Safety and Efficacy of Imatinib for Preserving Beta-cell Function in New-onset Type 1 Diabetes Mellitus

Version:8.0

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SAFETY AND EFFICACY OF IMATINIB FOR PRESERVING BETA-CELL FUNCTION IN NEW-ONSET TYPE 1 DIABETES MELLITUS

Version 7.0 (January 29, 2016)

IND # 117,644

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This clinical study is supported by JDRF and conducted by the University of California, San Francisco.

Protocol Approval

Trial ID:	**Protocol Version: 8.0**
	Dated: August 23, 2016
IND # 117,644	**Protocol Chairs:** Jeffrey Bluestone, PhD and Stephen Gitelman, MD

Title: Safety and Efficacy of Imatinib for Preserving ß-cell Function in New-onset Type 1 Diabetes Mellitus

I confirm that I have read the above protocol in the latest version. I understand it, and I will work according to the principles of good clinical practice (GCP) as described in the US Code of Federal Regulations (CFR)—45 CFR part 46 and 21 CFR parts 50, 56, and 312, and in the International Conference on Harmonization (ICH) document *Guidance for Industry: E6 Good Clinical Practice: Consolidated Guidance* dated April 1996. Further, I will conduct the study in keeping with local legal and regulatory requirements.

As the principal investigator, I agree to carry out the study by the criteria written in the protocol and understand that no changes can be made to this protocol without written permission of the study sponsor.

_____

Principal Investigator **(*Print*)**

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Principal Investigator **(*Sign*)** **Date**

Synopsis

Title	Safety and Efficacy of Imatinib for Preserving Beta-cell Function in New-onset Type 1 Diabetes Mellitus
IND Sponsor	Stephen E. Gitelman, MD
Conducted by	University of California, San Francisco
Protocol Chairs	Jeffrey Bluestone, Ph.D. and Stephen E. Gitelman, MD
Accrual Objective	Total of 66 participants will be enrolled during ~78 to 104 weeks.
Study Duration	Each participant will be in the study for 104 weeks.
Study Design	The study will be a multicenter, two-arm, double-blind, placebo-controlled, 2:1 randomly assigned, phase II clinical trial for individuals with recent-onset T1DM. The randomly assigned participants will receive either a 6-month course of imatinib or matching placebo tablets over a period of 26 weeks. Both treatment and placebo groups will undergo identical procedures and will be followed for 104 weeks. The primary endpoint, 2-hour C-peptide AUC in response to a mixed-meal tolerance test (MMTT), will be measured at 52 weeks. Safety, diabetes control, ß-cell function, and immune function will be assessed for 104 weeks. Both groups will receive intensive diabetes management. During the follow-up phase, participants will undergo serial clinical and immunologic assessments over the 104 week study period, and possible mechanisms of imatinib action will be assessed. Initial enrollment will be for subjects ages 18-45, with the goal to lower the age down to 12 upon acceptable safety review and prospect of benefit for this initial older cohort. When the first 10 enrollees have completed their week 26 assessment, the safety data will be reviewed by a subcommittee that includes the protocol chairs, the medical monitor, the clinical trial physician, and the DSMB. If the review concludes that significant safety concerns have been identified, then no further enrollment will occur pending further data review and evaluation by the DSMB. If the DSMB decides that the study may proceed then the FDA will be notified of study progress and determine if further age restriction is necessary, or if enrollment may be opened for subjects down to age 12. After the first 21 subjects have completed 6 months, a further safety review will occur for the DSMB. Additionally, an interim efficacy assessment will occur for the DSMB and FDA. The FDA will again be apprised of study progress, with further consideration for lowering the age of enrollees to 12, if this has not occurred earlier. If and when the age is lowered, an identical review procedure will be followed when the first 10 pediatric participants (ages 12-17) have completed their week 26 assessment. If the review is satisfactory, then enrollment will continue for subjects ages 12-45 until the study is fully enrolled. Adult participants will receive a single 400 mg tablet of imatinib or the corresponding placebo tablet daily for the first 6 months. Pediatric patients will receive 260 mg/m2/day or 400 mg/day (whichever is smaller) of active drug or placebo.
Primary Endpoint	MMTT--stimulated 2 hour C-peptide AUC at week 52.
Secondary Endpoints	**Efficacy:** • MMTT-stimulated peak and 4 hour C-peptide AUC at weeks 52 and 104.

- MMTT-stimulated 2 hour C-peptide AUC at week 104.
- MMTT-stimulated 2-hour C-peptide AUC assessed longitudinally at weeks -4, 13, 26, 52, 78, and 104.
- Insulin use in units per kilogram body weight per day at weeks 52 and 104.
- Major hypoglycemic events, as defined in section 8.2.1, occurring from randomization at weeks 0, 52 and 104.
- HbA1c levels at weeks 52 and 104

Safety:

- The rate of the following AEs in participants receiving imatinib or placebo:
  1. Myelosuppression.
  2. Gastrointestinal disorders.
  3. Infections.
  4. Hepatotoxicity.
  5. Cardiac toxicity and edema.
  6. Cutaneous reactions.
  7. Muscle cramps, bone pain, arthralgias.
  8. Fluid retention or peripheral edema.
  9. Bone metabolism and growth abnormalities (not including bone turnover markers).
  10. Frequency and severity of all AEs in participants receiving imatinib or placebo.

Mechanistic:

Immunological assessments described in section 7 will be compared with clinical outcomes to determine whether there is evidence of immune tolerance to diabetes-associated autoantigens.

Metabolic:

- Proportion of patients who are exogenous insulin-free (for at least 3 months) with an HbA1C $\leq$ 6.5% at weeks 52 and 104 in each treatment arm.
- Proportion of subjects who achieve a persistent reduction (for at least 3 months) in insulin dose to < 0.5 units/kg at weeks 52 and 104 in each treatment arm.
- Effects on insulin resistance as derived from data on MMTT, and change in adiponectin, proinsulin levels (and proinsulin/c-peptide ratio), and glucagon levels.

Inclusion Criteria

- Males and females age 12–45 years of age who meet the ADA standard T1DM criteria[1]. Positive for at least one islet cell autoantibody.
- Diagnosis of T1DM within 100 days of Visit 0.
- Peak stimulated C-peptide level >0.2 pmol/mL following an MMTT at screening.
- Participants of childbearing age who are sexually active must agree to effective contraception. For females, these contraceptive measures must be maintained throughout the study; for males these measures must be followed for a minimum of 3 months after discontinuation of imatinib therapy.

Exclusion Criteria

- Prior history of any significant cardiac disease such as congestive heart failure, myocardial infarction, arrhythmia, or structural defects or suspicion thereof.
- Leukopenia (<3,000 leukocytes/μL), neutropenia (<1,500 neutrophils/μL), or thrombocytopenia (<125,000 platelets/μL).
- Low Hemoglobin (baseline hemoglobin below lower limit of normal)
- Prior history of anaphylaxis, angioedema or serious cutaneous drug reactions
- Any sign of significant chronic active infection (e.g., hepatitis, tuberculosis, EBV, CMV, or toxoplasmosis), or screening laboratory evidence consistent with a significant chronic active infection (such as positive for HIV, PPD/IGRA, or HB_SAg). Significant acute infections must be resolved before treatment may commence, e.g., acute respiratory tract, urinary tract, or gastrointestinal tract infections.
- Anticipated ongoing use of diabetes medications other than insulin that affect glucose homeostasis, such as metformin, sulfonylureas, thiazolidinediones, glucagon-like peptide 1 (GLP-1) mimetics, dipeptidyl peptidase IV (DPP-IV) inhibitors, or amylin.
- Prior or current treatment that is known to cause a significant, ongoing change in the course of T1DM or immunologic status, including high-dose inhaled, extensive topical or systemic glucocorticoids.
- Evidence of liver dysfunction, with alanine aminotransferase (ALT) or aspartate aminotransferase (AST) >2.0 times the upper limit of normal persistent for 1 week or greater.
- Evidence of renal insufficiency as indicated by serum creatinine > 1.2 times the upper limit of normal and confirmed in a repeat test at least one week apart. Evidence of clinically significant metabolic bone disease (except adequately treated rickets).
- Females who are pregnant at the time of screening or unwilling to defer pregnancy during the 24-month study period.
- Prior treatment with imatinib or related tyrosine kinase inhibitor.
- Unable to avoid medications that affect CYP3A4: either inducers that may decrease imatinib levels, or inhibitors that may increase drug concentrations. (Refer to section 1.5.1.12 for a complete list of inducers and inhibitors.)
- Height standard deviation score ≥2 standard deviations below mean (participants of growing-age potential).
- Any sign of QT prolongation on Visit -1 noted on ECG (> 450 ms in males and > 470 ms in females).
- Known coagulation disorders or use of anticoagulants.
- Current and anticipated on-going treatment with drugs that may increase or decrease imatinib plasma concentrations (CYP3A4 family inhibitors or inducers) or drugs that may have their plasma concentration altered by imatinib (drugs metabolized by CYP3A4/5 and CYP2D6).
- Any condition that, in the investigator's opinion, may compromise study participation or may confound the interpretation of the study results.

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ABBREVIATIONS

ADA	American Diabetes Association
AE	Adverse event
ALT	Alanine aminotransferase
ANCA	Anti-neutrophil cytoplasmic autoantibodies-associated vasculitis
AST	Aspartate aminotransferase
ATG	Antithymocyte globulin
ATP	Adenotriphosphate
AUC	Area under the curve
CBC	Complete blood count
CFR	Code of Federal Regulations
CML	Chronic myeloid leukemia
CMV	Cytomegalovirus
CRF	Case report form
CRO	Contract research organization
CTL	Cytotoxic T cell
CY	Cyclophosphamide
DB/DB	Diabetic dyslipidemia
DC	Dendritic cell
DCCT	Diabetes Control and Complications Trial
DPT-1	Diabetes Prevention Trial of Type I Diabetes
DSMB	Data and Safety Monitoring Board
EBV	Epstein-Barr virus
EDIC	Epidemiology of Diabetes Interventions and Complications Study
FDA	US Food and Drug Administration
GAD	Glutamate decarboxylase

GCP	Good clinical practice
GIST	Gastrointestinal stromal tumors
HbA_{1C}	Hemoglobin A1C
$HBsAg$	Hepatitis B surface antigen
HIV	Human immunodeficiency virus
ICH	International Conference on Harmonisation
IFNγ	Interferon gamma
IGRA	Interferon Gamma Release Assay
IRB	Institutional Review Board
ITN	Immune Tolerance Network
LFTs	Liver function tests
MedDRA	Medical Dictionary for Regulatory Activities
MHC	Major histocompatibility complex
MMTT	Mixed-meal tolerance test
NCI-CTCAE	National Cancer Institute *Common Terminology Criteria for Adverse Events*
NFkB	Nuclear factor kappa B
NK	Natural killer cell
NKT	Natural killer T cell
NOD	Nonobese diabetic
PCR	Polymerase chain reaction
PDGFR	Platelet-derived growth factor receptor
PPD	Purified protein derivative test
PTH	Parathyroid hormone
IGRP	Islet-specific glucose-6-phosphatase catalytic subunit related protein
SAE	Serious adverse event

SAP	Statistical analysis plan
STZ	Streptozotocin
T1DM	Type 1 diabetes mellitus
TCR	T cell receptor
TK	Tyrosine blocking
TNFα	Tumor necrosis factor alpha
Tregs	Regulatory T cells
TUNEL +	TdT-mediated dLJTP-biotin nick-end-labeling positive cells
WHO	World Health Organization

1. BACKGROUND AND RATIONALE

1.1 OVERVIEW

Type 1 diabetes mellitus (T1DM) results from the autoimmune destruction of insulin-producing ß cells. Although exogenous insulin is widely available, it is not possible for affected individuals to consistently achieve euglycemia with current technology, and thus they are at risk for devastating long-term complications. This phase II study is designed to evaluate the safety and efficacy of imatinib mesylate as a novel therapy for new-onset T1DM. Imatinib is a first–in-class tyrosine kinase inhibitor that has had remarkable success as a therapy for several cancers, including chronic myelogenous leukemia (CML). In preclinical studies, imatinib is one of only a handful of agents that has been shown in the nonobese diabetes (NOD) mouse to induce a durable remission of new-onset diabetes without continuous ongoing therapy. These findings have been extended to the clinical arena, where case reports and smaller studies have shown positive effects of imatinib in patients with autoimmune conditions and for type 2 diabetes. Although the mechanism whereby this agent functions has not been fully elucidated, the possibility that short-term therapy with imatinib can induce tolerance and lead to a durable long-term remission makes it a very attractive potential therapy for new-onset T1DM.

