination (s)

Criteria to move on**:** mIAA **and** one other antibody positive

If eligible ↓

Natural History Study Monitoring Stage Procedures	
Criteria to move on to Oral Trial	Confirmation of autoantibody status[1], OGTT, HLA, **Age 3-45 at time of randomization if proband 1st degree relative** **or** **Age 3-20 at time of randomization if proband second or third degree relative** **Autoantibodies (AA)** mIAA confirmed At least one other antibody positive on two samples **OGTT**[2] Fasting Plasma Glucose < 110 mg/dL (6.1 mmol/l) 2-hr Plasma Glucose < 140 mg/dL (7.8 mmol/l) 30, 60, 90 minute Plasma Glucose < 200 mg/dL (11.1 mmol/l) **HLA** Subject without HLA DQB1*0602

If eligible[3] ↓

Oral Trial Initial Visit Procedures: IVGTT[4], History[5], PE[5], volunteer quiz

If eligible ↓

Oral Insulin Randomization and Baseline[6]

Procedures (IVGTT)[7], Study medication dispensed

1 If autoantibodies are not confirmed positive on the second test done as part of Natural History, a tiebreaker draw will be required.
2 If Diabetes on first OGTT, confirmatory OGTT will be offered for subject information; however, if first OGTT is consistent with Diabetes, the subject is NOT eligible for oral trial regardless of results of subsequent OGTT.
3 Subjects not eligible for Oral Insulin Trial will be eligible for follow-up in Natural History study.
4 IVGTT may be done as soon as 24 hours after OGTT in which meter glucose values are consistent with NGT while formal results are pending.
5 History, PE, may be done at IVGTT initial visit or at baseline.
6 Randomization must occur within 7 weeks of OGTT.
7 If first IVGTT is below threshold, repeat IVGTT will be done at randomization/baseline visit.

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