1.2 BACKGROUND

T1DM is a chronic autoimmune disease in which insulin-producing ß cells are completely destroyed, resulting in a lifelong dependence on exogenous insulin.[2-5] Current management of T1DM is not optimal. To avoid long-term complications, affected individuals must maintain near-normal glycemic control by frequent glucose monitoring, and by taking multiple daily doses of insulin (via injection or pump) adjusted for variations in diet and exercise.[6] Such strict glycemic control is rarely achieved with current T1DM management, and can result in recurrent severe hypoglycemia. Thus, it is not possible to fully mimic ß-cell function with current therapies, and there are currently no established treatments that can prevent the autoimmune ß-cell destruction before diagnosis.

After clinical presentation, affected individuals often enter a “honeymoon,” or remission, phase when they are still able to make substantial amounts of insulin.[7-9] Nevertheless, endogenous insulin secretion continues to slowly deteriorate over the first few years of disease, eventually becoming undetectable and necessitating increasing reliance on exogenous insulin. Past studies have demonstrated that preservation of even modest endogenous insulin secretion dramatically improves metabolic control of T1DM, which, in turn, is associated with reduced morbidity and mortality.[10-13] The ultimate goal of this study is to identify a means of blocking further autoimmune destruction of the ß cells, retaining endogenous insulin secretion and thereby improving metabolic control. Eventually, such a therapy may also be used at an even earlier stage to prevent T1DM.

T1DM occurs in those who have an underlying genetic risk in synergy with one or more environmental exposures.[2-5] Increasing evidence suggests that both adaptive and innate immunity may play a role.[14,15] This process is mediated by the progression of a destructive T-cell infiltration of insulin-producing ß cells. Both CD4+ and CD8+ T cells cooperate in the initiation of insulitis and ß-cell destruction occurs via cytokines (e.g., IFNγ, TNFα) and direct cytolytic activity.[2-4,16-19] Development and propagation of this autoimmune destruction is not solely dependent on T cells, with potential roles ascribed to B cells, NK cells, NKT cells, dendritic cells (DCs), and macrophages.[3,20-24] For example, B-cell depletion in the NOD mouse has been shown to prevent and reverse diabetes,[25,26] and in a recent clinical trial subjects with new-onset T1DM treated with rituximab, an anti-CD20 monoclonal antibody (mAb) had slower decline in ß-cell loss than controls.[27] Thus, agents affecting more than one cell type of the immune system, or with effects on inflammation, may have greater efficacy in modulating the autoimmune response.

Several promising studies have shown that interventions shortly after the time of T1DM diagnosis can alter the natural course of the honeymoon phase. Cyclosporine prolongs endogenous insulin secretion, and some participants experienced complete remission.[28,29] However, long-term treatment is limited by toxicity and by the transience of the effects.[30-32] More targeted therapy with a short course of two different preparations of an anti-CD3 mAb have been promising, although not all respond, and the effects wane over time.[33-36] Recent new onset trials with other agents have had mixed results, with some agents causing acute worsening in beta cell function (IL-2 with rapamycin, Long et al, Diabetes, in press), some showing no effect[37,38] and some showing initial preservation but without sustained effects.[27,39] Despite these early successes, there is ongoing concern with the durability of the response, and the safety and tolerability of the agents offered. Thus, there is currently no established, acceptable immunotherapy for new-onset T1DM to preserve endogenous insulin secretion. The limitations may stem from the need for a multi-faceted approach to interdicting the autoimmune response, using either a combination of drugs[40], or a drug that affects multiple different cell types or pathways. The drug proposed in this trial, imatinib, falls into this latter camp.

1.3 SCIENTIFIC RATIONALE

Imatinib mesylate, also known as CGP57148B, STI-571, Gleevec®, or Glivec® (Novartis), is a specific inhibitor of the Abl protein tyrosine kinases (v-Abl, Bcr-Abl, and c-Abl).[41-43] Imatinib's activity against cells bearing the Bcr-Abl translocation, created by the Philadelphia chromosome abnormality, has resulted in a unique niche for the treatment of CML.[44-48] However, imatinib's activity is not limited to Abl, as it also inhibits other constitutively activated tyrosine kinases, such as platelet-derived growth factor receptor (PDGFR), c-kit (CD117), macrophage colony stimulating factor receptor (c-fms), Abl-related gene, and Lck, whereas other kinases are probably not affected.[49-54] The inhibitory activity against c-kit and PDGFR has resulted in imatinib's

expanded use as a therapy for gastrointestinal stromal tumors (GIST), [55] eosinophilic disorders, [56] and systemic mast cell disease.[56,57]

In addition to its remarkable effect on malignant cells, imatinib has been shown to affect various arms of the immune system. Imatinib treatment may lead to impaired T-cell function and decreased DC number in CML patients,[58-60] although other studies have not documented such effects.[61-64] Similarly, while some reports suggest that imatinib enhances DC function both in human[65,66] and mouse,[67-69] other studies demonstrated that relatively high concentrations (1–5 μM) inhibited the development and the functional capacity of human or mouse DCs,[70-72] as well as human monocytes/macrophages.[73-75] Several investigators have now shown that imatinib has significant anti-inflammatory properties in different mouse models. Wolf et al demonstrated in a mouse model of acute hepatic inflammation that imatinib inhibits tumor necrosis factor-alpha (TNFα) production in macrophages, conferring a strong anti-inflammatory effect.[76] Dietz et al noted that delayed-type hypersensitivity was reduced in mice treated with imatinib.[77]

Most recently, it has become clear that imatinib can function as an effective immunosuppressive drug. In preclinical studies, imatinib efficiently prevents disease and induces remission of mouse and rat models of autoimmune arthritis.[78-80] Moreover, this drug inhibits collagen-induced arthritis effectively in mice and blocks PDGFR-mediated signaling in synovial fibroblasts obtained from rheumatoid arthritis patients.[81-83] Efficacy in rheumatoid arthritis may also result from effects on mast cells: treatment of rheumatoid synovia in vitro with imatinib inhibits c-kit and induces apoptosis of mast cells.[84] Furthermore, imatinib treatment ameliorates autoimmune nephritis in two mouse models of lupus.[85,86] In studies of scleroderma, Soria et al reported that imatinib limits dermal fibroblast proliferation.[87] Imatinib reduces production of extracellular matrix and suppresses experimental fibrosis,[88] and prevents fibrosis in different models of systemic sclerosis.[87] Imatinib inhibits PDGF-mediated responses related to the development of intimal hyperplasia in giant cell arteritis.[89] Imatinib may also be effective in the treatment of anti-neutrophil cytoplasmic autoantibodies-associated vasculitis (ANCA), based on inhibition of T-cell activation in samples taken from affected patients.[90]

These findings have been extended to the clinical arena, where a few case reports and a phase I study show positive effects of imatinib in patients with rheumatoid arthritis.[79,91-94] Early studies suggest that imatinib may have a role in the treatment of systemic sclerosis.[95-98] Imatinib therapy improved cutaneous sclerosis and inflammatory manifestations of the disease in 3 treated subjects, [96,97] and a specific gene signature of imatinib response has been identified.[96] Seven trials evaluating the efficacy of Imatinib in systemic sclerosis are now posted on the clinicaltrials.gov web site. Imatinib was also effective and well tolerated in a phase I study of 6 patients with active spondyloarthritis.[99] There is a case report of a patient with bullous pemphigoid, the most frequent autoimmune blistering dermatosis, with eosinophilia and a deletion

consistent with hypereosinophilic syndrome, who responded to imatinib therapy.[100] There is one case report of imatinib therapy inducing a long-standing remission in a patient with Crohn's disease, without significant side effects.[101] Imatinib has also been reported to treat intractable psoriasis in a patient who was receiving this agent for GIST.[102]

Although the aforementioned studies suggest efficacy, the mechanisms are unclear. *In vitro* studies suggest that imatinib may have direct effects on T-cell, B-cell, and antigen-presenting cell signaling and function. Imatinib can inhibit human T-cell proliferation in vitro without inducing apoptosis,[77,103,104] and analysis of the TCR-induced signaling cascade revealed a reduced phosphorylation of ZAP-70, LAT, Lck and ERK1/2, as well as reduced levels of activated NFκB.[77,104,105] Interestingly, Lck has been suggested as a direct target of imatinib.[52] Studies in the mouse demonstrated that imatinib could impair memory CD8+ T-cell development but did not affect the primary response and cytotoxic activity or induce apoptosis.[106-108] However, lytic function of human CD8+ T cell has recently been shown to be inhibited by imatinib.[109]

The proposed trial represents a unique approach to the treatment of T1DM and will provide critical validation of the hypothesis that a new class of kinase inhibitors, represented by the first-in-class compound imatinib, will demonstrate a novel therapy for T1DM. The possibility that short-term therapy with imatinib can induce tolerance and lead to a durable long-term remission makes it a very attractive potential therapy for patients with new-onset T1DM. If this therapy is safe and effective, then its use can be expanded to other autoimmune disorders, and possibly to T1DM prevention.

1.4 PRECLINICAL AND CLINICAL EXPERIENCE

1.4.1 Preclinical Studies

1.4.1.1 *Studies in the NOD Mouse*

The known mechanisms of imatinib action and observations in other autoimmune settings suggest that imatinib may have an important role in treating T1DM. Hagerkvist et al noted that imatinib prevents T1DM in the NOD mouse when administered from 9 to 35 weeks of life; it did not affect the extent of islet inflammation when given from 3 to 9 weeks of age or when treated out to 35 weeks, but the ß-cell area is significantly greater in treated animals.[110] They also examined specific effects of imatinib on the ß cell. Three doses of imatinib partially protect against T1DM in streptozotocin (STZ)-injected mice. It also protects cultured islets from various apoptosis-promoting agents, including pro-inflammatory cytokines, nitric oxide, and STZ.[110,111] Thus, imatinib appears to act on multiple levels in diabetes, including reduction of insulin resistance and prevention of ß-cell apoptosis. The authors concluded that imatinib enhances ß-cell survival via a mechanism similar to ischemic preconditioning, as they found NFkB activation, increased nitric oxide and reactive oxygen species production, and depolarization of the inner mitochondrial membrane.

1.4.1.1.1 Prevention of T1DM

Treatment of prediabetic NOD mice starting at 12 weeks of age (a stage when insulitis is firmly established)[112] with imatinib orally once a day at 1.5 mg/mouse for 7 weeks led to complete protection from hyperglycemia during treatment, whereas half of the control mice developed diabetes. After cessation of treatment, only ~40% of the imatinib-treated animals became progressively diabetic. The majority of the mice remained nondiabetic at more than 50 weeks of age. Treatment of mice with cyclophosphamide (CY)-induced diabetes (a more robust model with rapid onset of disease) resulted in only ~30% of imatinib-treated animals developing diabetes within 3 weeks after CY injection, in contrast to 85% of the control mice. These results confirmed that imatinib can prevent T1DM.

1.4.1.1.2 Remission Induction

Many agents prevent T1DM in the NOD mouse before the development of insulitis,[20,113] but only a handful of agents are successful in inducing remission in overtly diabetic NOD mice. Louvet et al[112] found that only 1 week after initiation of treatment, imatinib reversed diabetes in more than 40% of diabetic animals (Figures 1*A* and 1*C*) and after 2 weeks the remission rate jumped to ~80% of the mice (n = 28). The effect of imatinib was most notable in mice with glucose levels that were modestly elevated at diagnosis (250–350 mg/dL) but was also effective in mice with glucose levels > 300–450 mg/dL, although less so. Mice treated with vehicle alone did not reverse diabetes (Figures 1*B* and 1*C*).

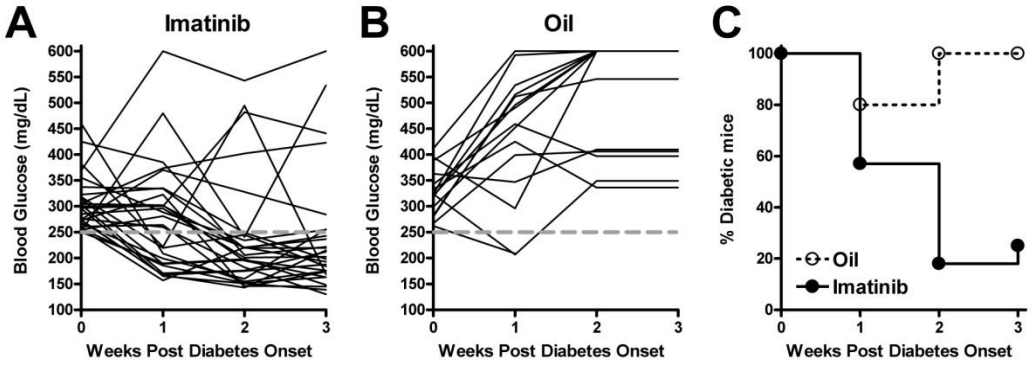

Figure 1. Induction of diabetes remission. Imatinib or oil treatment was initiated at the time of disease onset (blood glucose >250 mg/dL) and continued for 3 weeks. Individual glucose readings for imatinib- (*A*) and oil-treated mice (*B*) are shown. (*C*) The percentage of diabetic mice is shown for each group : imatinib n=28, oil n=15. From Louvet et al.[112]

1.4.1.1.3 Tolerance Induction

With cessation of imatinib therapy at 3 weeks, all mice became hyperglycemic within 10 weeks (Figure 2*A*). However, by increasing treatment duration from 3 weeks to 10 weeks the majority of the mice remained euglycemic (Figure 2*B*).[112] Thirty-five weeks

after initiation of therapy, ~50% of imatinib-treated animals had reversed from diabetes into a state of long-term tolerance without the need of continuous treatment.

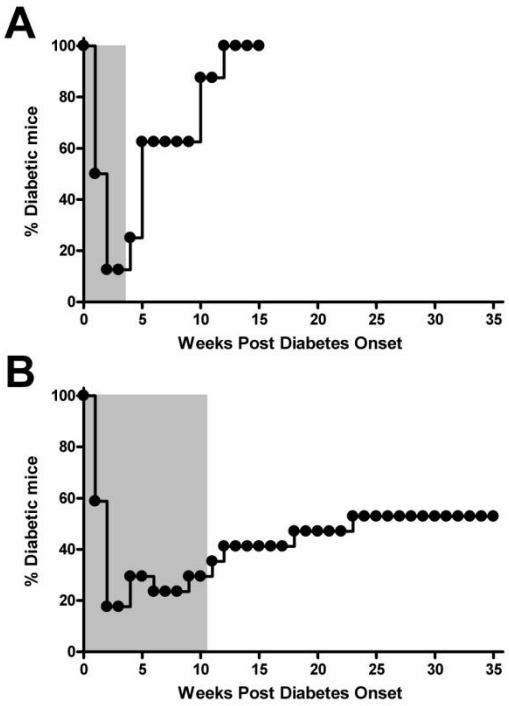

Figure 2. Long-lasting remission of diabetic NOD mice. Imatinib treatment (*gray shaded area*) was initiated at the time of disease onset and continued for (*A*) 3 weeks (n=8) or (*B*) 10 weeks (n=17). From Louvet et al.[112]

1.4.1.1.4 Leukocyte Infiltration of the Pancreas

Treatment of prediabetic NOD mice for variable periods of time with imatinib did not prevent insulitis.[112] There were subtle differences in severe and mild insulitis in the drug-treated versus control islets, but these findings were not statistically significant, in accord with prior studies.[110] Similarly, pancreatic islets from long-term normoglycemic mice after cessation of imatinib treatment still harbored significant insulitis. Thus, while preventing clinical disease, imatinib does not eliminate leukocyte infiltration of the pancreas. It should be noted that this observation is different from what has been observed for anti-T-cell agents such as Thymoglobulin and anti-CD3, which clear the islets of infiltration, suggesting a different mechanism of action.[114,115]

1.4.1.1.5 Relevance of Tyrosine Kinase–blocking Activity

As documented previously,[116] imatinib can affect a series of tyrosine kinases *in vitro* including abl, c-kit, c-fms, and PDGFR. To pinpoint the relevant kinases in T1DM therapy, different TK-blocking agents that share targets with imatinib have been tested.[112] These studies suggest that the dramatic effects of imatinib in the reversal of diabetes are not due to a single drug activity but due to the combination of the anti-

inflammatory effects short term, due at least in part to its PDGFR antagonism, combined with longer term effects, perhaps due to its c-abl- and c-kit-specific activities. A clinical case report (see section 1.3.2) lends further support for this proposal.[117]

1.4.1.2 *Studies in Rat and Mouse Models of T2DM*

Hagerkvist et al reported that imatinib normalizes peripheral insulin resistance in rats fed a high-fat diet.[118] Han et al extended these observations in the diabetic dyslipidemia (db/db) mouse, noting that imatinib reduces insulin resistance and induces diabetes remission.[119] Liver steatosis and serum transaminitis are markedly reduced in treated animals. Imatinib also decreased TUNEL+ apoptotic ß cells and increased BrdU+ ß-cell numbers. A unifying explanation for these effects is that imatinib ameliorates endoplasmic reticulum (ER) stress: ER stress appears to play an important role in insulin resistance, and Abl kinase, an imatinib target, has been reported to play a role in this process.[120] Indeed, a variety of ER stress markers in the liver and adipose tissue were reduced by imatinib treatment of these animals. Recent findings that kinase inhibition of IRE1α ameliorates ER stress and consequent apoptosis[121] lends additional support to the potential role of imatinib in this process.

1.4.2 Clinical Studies in Patients with Diabetes

Since approved for treatment of CML, several case reports and small case series have appeared describing patients with T2DM who have experienced significant improvement or resolution on imatinib.[122-124] Reductions in cholesterol and triglycerides have also been described in treated patients.[125]

These changes have not been ascribed to significant effects with decreased appetite or weight loss. Fitter et al[126] have further evaluated mechanisms whereby metabolic control improves for subjects with T2DM treated with imatinib. They have noted that adiponectin levels increase ~ 3-fold within 3 months of initiating imatinib, and remain elevated with on-going therapy out to 12 months. This adipokine increases glucose uptake and fatty acid oxidation in muscle, and decreases hepatic gluconeogenesis; low adiponectin levels are associated with increased insulin resistance and higher risk for T2DM.

In a case report of another tyrosine kinase inhibitor, a 64-year-old man with metastatic renal cell carcinoma was started on sunitinib 8 months after the diagnosis of diabetes.[117] He was presumed to have T1DM, based on strongly positive GAD antibody titers. After ~15 weeks on sunitinib, the insulin dose was decreased, until he discontinued insulin altogether at ~45 weeks on sunitinib. Sunitinib was discontinued at about that same time, but glycemic control has remained near normal over the ensuing 30 weeks, with persisting elevation of GAD antibody titers. His weight remained stable over the treatment period.

1.5 SUMMARY OF KNOWN AND POTENTIAL RISKS AND BENEFITS FOR HUMAN PARTICIPANTS

1.5.1 Risks

1.5.1.1 *Overview*

There is an extensive safety experience in patients treated with imatinib for CML and GIST (see larger clinical trials in Refs. [45,47,55,127-131] and the package insert at http://www.pharma.us.novartis.com/product/pi/pdf/gleevec_tabs.pdf) Overall, imatinib has been well tolerated in clinical trials. Adverse events (AE) are typically mild to moderate (grades 1 to 2) and manageable without dosage reduction or permanent discontinuation of therapy. The most common side effects include gastrointestinal reactions (nausea, vomiting, and diarrhea), edema, muscle cramps, and rash. Hematological toxicities (thrombocytopenia, neutropenia, and anemia) are dose-dependent and reversible, and noted much more frequently in CML than in GIST, suggesting they relate in large part to the underlying disease. Specific issues are discussed in more detail below. These side effects tend to be more noticeable in older and sicker patients who are on higher doses of imatinib. Many of these adverse events may improve spontaneously while continuing therapy at the same dose. It is difficult to extrapolate fully from the past safety and tolerability experiences in cancer to a diabetes setting, in which our study participants will be younger, will be carefully selected so as not to have concurrent or chronic medical problems, and will be on lower doses of the drug and for a shorter duration. Our expectation, however, is that the therapy will be even better tolerated than in other settings in which it has been traditionally used (B. Druker, Oregon Health Sciences University, personal communication). The published and unpublished experience has been that imatinib is generally better tolerated in younger patients, including children.[132-135] No AEs unique to children have been reported, with the possible exception of transient, reversible effects on growth rate (as detailed in section 1.5.1.11).

1.5.1.2 *Gastrointestinal Reactions*

Nausea constitutes the most common side effect of therapy. It has been noted in up to 40%–60% of subjects with CML or GIST, and it is usually mild, grade 1, and dose related. Nausea occurs much more commonly when the drug is taken on an empty stomach, and can be avoided when taken with food. Ingestion with food has not been shown to affect pharmacokinetic parameters. Diarrhea is also experienced by some patients, possibly from imatinib blockade of c-kit, and is often dose related.

1.5.1.3 *Edema*

Edema is present in about half of patients on imatinib. It is usually superficial, mild to moderate in nature, and does not require specific therapy. It usually manifests as periorbital edema and is most noticeable first thing in the morning. Edema in the lower extremities is noted much less frequently. More marked issues with fluid retention, such as pulmonary edema or ascites, occur rarely, and have typically been limited to those

with more advanced stages of CML. This issue generally occurs at higher doses, and in elderly patients, and has been less common in children.

1.5.1.4 *Cutaneous Reactions*

Cutaneous reactions have been reported in ~30% of patients during imatinib treatment. The rashes are often pruritic, and most commonly erythematous, maculopapular lesions on the forearms and trunk, and sometimes also involve the face. The rashes are usually mild (grades 1–2) and self-limited. About 3% are higher grade, and again are usually associated with higher doses of therapy. Severe exfoliative rashes have been noted in 1 in 500 treated subjects, and they usually occur early in the course of treatment. Imatinib treatment has been associated on rare occasions with induction of vitiligo-like lesions, which may be related to blockade of c-kit in melanocytes.[136,137] This finding appears to increase with dose and duration of treatment, and is reversible with dose reduction or discontinuation. Paradoxically, a patient with vitiligo has been described who had repigmentation of his lesions while on imatinib treatment for GIST.[138]

1.5.1.5 *Muscle Cramps, Bone Pain, Arthralgias*

Arthralgias affect 25%–50% of treated patients. As with other issues, this is usually mild to moderate, and manageable without reduction of the drug dose. Cramps usually occur in the hands, feet, calves and thighs, and tend not to change over time in terms of location, frequency, and intensity. Ionized calcium and magnesium levels are normal. Bone pain and arthralgias are reported typically early in the course of therapy and usually subside after several months.

1.5.1.6 *Hepatotoxicity*

Liver toxicity, if an issue, usually emerges during the first few months of therapy, but can be a late finding. Frequent monitoring of liver function is suggested during therapy. Grade 1 elevations in transaminases occur in up to 10% of treated patients and often normalize while continuing on therapy. Grade 3 events have been reported in 2%–5% of subjects in GIST and CML trials, but these events may be confounded by a variety of other factors, including leukemic liver infiltration. For grade 3 events in oncology settings, therapy is temporarily interrupted. Imatinib is permanently discontinued for grade 3 events that recur with drug re-challenge.

1.5.1.7 *Myelosuppression*

The development of myelosuppression is much more common in CML than GIST, and may relate to the underlying effects of the cancer on the bone marrow. In the phase II study of imatinib for GIST, anemia (9% of subjects, 2% grades 3 or 4), neutropenia (7% of subjects, 5% grade 3 or 4), and leukopenia (5% of subjects, 1% grades 3 or 4) were the most commonly reported findings; grades 3 or 4 thrombocytopenia was noted in <1%. Complete blood counts with differential will be monitored at the same time points as liver function tests. For myelosuppression of grade 3 or higher severity in oncology

settings, therapy is temporarily interrupted, and permanently discontinued for grade 3 events that recur with drug re-challenge.

1.5.1.8 *Cardiac Toxicity*

Prior concern was raised that imatinib may induce cardiac dysfunction, based on a report of 10 subjects with left ventricular dysfunction and congestive heart failure.[139] The ejection fraction for these subjects dropped significantly during the course of imatinib therapy, but the authors did not report the size of the underlying cohort from which these cases were derived, nor did they report baseline cardiac function, or other physiological risk factors that might be associated with cardiac dysfunction. A subsequent retrospective review of a large database of 942 GIST patients treated with 400–800 mg of imatinib for a median of 24 months revealed only 2 patients (0.2%) who may have had a cardiotoxic effect from this drug.[140] Atallah et al reported that 0.6% of 1276 patients (median age 70) treated with imatinib developed congestive heart failure, and half of these continued therapy.[141] The cases typically occurred in elderly patients with preexisting cardiac conditions. A recent study in knockout mice revealed that PDGFR-β signaling is required for the cardiac response to pressure overload-induced stress.[142] This provides a mechanistic basis for the observation that cardiac toxicity induced by PDGFR inhibitors, such as imatinib, is generally observed only in patients with preexisting cardiac conditions. Druker (personal communication) has not noted any cardiac issues in treatment of over 1000 patients with imatinib, with the exception of 2 patients over age 65 who developed congestive heart failure; thus, aside from a careful past medical history he does not suggest routine cardiac studies for subjects on imatinib. The relatively short course of proposed treatment and younger, otherwise healthy population would suggest that this will not be an issue in this trial. Subjects enrolled in this trial will have careful baseline history and physical examination to ensure no preexisting cardiac problems.

1.5.1.9 *Infectious Disease*

Imatinib has been well tolerated, and patients receiving chronic therapy do not appear to be abnormally susceptible to viral infections or opportunistic infections.[45,47,55,127,128,131] Further reassurance comes from evaluation in a mouse model in which imatinib selectively impaired expansion of memory cytotoxic T cells but the expansion of naïve cytotoxic T-cells (CTLs) in response to viral infection in vitro and in vivo was not impaired.[107]

1.5.1.10 *Reproductive Toxicity*

Despite the growing clinical experience with imatinib in CML and GIST, there is still only limited data on the effects of imatinib on fertility and/or pregnancy. Animal studies have shown that this drug is teratogenic in rats but not rabbits (Gleevec IB). Spermatogenesis is impaired in rats, dogs, and monkeys. Female rats receiving imatinib doses of 45 mg/kg, which approximates the usual clinical dose of 400 mg/day, experience significant post-implantation loss, including increased fetal resorption,

stillbirth, and early pup mortality. It does not appear to cause chromosome damage. Exposure to doses of 100 mg/kg (approximating a clinical dose of 800 mg/day) during organogenesis results in teratogenic effects, with exencephaly or encephalocele, and absent or reduced frontal and parietal bones. Based on these studies, it has been classified as a class D drug. However, there have been a series of case reports and small series reporting normal outcomes following pregnancies.[143-151] The largest compilation of clinical experience is from a retrospective chart review of 180 women exposed to drug during pregnancy; outcome data were available for 125.[150] Of these, 50% delivered normal children and 28% had elective terminations (3 following the identification of abnormalities). Twelve infants had abnormalities identified, 3 with a similar complex of malformations. There did not appear to be a higher rate of spontaneous abortion. Thus, although most fetuses exposed to imatinib appear to be unaffected, there is a risk for potential malformation. In light of these findings, investigators need to ensure that all participants avoid pregnancy.

1.5.1.11 *Bone and Mineral Metabolism*

One small sub-study has noted hypophosphatemia with increased PTH, low normal serum calcium, and increased urinary phosphorous in patients treated with imatinib.[152] The authors postulate that this is an effect mediated by PDGFR inhibition. Further studies in vivo and in vitro suggest that short-term therapy with imatinib (on the order of 3-6 months) causes an uncoupling of bone turnover, with activation of bone formation via promotion of osteoblast differentiation, and inhibition of resorption by inhibition of osteoclastogenesis and promotion of osteoclast apoptosis.[153]

A recent study in rats indicated that imatinib treatment (4–12 weeks duration) resulted in narrowing or premature closure of the growth plate in the proximal tibia, although the animals were not assessed for a follow-up period after drug was withdrawn.[154] Three recently published case studies report decelerated growth in juvenile CML patients undergoing imatinib therapy.[155-157] Notably, one of the patients progressed through puberty and reached appropriate final adult height for family while being treated continuously with imatinib[156]; another patient experienced growth acceleration following discontinuation of imatinib[155], but final height was not reported [155]

In the initial reports from the pediatric CML trials with imatinib, no growth or bone metabolism issues were reported.[132-135,158] Following the case reports noted above, Japanese investigators performed a retrospective analysis of 48 children with CML treated with imatinib as a first line therapy for a median of 34 months[159]. They noted that growth rate decreased in pre-pubertal children (defined as girls < age 9 years, boys < 11 years), with a median decrease after 1 year of ~0.5 in height standard deviation score (Ht-SDS) for chronological age, and further progressive decrement with on-going therapy. However, the growth rate improved during puberty, and children who initiated therapy at a pubertal age appeared to have little or no impact on growth rate. These growth issues may be confounded by a variety of issues associated with a chronic illness

such as CML, which in and of itself may impact growth rate, and one should note that decreased growth rate is associated with alternate therapies for CML, such as bone marrow transplantation[160].

1.5.1.12 *Drug Interactions*

Imatinib is metabolized by the CYP3A4/5 cytochrome P450 enzyme system. Drugs that induce or inhibit this P450 will affect imatinib levels, and should be used with caution, and if possible avoided, during the course of imatinib therapy. Major CYP3A4/5 inducers may decrease imatinib levels; these include carbamazepine, dexamethasone, phenytoin, phenobarbital, progesterone, rifampin, and St. John's wort. Major CYP3A4/5 inhibitors, which will serve to increase imatinib levels, include cimetidine, erythromycin, fluoxetine, ketoconazole, ritonavir, itraconazole, verapamil, and grapefruit juice. Participants who require chronic therapy with any of these agents will be excluded from participation..

1.5.1.13 *Malignancies*

On-going pharmaco-vigilance data is being collected to assess risk for malignancy following imatinib exposure (see data on file, Gleevec IND 55,666). An integrated review of safety data from imatinib oncology clinical trials suggests that there may be an increased frequency of genito-urinary malignancies. However, the effect is not statistically significant, and it is limited to those subjects treated for > 2 years. Thus, we will exclude any subjects with prior malignancy, and will carefully monitor genito-urinary risk throughout the study with serial urinalyses and prostate specific antigen (for male participants)

When rats are treated with imatinib for 2 years, they had a shortened lifespan, and higher risk for developing cancers in the kidneys, bladder, urethra, clitoris, small intestine, parathyroid glands, adrenal glands, and stomach. The relevance of these findings to humans is not known.

1.5.2 Potential Risks and Benefits of Trial Participation for Children

Imatinib has been used to treat Ph+ CML in children as young as age 3, and is FDA approved for this indication .[132,134,135] Smaller trials have also been conducted in children with Ph positive acute lymphoblastic leukemia and some brain tumors[133,161,162] . Doses of 260-340 mg/m^2 of imatinib in the pediatric population achieved a similar area under the curve (AUC) to the 400-mg dosing in adults; thus, we plan to employ such dosing in younger or smaller participants enrolled in this trial, offering whichever is the smaller of these two doses to pediatric participants. The safety profile was similar to that in adults, except that there were fewer reports of musculoskeletal pain and peripheral edema. As described in section 1.5.1.11, there is a potential concern about the effects of imatinib on growth, based on preclinical data several case reports, and a retrospective study of pre-pubertal Japanese children with CML (defined as girls < 9 and boys < 11 years of age)[159]. With these initial observations in mind, we will limit

enrollment to ages 12 and older for this trial. At age 12, girls have completed on average 92% of their growth, and boys 83% of their final adult height[163], thus limiting potential effects of drug on final height. Furthermore, as opposed to the studies referenced above in cancer, subjects in this trial will only have a brief 6-month exposure period on study drug, at a modest dose, and thus will not have the long term continuous exposure that has been associated with progressive slowing in growth rate for pre-pubertal children. As detailed in section 5.2.9, effects on growth rate will be carefully assessed in subjects with significant remaining growth potential at study entry, and all subjects will have frequent monitoring of bone metabolism and bone turnover markers. One of the study stopping rules relates to impact of imatinib on growth rate (Section 3.7).

As one of the most common chronic childhood diseases, T1DM is a particular burden to children and their families. While T1DM can occur into adulthood, the worldwide incidence of T1DM is increasing rapidly in children younger than 15 years, as recently documented by the WHO Diamond Project.[164] During the period 1995–1999, the global annual increase in childhood T1DM was 3.4% but was as high as 5.3% in North America.[164] In Europe, the EURODIAB study group has found the greatest rate of increase in the 0-4 years age group and is predicting a doubling in the number of new cases in children younger than 5 years in the next decade.[165] Overall, prevalence of T1DM in children under age 15 in Europe is predicted to rise from 94,000 in 2005 to 160,000 in 2020.[165] In contrast, the incidence of T1DM in young adults over age 15 is not increasing.[165] For these reasons, there is considerable interest in identifying safe and effective interventions that can modify the course of T1DM in pediatric populations. Successful initial preservation of β-cell function in pediatric populations has recently been reported for several treatment modalities.[27,33-36,39]

Evaluation of new interventions for T1DM in adults may not be informative about their success in children. It is known that the rate of β-cell decline is different in children versus adults (Greenbaum et al, Diabetes)[178], and therefore lack of efficacy of a treatment in adults is not necessarily predictive of efficacy in children.[27,36] There are currently no approved interventions for new-onset T1DM. In contrast to many interventions currently being studied in the pediatric population, imatinib is approved for use in children and has an extensive clinical safety record. Imatinib is also unique among agents being studied for new-onset T1DM in that is an oral drug, which has special relevance in the pediatric age group.

1.5.3 Potential Benefits

In this study, all participants will receive intensive diabetes management aimed at achieving near-normal metabolic control per the standard American Diabetes Association (ADA) guidelines.[1] Although intensive diabetes management is recommended for all patients with T1DM, it is not always available to all subjects in the community. The Diabetes Control and Complications Trial (DCCT) research group documented that improved metabolic control lowers the risk for long-term complications.[6] The means to achieve this improved control in the DCCT has become

the idealized standard of care, with clinical management and education provided by a diabetes specialty team. It should be noted that such care may not necessarily be available to those outside the study, and those who are not seen by a diabetes specialty team may have worse outcomes over time.[166]

Improved metabolic control early in the course of T1DM will have a long-standing effect on lowering the risk for long-term complications for many years to follow; i.e., there appears to be a "metabolic memory" that influences later risk. This effect has been documented in the Epidemiology of Diabetes Interventions and Complications (EDIC) study, the long-term follow-up of the DCCT cohort. [6,12,167-170] Although the conventional group (with less stringent metabolic control) and the intensive group (with near-normal metabolic control) have had comparable HbA_{1C} levels since the end of the formal DCCT study, the intensive group continues to have significantly lower risk for complications 10 years later.

An additional benefit that may be realized by all participants is that maintaining near-normal glycemic control through intensive diabetes management may, in and of itself, lead to the preservation of ß-cell function.[10,11,13,171] The benefits of endogenous insulin secretion, even if one needs to continue exogenous insulin therapy, have been demonstrated in a number of studies, including the DCCT, where those subjects with residual C-peptide had improved metabolic control, with lower risk for severe hypoglycemia and less likelihood of microvascular complications. Finally, the treatment group may have significant benefits from participation even if imatinib therapy has only modest effects on the preservation of endogenous insulin secretion, which is what was noted in the new-onset T1DM studies with anti-CD3 mAb therapy.[34,172]

2. OBJECTIVES

2.1 PRIMARY OBJECTIVE

Determine whether imatinib will slow the progression of the autoimmune destruction of ß cells and lead to the preservation of C-peptide secretion in T1DM.

2.2 SECONDARY OBJECTIVES

Diabetes-related objectives:

1. Assess whether the drug has prolonged clinical efficacy.
2. Assess the effect of imatinib on selected secondary clinical outcomes.

Safety:

1. Determine the safety of imatinib in participants with T1DM, especially with respect to hepatic, bone marrow, cardiac, and cutaneous toxicity.

Mechanistic studies:

1. Characterize how imatinib alters general and diabetes-specific immune responses.
2. Gain a better understanding of the mechanism of action for imatinib in the maintenance of ß-cell function and determine whether the loss of tolerance associated with this disease is reversed.
3. Assess effects of imatinib on insulin sensitivity.

3. STUDY DESIGN

3.1 DESCRIPTION

The study will be a multicenter, two-arm, double-blind, placebo-controlled, 2:1 randomly assigned, phase II clinical trial for individuals with recent-onset T1DM. The randomly assigned participants will receive either a 6-month course of imatinib or matching placebo tablets over a period of 26 weeks. Both treatment and placebo groups will undergo identical procedures and will be followed for 104 weeks. The primary endpoint, 2-hour C-peptide AUC measured at 52 weeks in response to a mixed-meal tolerance test (MMTT), will be measured at 52 weeks. Safety, diabetes control, ß-cell function, and immune function will be assessed for 104 weeks. Both groups will receive intensive diabetes management. During the follow-up phase, participants will undergo serial clinical and immunologic assessments over the 104 week study period, and possible mechanisms of imatinib action will be assessed.

Initial enrollment will be for subjects ages 18-45, with the goal to lower the age down to 12 upon acceptable safety review and prospect of benefit for this initial older cohort. When the first 10 enrollees have completed their week 26 assessment, the safety data will be reviewed by a subcommittee that includes the protocol chairs, the medical monitor, the clinical trial physician, and the DSMB. If the review concludes that significant safety concerns have been identified, then no further enrollment will occur pending further data review and evaluation by the DSMB. If the DSMB decides that the study may proceed then the FDA will be notified of study progress and determine if further age restriction is necessary, or if enrollment may be opened for subjects down to age 12. After the first 21 subjects have completed 6 months, a further safety review will occur for the DSMB. Additionally, an interim efficacy assessment will be conducted for the DSMB and FDA. The FDA will again be apprised of study progress, with further consideration for lowering the age of enrollees to 12, if this has not occurred earlier. If and when the age is lowered, an identical review procedure will be followed when the first 10 pediatric participants (ages 12-17) have completed their week 26 assessment. If the review is satisfactory, then enrollment will continue for subjects ages 12-45 until the study is fully enrolled.

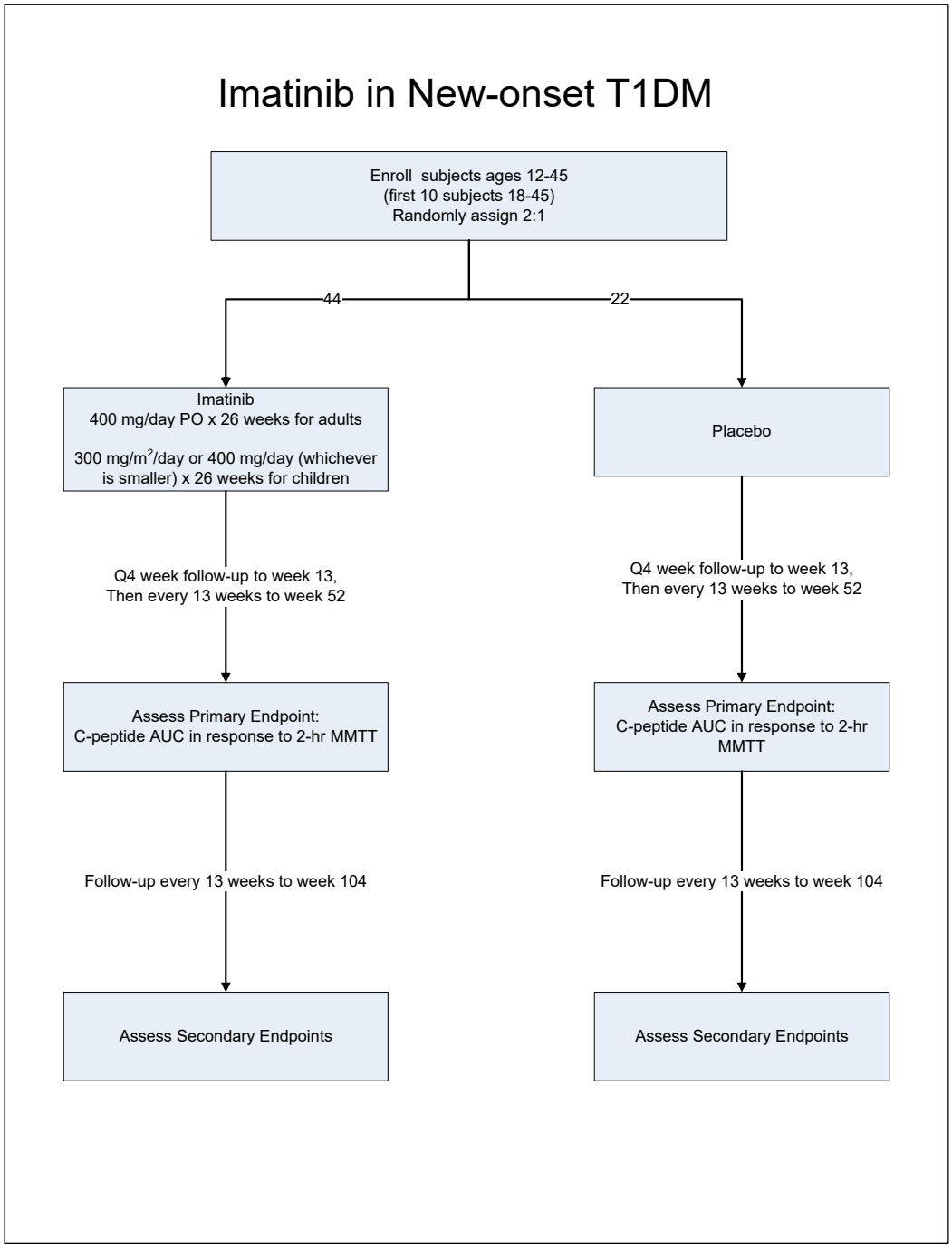

Figure 3. Study Schema

3.2 STUDY DURATION AND PACE OF ENROLLMENT

Total study duration is expected to be approximately 4 years. Recruitment is anticipated to be completed in 18 to 24 months. Individual study participation is 24 months.

3.3 STUDY ENDPOINTS

3.3.1 Primary Endpoint

The primary endpoint is an MMTT-stimulated 2 hour C-peptide AUC at week 52.

3.3.2 Secondary Endpoints

Efficacy:

1. MMTT-stimulated peak and 4 hour C-peptide AUC at weeks 52 and 104.
2. MMTT-stimulated 2 hour C-peptide AUC at week 104.
3. MMTT-stimulated 2-hour C-peptide AUC assessed longitudinally at weeks -2, 13, 26, 52, 78, and 104.
4. Insulin use in units per kilogram body weight per day at weeks 52 and 104.
5. Major hypoglycemic events, as defined in section 8.2.1, occurring from randomization at weeks 0, 52 and 104.
6. HbA1c levels at weeks 52 and 104.

Safety:

The rate of the following AEs in participants receiving imatinib or placebo:

1. Myelosuppression.
2. Gastrointestinal disorders.
3. Infections.
4. Hepatotoxicity.
5. Cardiac toxicity and edema.
6. Cutaneous reactions.
7. Muscle cramps, bone pain, arthralgias.
8. Fluid retention or peripheral edema.
9. Bone metabolism and growth abnormalities
10. Frequency and severity of all AEs in participants receiving imatinib or placebo.

3.3.3 Exploratory Endpoints

Mechanistic:

Immunological assessments described in section 7 will be compared with clinical outcomes to determine whether there is evidence of immune tolerance to diabetes-associated autoantigens.

Metabolic (see section 6.1.1 for further discussion):

1. Proportion of patients who are exogenous insulin-free (for at least 3 months) with an $HbA_{1C} \leq 6.5\%$ at weeks 52 and 104 in each treatment arm.
2. Proportion of subjects who achieve a persistent reduction (for at least 3 months) in insulin dose to < 0.5 units/kg at weeks 52 and 104 in each treatment arm.
3. Effects on insulin resistance will be evaluated using modeling from data obtained on MMTTs, and by assessing change in adiponectin, proinsulin levels (and proinsulin/c-peptide ratio), and glucagon.

3.4 RATIONALE FOR CLINICAL TRIAL DESIGN

The proposed trial will evaluate imatinib, a tyrosine kinase inhibitor, as a novel tolerance-inducing intervention in new-onset T1DM. As with ITN and TrialNet new-onset T1DM studies, endogenous insulin secretion will be assessed serially by measuring C-peptide AUC in response to a 4-hour MMTT. The proposed primary endpoint is a 2-hour C-peptide AUC in response to MMTT at week 52. This endpoint is in accord with an ADA workshop,[173] and the TrialNet consensus guidelines for new-onset T1DM studies. Other clinical outcome measures of efficacy include insulin use, HbA_{1C}, and major hypoglycemic events. The safety of patients in this study will be closely monitored, and expected adverse events related to imatinib have been selected as the secondary endpoints for safety (see section 3.3.2).

In accord with other ITN and TrialNet new-onset T1DM studies, we plan to randomly assign participants 2:1 (treatment vs. placebo). The greater chance of enrolling into a drug treatment arm has helped facilitate recruitment for such trials. Participants between ages 12 and 45 years will be recruited. T1DM can occur at any age, but it occurs with dramatically increased incidence in children. The average age of children at presentation of this disease is approximately 13 years, and most individuals present when they are under the age of 18 years. The incidence of T1DM is increasing at approximately 3% per year, mainly in the younger population.[165] Beta cell function declines faster in those < 21 years of age, relative to those > age 21 (Greenbaum et al, Diabetes) [178]. Many new-onset T1DM studies enroll up to age 45; the upper age limit is restricted in this study to45 so that the trial will enrich for a group of subjects who are more likely to have a faster rate of decline in beta cell function, and therefore enable a more pronounced difference between those in the treatment versus placebo group, assuming the therapy will have an effect. Imatinib is approved for the treatment of chronic myeloid leukemia in children down to age 3 years. The dosing is adjusted for

body surface area, and the safety spectrum appears to be comparable to that in adults. Additional justification for the inclusion of children is presented in section 1.5.2.

We will treat participants within 100 days of their T1DM diagnosis. Past studies have suggested that subjects respond better with this earlier enrollment ,[29,36] and currently all recent-onset T1DM immunotherapy trials conducted by TrialNet , ITN, and many pharmaceutical studies enroll patients within 100 days of diagnosis[36,38]. We have elected to have a 52-week primary endpoint for this trial, again in accord with ITN and TrialNet new-onset studies. It is important to consider whether the degree of C-peptide decline in the placebo group with optimal diabetes management is significant enough during the first year of diagnosis to detect a difference between the imatinib group and the placebo group. Steele et al. demonstrated that those with new-onset T1DM have steady, progressive loss in ß-cell function from the time of diagnosis, as assessed by C-peptide AUC in response to an MMTT, the measure to be employed in this study.[9] This finding has been corroborated by others in a series of new onset T1DM trials.[27,34,36-39,174,175]

The metabolic and immunological / tolerogenic effect of imatinib will continue to be assessed during the follow-up phase (months 12–24). Preservation of C-peptide levels at the end of the follow-up phase in the imatinib group will be an indication of whether imatinib induces tolerance. A host of immunologic assays performed throughout the study will also provide insight into the mechanism of imatinib action and its possible tolerogenic properties (Refer to section 7 for a complete discussion of immunologic assays.)

3.5 RATIONALE FOR SELECTION OF DRUG, ROUTE, DOSE, AND REGIMEN

There have been a series of studies to define dose-response relationships with imatinib, beginning with the early phase 1 studies in CML.[46,47,176] Doses between 200 to 300 mg/day appear to be on the steep part of the dose response curve. A dose that achieves concentrations of 1 μmol/L, which inhibits Bcr-Abl kinase activity, appears to be optimal for inducing apoptosis, and these are the trough levels achieved in phase I studies of subjects on 260 mg/day. Thus, 260 mg has been considered a threshold dose for inducing optimal therapeutic responses. To ensure that the majority of treated subjects were above this threshold, 400 mg/day was adopted as the dose for phase II studies of patients with chronic disease, and with a half-life of 13–16 hours, once daily dosing was considered adequate. This dosing regimen has proven to be highly successful in treating CML and GIST.[44-48] Doses higher than 400 mg/day may achieve better responses, particularly for those in accelerated and blast crisis studies. Doses of up to 1000 mg have been reached without convincing dose-limiting toxicity but, as noted above, the frequency and grade of adverse events increases, particularly above 600 mg/day.

The responses in autoimmunity noted above were achieved in subjects receiving at least 400 mg/day, and sometimes higher imatinib doses. Thus, 400 mg/day appears to be an effective dose in autoimmune diseases.

It is difficult to extrapolate exactly from the mouse dose employed by Louvet et al[112] to humans. They were successful with doses of 50 mg and 100 mg/kg and 1.5 to 3 mg/mouse, and ultimately used 1.5 mg/mouse. Druker considers this dose roughly comparable to 400 mg/day used in clinical studies (personal communication).

A common question with all studies is whether or not dose should be adjusted for a participant's size. Peng et al conducted a study to determine whether obesity had an effect on plasma levels of imatinib, and concluded that there was no evidence that patient size needed to be taken into consideration.[177] Deininger et al also reported that flat dosing was appropriate in a small number of obese subjects.[129] Considerations for children were reviewed in section 1.5.2, and we will follow the standard dosing of 260 mg/m^2 or 400 mg, whichever is lower, stated in the Gleevec package insert.

One additional consideration with regard to dosing is how long to continue drug therapy. Longer-term treatment (10 weeks versus 3 weeks) clearly resulted in a more robust and durable response in the NOD mouse. Based on this, we propose a 6-month treatment period—6 months strikes the appropriate balance between too short a period, with loss of long-term efficacy, and longer treatment (such as 12 months) with ongoing risk from the therapy. For its approved indications, Gleevec therapy is typically administered for years and even lifelong in some patients, but in this trial we will be assessing its role as a tolerizing agent after 6 months of therapy.

3.6 MEASURES TO MINIMIZE BIAS

The proposed trial has a double-blind design. One salient issue is whether or not the side effect profile will be such that it will serve to unmask a significant number of participants and study personnel. We expect that the side effects will be milder and less frequent than seen in oncology settings, as we will be enrolling a younger and healthier patient population who will be treated at the lowest therapeutic imatinib dose (B. Druker, personal communication). Both the treatment and placebo groups will require close, regular monitoring, particularly during the treatment phase. Because endogenous insulin secretion is the primary outcome variable (i.e., C-peptide), and this in turn is affected by glycemic control, we will aim for comparable glycemic control in both groups, treating to established targets. The health care professionals managing T1DM will be blinded to treatment assignment.

3.7 STOPPING RULES

3.7.1 Ongoing Review

The progress of the study will be monitored by a Data and Safety Monitoring Board (DSMB), which will review safety data and make recommendations regarding

continuation, termination, or modification of the study. Based on an 18- to 24-month recruitment period and an additional study period of 24 months, the DSMB will formally review the safety data at least yearly. The number of subjects who discontinue study treatment will also be included in the reports prepared for the DSMB.

In addition, safety data will be reviewed by the DSMB when an event occurs that is of sufficient concern to the medical monitor, clinical trial physician, or protocol chairs to warrant review, or when an event occurs that contributes to a stopping rule listed in Section 5.3.

3.7.2 Stopping Rule Guidance

3.7.2.1 Study-related adverse events

If any of the following events occur, enrollment will be suspended and the DSMB chair will be notified such that a review of safety data will be conducted to determine if enrollment in the study will be stopped and/or administration of investigational study medication should be halted:

- Any death except those assessed as not related to study treatment on review by the protocol chairs, the clinical trial physician, and the medical monitor.
- One or more participants experience treatment-emergent, clinically significant cardiac toxicity.
- Two or more of the first 10 treated participants or ≥20% of all treated participants experience a clinically significant, drug-related adverse event resulting in the permanent discontinuation of study treatment, as defined in section 5.3.
- If two or more of the first 10 treated growing-age participants, as defined in section 5.2.9, or greater than 20% of all treated growing-age participants, exhibit a decrease in Height Standard Deviation Score (Ht-SDS) of > 0.5 from baseline at 6 months, or > 0.8 from baseline at 12 months, then enrollment will pause for growing-age participants. Study enrollment will continue for participants who are not growing-age. DSMB review will determine whether imatinib treatment can resume in growing-age participants based on available growth and bone-metabolism data, as well as bone-age radiographs.

4. ELIGIBILITY

4.1 INCLUSION CRITERIA

Patents must meet *all* of the following criteria:

1. Males and females age 12–45 years of age who meet the ADA standard T1DM criteria.
2. Positive for at least one islet cell autoantibody (glutamate decarboxylase; insulin, if obtained within 10 days of the onset of insulin therapy; ICA 512-antibody; and/or ICA or ZnT8).
3. Diagnosis of T1DM within 100 days of Visit 0.
4. Peak stimulated C-peptide level >0.2 pmol/mL following an MMTT.
5. Participants of childbearing age who are sexually active must agree to use an effective form of birth control (e.g., barrier method, oral contraception, or surgery). For females, these contraceptive measures must be maintained throughout the study; for males these measures must be followed for a minimum of 3 months after discontinuation of imatinib therapy.

4.2 EXCLUSION CRITERIA

Patients must *not* meet any of the following criteria:

1. Prior history of any significant cardiac disease such as congestive heart failure, myocardial infarction, arrhythmia, or structural defects or suspicion thereof.
2. Leukopenia (<3,000 leukocytes/µL), neutropenia (<1,500 neutrophils/µL), or thrombocytopenia (<125,000 platelets/µL).
3. Low Hemoglobin (baseline hemoglobin below the lower limit of normal)
4. Prior history of anaphylaxis, angioedema or serious cutaneous drug reactions
5. Any sign of significant chronic active infection (e.g., hepatitis, tuberculosis, EBV, CMV, or toxoplasmosis), or screening laboratory evidence consistent with a significant chronic active infection (such as positive for HIV, PPD/IGRA, or HB_SAg). Significant acute infections must be resolved before treatment may commence, e.g., acute respiratory tract, urinary tract, or gastrointestinal tract infections.
6. Anticipated ongoing use of diabetes medications other than insulin that affect glucose homeostasis, such as metformin, sulfonylureas, thiazolidinediones, glucagon-like peptide 1 (GLP-1) mimetics, dipeptidyl peptidase IV (DPP-IV) inhibitors, or amylin.
7. Prior or current treatment that is known to cause a significant, ongoing change in the course of T1DM or immunologic status, including high-dose inhaled, extensive topical or systemic glucocorticoids.

8. Evidence of liver dysfunction, with alanine aminotransferase (ALT) or aspartate aminotransferase (AST) >2.0 times the upper limit of normal persistent for 1 week or greater.
9. Evidence of renal insufficiency as indicated by serum creatinine of >1.2 times the upper limit of normal confirmed in a repeat test at least 1 week apart.
10. Evidence of clinically significant metabolic bone disease (except adequately treated rickets).
11. Females who are pregnant at the time of screening, breastfeeding or unwilling to defer pregnancy during the 24-month study period. (Female participant must be at least 100 days postpartum before enrollment into study).
12. Prior treatment with imatinib or related tyrosine kinase inhibitor.
13. Unable to avoid medications that affect CYP3A4: either inducers that may decrease imatinib levels, or inhibitors that may increase drug concentrations. (Refer to section 1.5.1.12 for a complete list of inducers and inhibitors.)
14. Height standard deviation score ≥2 standard deviations below mean (participants of growing-age potential)
15. Any sign of QT prolongation on Visit -1 ECG (> 450 ms in males and > 470 ms in females).
16. Known coagulation disorders or use of anticoagulants
17. Current and anticipated on-going treatment with drugs that may increase or decrease imatinib plasma concentrations (CYP3A4 family inhibitors or inducers) or drugs that may have their plasma concentration altered by imatinib (drugs metabolized by CYP3A4/5 and CYP2D6).
18. Any condition that, in the investigator's opinion, may compromise study participation or may confound the interpretation of the study results.

4.3 PREMATURE TERMINATION OF A PARTICIPANT FROM THE STUDY

Prematurely terminated participants will be asked to remain in the study and participate in follow-up. If study treatment is discontinued, the medical monitor should be notified. Participants who prematurely terminate from the study will not be replaced.

Withdrawal of consent. Participants who withdraw consent will be asked to participate in follow-up. If the participant does not consent to follow-up visits, they should complete an end-of-study visit, which will include all the assessments listed for Visit 11 in Appendix 1.

Failure to return. Participants who do not return for visits and who do not respond to repeated attempts by the site staff to have them return will be considered *lost to follow-up*.

Investigator judgment. A severe or serious AE occurs, which, based on the medical judgment of the investigator, prevents completion of participation in the study.

5. STUDY MEDICATIONS

5.1 INVESTIGATIONAL MEDICATION

5.1.1 Formulation and Packaging

Imatinib mesylate (Gleevec; Novartis, East Hanover, NJ) is produced as film-coated tablets, equivalent to 100 mg of imatinib free base. The placebo group will receive an identical pill that is manufactured and distributed by a designated drug distributor. If necessary to prevent unblinding, the distributor may need to overencapsulate the imatinib tablets.

5.1.2 Dosage, Preparation, and Administration

Adult participants will receive four 100 mg tablets of imatinib or the corresponding placebo tablets daily for the first 6 months. Pediatric participants will receive 260 mg/m^2/day (rounded up or to the closest 100 mg) or 400 mg/day (whichever is smaller) of active drug or placebo.

5.1.3 Recommended Storage Conditions

- Store at 25°C (77°F); excursions permitted to 15–30°C (59–86°F).
- Protect from moisture.
- Dispense in a tight container, USP.

5.2 DOSE MODIFICATION AND MANAGEMENT OF ADVERSE EVENTS

With the use of a relatively low dose of imatinib in an otherwise healthy population, we hope to minimize the nature and extent of AEs experienced. It is important to note here, as in section 1.4, that the AEs referenced below, if observed, will usually occur early in the course of treatment, are often self-limited, and do not usually require dose adjustment or discontinuation of imatinib. Nonetheless, the investigative team recognizes that cancer treatment is clearly different than the proposed elective clinical trial: side effects considered acceptable and tolerable in a cancer setting may not be considered in the same light for subjects in this study. Every effort will be made to carefully balance the intent to provide the maximum total prescribed dose of study drug to subjects, versus the discomfort and risk that may be accompanied with imatinib administration. The “art” of subject management in this study will be to minimize the number of subjects that drop out of the trial due to these side effects, working in close concert with the investigative team to either adjust study drug dose and/or provide symptomatic relief with concomitant medications. General guidelines are outlined below, but may need to be modified as deemed necessary, in consultation with the trial physicians and medical monitor. In general, when a dose reduction is required, the daily dose of imatinib or placebo will be halved: For adults, the dose will be reduced from

400 mg/day to 200 mg/day. Unless otherwise stated, the equivalent dose reduction in children will be from 260 mg/m^2/day to 130 mg/m^2/day. Investigators will be instructed to always round up to the nearest 50 mg increment for the half-dose dosage.

5.2.1 Gastrointestinal Reactions

Nausea. To minimize issues with nausea, participants will be advised to take study medication with the largest meal of the day. If the problem persists, they may need to split the study medication into twice daily dosing.

For recurrent, persistent vomiting, grades 1 and 2, lasting for 48 hours, investigators will consider the use of anti-emetics such as prochloropherazine or ondansetron. Subjects usually respond promptly, and dose reduction is seldom needed.

For grade 2 vomiting that persists for 48 hours despite the use of antiemetic drugs, imatinib will be reduced to 50% of the initial daily dose. If vomiting resolves over the ensuing week, then the full dose will be restored. If vomiting recurs, then the subject will need to remain on the 50% tolerated lower dose, and re-evaluated weekly, with return to full dose reconsidered.

For vomiting that is grade 3, or grade 2 that does not improve with anti-emetics and dose reduction for 48 hours,, imatinib will be suspended until the problem is resolved. Electrolytes should be obtained. Imatinib will then be reintroduced at 50% of the initial daily dose. If vomiting does not recur over the ensuing week, the initial dose will be restored. If vomiting of grade 3 recurs, then the drug will be permanently discontinued. If nausea or vomiting resolve and then occur again at a later time, the same procedures may be followed as outlined above, depending on the grade.

Diarrhea. If it occurs, diarrhea is often mild and requires no specific therapy. For persistent diarrhea (1 week or greater) of grades 1 and 2 the participant may be treated with antidiarrheal and antispasmotic drugs, such as Imodium®, Lomotil®, or paregoric, at the discretion of the investigator.

For grade 2 diarrhea that persists for 1 week despite the use of antidiarrheal drugs, imatinib will be reduced to 50% of the initial dose. If diarrhea improves to less than or equal to grade 1 for at least 1 week, the initial dose will be restored. If diarrhea persists despite use of antidiarrheal drugs and dose reduction, permanent discontinuation of imatinib will be considered.

For diarrhea of grade 3 or higher, imatinib will be suspended until the problem is resolved. Electrolytes should be obtained. Imatinib will then be reintroduced at 50% of the initial dose for 1 week. If diarrhea does not recur, the initial dose will be restored. If diarrhea greater than grade 2 recurs, imatinib will be permanently discontinued. If diarrhea resolves and then occurs again at a later time, the same procedure may be followed.

Chronic diarrhea, of 14 or more days in duration, should be evaluated to determine if there is an infectious cause. Evaluation will be conducted in consultation with local infectious disease consultants, and will include a thorough clinical and epidemiological evaluation, including travel history and any possible exposures. Stool culture and other evaluation may be required, and anti-microbial therapy offered as indicated. Other potential causes of diarrhea, such as lactase deficiency, should be considered.

5.2.2 Muscle Cramps, Bone Pain, Arthralgias

Mild to moderate cramps may respond to calcium and magnesium supplementation, and some have responded to quinine. Mild to moderate bone pain and arthralgia can usually be relieved with nonsteroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen and naproxen.

For grade 2 muscle cramps, bone pain or arthralgia that persist for 1 week despite the use of symptom-relieving drugs, the dose of imatinib will be reduced to 50% of the initial daily dose. If symptoms improve to less than or equal to grade 1 for at least 1 week, the initial dose will be restored. If symptoms persist despite symptomatic treatment and dose reduction, permanent discontinuation of imatinib will be considered.

For grade 3 muscle cramps, bone pain or arthralgia, imatinib will be suspended until the problem is resolved. Imatinib will then be reintroduced at 50% of the initial dose for 1 week. If symptoms do not recur, the initial dose will be restored. If symptoms greater than grade 2 recur, imatinib will be permanently discontinued.

5.2.3 Edema

Edema associated with imatinib is usually mild and self-limited, and often does not require therapy. Affected participants may consider a reduction in salt intake. Patients with periorbital edema may benefit from topical 0.25% phenylephrine or 1% hydrocortisone.

Localized edema. Grade 2 localized edema, including periorbital edema and limb edema, that persists for longer than 1 week may be treated with diuretics. For grade 2 edema that persists despite the measures described above, the dose of imatinib will be reduced to 50% of the initial daily dose. If edema improves to less than or equal to grade 1 for at least 1 week, the initial dose will be restored. If edema persists despite reductions in salt intake, use of diuretics, and imatinib dose reduction, permanent discontinuation of imatinib will be considered.

For edema of grade 3 or higher, imatinib will be suspended until the problem is resolved. Imatinib will then be reintroduced, with ongoing diuretic use, at 50% of the initial daily dose for 1 week. If edema does not recur, the initial dose will be restored. If edema greater than grade 2 recurs, imatinib will be permanently discontinued. If localized edema resolves and then occurs again at a later time, the same procedure may be followed.

Generalized fluid retention. Participants will be monitored for signs and symptoms of generalized fluid retention (including pulmonary and cardiac issues), and will be weighed regularly at each study visit to detect the early onset of fluid retention. If sudden increases in weight occur, participants should be examined for signs of pulmonary edema, pleural effusion, pericardial effusion, and ascites. If any signs of generalized fluid retention are found, imatinib should be suspended and diuretic therapy started. Electrolytes should also be obtained if greater than grade 2. If these measures lead to a prompt resolution of fluid retention, imatinib may be reintroduced at 50% of the initial daily dose. If fluid retention does not recur, the initial dose may be restored. If generalized fluid retention recurs, imatinib will be permanently discontinued.

5.2.4 Cutaneous Reactions

Rash. Investigators should be prepared for rashes early in the course of therapy, when they are most likely to occur. These are usually grades 1 or 2 and are self-limited. Symptomatic treatment with antihistamines, salves, and coal tar preparations has been helpful.

For grade 2 rash that persists for 1 week despite the measures described above, the dose of imatinib will be reduced to 50% of the initial daily dose. If the rash improves to less than or equal to grade 1 for at least 1 week, the initial dose will be restored. If the grade 2 rash persists despite symptomatic treatment and imatinib dose reduction, permanent discontinuation of imatinib will be considered. If grade 2 rash resolves and then occurs again at a later time, the same procedure may be followed.

For a rash of grade 3 or higher, imatinib will be suspended until the problem is resolved. Imatinib will then be reintroduced at 50% of the initial dose for 1 week. If the rash does not recur, the initial dose will be restored. If a rash greater than grade 2 recurs, imatinib will be permanently discontinued.

Bullous dermatologic reactions. Rare cases of bullous dermatologic reactions, including erythema multiforme and Stevens-Johnson syndrome, have been reported with imatinib treatment.

In cases of less than or equal to grade 1 erythema multiforme, imatinib will be suspended. If the condition resolves promptly, reintroduction of imatinib may be considered, starting at the lowest dose (100 mg/day) with gradual dose escalation, if tolerated. If the condition recurs at any grade, imatinib will be permanently discontinued.

If a participant presents with Stevens-Johnson syndrome (any grade), toxic epidermal necrolysis (any grade), or > grade 1 erythema multiforme, imatinib will be permanently discontinued and supportive therapy, including systemic glucocorticoids, started immediately.

5.2.5 Hepatotoxicity

In general, guidelines established for imatinib use in CML and GIST will be followed,[129,130] but a more conservative approach will be used, as follows. Liver function tests (LFTs) will be obtained every other week during the first month of therapy, and monthly thereafter.

For grade-2 transaminase (ALT or AST > 3.0 × ULN) or bilirubin (> 1.5 × ULN) elevations, imatinib must be suspended, and the LFTs monitored weekly. When they have fallen to grade 1 or normalized, imatinib will be restarted at 50% of the initial daily dose. If the liver toxicity does not recur within 6–12 weeks, then the initial dose will be restored. If after resumption of treatment grade 2 transaminase or bilirubin elevations recur, study drug will be permanently discontinued.

For grade-1 abnormalities (ALT or AST greater than or equal to 1.0–3.0 × ULN; bilirubin greater than or equal to1.0-1.5 × ULN), imatinib treatment can be continued but LFTs will be monitored weekly for 2 weeks and then, if no worsening is observed, every other week. Concomitant hepatotoxic agents, such as alcohol or acetaminophen, should be removed. Persisting grade 1 abnormalities should be managed by dose reduction, biweekly monitoring, and further evaluation in conjunction with gastroenterology consultation.

5.2.6 Myelosuppression

In general, recommendations will be a conservative algorithm, derived from Deininger et al. for the chronic phase of CML will be followed.[129] For grade 1 neutropenia (absolute neutrophil count [ANC] <LLN-1500/mm^3) and thrombocytopenia (<LLN-75,000/ mm^3), the dose of imatinib will be reduced to 50% of the initial dose. Counts will be monitored bi-weekly. The initial dose will be restored when myelosuppression resolves.

For grade 2 or greater events (ANC less than 1,500/mm^3, or platelets less than 50,000/mm^3) imatinib should be suspended and the counts will be repeated weekly until they have recovered (ANC greater than 1,500/mm^3, platelets greater than 75,000/mm^3). For grade 1 neutrophil or platelet levels, imatinib will be restarted at 50% of the original dose, and the counts will be monitored biweekly; imatinib will be restored to the full dose when the counts normalize. If myelosuppression resolves and then occurs again at a later time, the same procedure may be followed.

5.2.7 Infectious Disease Risk

In general, higher risk for infections has not been a concern during the clinical use of imatinib. Nevertheless, all participants will undergo careful surveillance during the initial phase of the study. Baseline analyses will determine whether the participant has had a primary infection with EBV, CMV, or varicella. At each visit, participants will be assessed for signs and symptoms of new or reactivating infections. If there are concerns, including pyrexia of unknown origin, participants will be evaluated for

opportunistic infections, including PCR monitoring for EBV and CMV. If a diagnosis of a new or reactivating infection that is clinically significant is confirmed, imatinib treatment will be discontinued until successful treatment and resolution of the infection. In conjunction with an infectious disease consultation, reintroduction of imatinib will be considered.

5.2.8 Pregnancy

Site staff will regularly review the concerns regarding avoidance of pregnancy with participants during the course of the study, especially during the initial 6 months on therapy. Urine pregnancy tests will be conducted at baseline and every following visit while on study drug. Male participants are to use birth control at least 3 months after study drug discontinuation. If, despite these measures, a participant becomes pregnant, imatinib will be permanently discontinued and the participant will be counseled on potential risks to the fetus. Pregnancies of partners of male participants will also be followed.

Given the potential risk to the fetus, while on study drug/placebo, women of child-bearing potential, defined as all women physiologically capable of becoming pregnant, must agree to use highly effective methods of contraception.. This contraception should start1 week or more prior to the start of study drug/placebo (noting that the terminal half-life of imatinib is approximately 18 hours, and 5 times this duration is 90 hours or ~4 days)) and continue during the subsequent 6 months of study drug/placebo dosing ***Highly effective contraception methods include:***

- Total abstinence (when this is in line with the preferred and usual lifestyle of the subject). Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception
- Female sterilization (have had surgical bilateral oophorectomy with or without hysterectomy) or tubal ligation at least six weeks before taking study treatment. In_case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow up hormone level assessment
- Male sterilization (at least 6 months prior to screening). For female subjects on the study the vasectomized male partner should be the sole partner for that subject
- Combination of any two of the following (a+b or a+c, or b+c):
    a. Use of oral, injected or implanted hormonal methods of contraception or other forms of hormonal contraception that have comparable efficacy (failure rate <1%), for example hormone vaginal ring or transdermal hormone contraception
    b. Placement of an intrauterine device (IUD) or intrauterine system (IUS)

c. Barrier methods of contraception: Condom or Occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/vaginal suppository

In case of use of oral contraception women should have been stable on the same pill for a minimum of 3 months before taking study treatment.

5.2.9 Bone and Mineral Metabolism

Small clinical studies in oncology have identified effects on bone metabolism. Further, in a pilot animal study there is a possible effect of imatinib on growth plate architecture, and in a few case reports and a retrospective analysis of children with CML there is a possible effect of therapy on growth rate (see section 1.5.1.11). Therefore, biochemical markers of bone metabolism will be measured in all subjects. At Visit 0, all subjects less than 18 years of age will have their bone age assessed using the Fels method (plain film) by a central reader who is an expert in skeletal maturation in order to determine their bone age and growth potential. Female subjects with a bone age of less than 14 years and male subjects with a bone age less than 16 years will be considered to have significant remaining growth potential at study entry and referred to in this protocol as a "growing-age participant". Growth assessments will be performed according to the Schedule of Events.

Growing-age participants will have their Ht-SDS determined via standard reference charts published by CDC (2001). Additional factors that commonly affect growth will be evaluated at regular intervals, to distinguish the potential contribution from these issues versus those that may be attributed to study drug.

Phosphorous and vitamin D supplementation may be needed if any deficiencies are noted, as suggested by Berman et al.[152] Given the relatively short-term course of therapy, we do not expect any long-term problems with bone metabolism.

5.2.10 QT Prolongation

All participants will have electrocardiograms (ECGs) performed at Visit -1 (screening) and Visit 1. Participants will not be eligible for the study if QTc is > 450 ms in males or >470 ms in females at Visit -1, If QTc is >480 ms or there is > 60 ms change from the Visit -1 ECG, then study drug will be discontinued. Participants experiencing dizziness or syncope greater than a grade 2 should promptly seek medical attention and obtain an ECG and electrolytes.

5.3 DISCONTINUATION OF STUDY MEDICATION IN AN INDIVIDUAL PARTICIPANT

Study medication according to study specifications will be discontinued for an individual participant if *any* of the following occurs:

- A discontinuation criterion for imatinib, as noted in section 5.2, is met.
- Clinical evidence of cardiac toxicity, such as congestive heart failure or significant cardiac arrhythmias, is observed.
- Pregnancy occurs.
- A severe or serious AE occurs, which, based on the medical judgment of the investigator, prevents a participant from completing the study treatment (see section 8 for classification of AEs).
- The investigator determines that it is in the participant's best interest to discontinue treatment.
- The participant, or participant's legal representative, requests that treatment be halted.

Further care will be provided according to the judgment and practice of the investigator.

The participant will be asked to remain in the study and participate in follow-up. If study treatment is discontinued, the medical monitor should be notified.

5.4 CONCOMITANT MEDICATIONS

Any participants who need to start a concomitant medication, for whatever reason, should contact the study team to review its potential effects on imatinib metabolism. Those who require an emergency therapy will be encouraged to pursue the necessary clinical care, but then contact the study team within 24 hours to review the potential effects on imatinib metabolism. Whenever possible, the recommendation will be to use concomitant medications that do not alter imatinib levels.

5.4.1 Prohibited Medications

Use of cytochrome P450 inducers/inhibitors, unless considered essential by the investigator, are prohibited during the treatment phase. Imatinib is metabolized by the CYP3A4/5 cytochrome P450 enzyme system, and drugs that induce or inhibit this enzyme may alter imatinib levels (see section 1.5.1.12 for a list of inducers and inhibitors).

5.5 DRUG ACCOUNTABILITY

Under federal regulations (21CFR 312.62) an investigator is required to maintain adequate records of the disposition of the investigational product, including the date and quantity of drug that was received, the participants to whom drug was dispensed

(participant by participant accounting), and an account of any drug accidentally or deliberately destroyed. The investigator will ensure that the investigational product supplies are stored as specified in the protocol and pharmacy manual in a secured area, with access limited to authorized study personnel as described in the clinical study agreement.

Records for receipt, storage, use, and disposition of the study drug will be maintained by the study sites. A drug-dispensing log will be kept current for each participant and will contain the identification of each participant and the date and quantity of drug dispensed. All remaining unused investigational product will be returned to the sponsor or sponsor's representative after study termination, or destroyed with the permission of the sponsor in accordance with applicable law and study site procedures. If investigational product is to be destroyed locally, the investigator will provide documentation in accordance with sponsor's specifications.

All records regarding disposition of the investigational product will be available for inspection by the clinical trial monitor.

5.6 ASSESSMENT OF COMPLIANCE WITH STUDY MEDICATION

Pill counts will be used to assess participant compliance with daily doses of the study medication.

6. STUDY PROCEDURES

6.1 INTENSIVE DIABETES MANAGEMENT

During the study, all participants will receive "intensive" management of their diabetes, and HbA_{1C} will be assessed every 3 months to evaluate metabolic control. The goal of treatment will be to maintain the HbA_{1C} level as close to normal as possible, without frequent occurrence of hypoglycemia. All individuals should strive for targets in accordance with current ADA recommendations, with HbA_{1C} levels of less than 7% in adults and less than 7.5% in adolescents (age 12–17 years), and with preprandial glucose levels of 90–130 mg/dL (plasma), postprandial levels of less than 180 mg/dL, and bedtime levels of 110–150 mg/dL.[178] All participants will be expected to take a sufficient number of daily insulin injections to meet the glycemic targets. In general, the expectation is that all participants will receive at least three injections of insulin daily, including short- and long-acting insulin preparations, or will utilize continuous subcutaneous insulin infusion (CSII insulin pump). Glucose levels should be checked at least four times daily. After reviewing these records, the diabetes management team will contact the treating physician about possible adjustments in the insulin regimen, referral to a registered dietitian, or other approaches that the diabetes management team believes would improve the glucose control if necessary. Records of glucose measurements and communication with the participant will be kept as source documentation. Participants will be contacted by the diabetes educator every 2 weeks

between visits to assess their diabetes. In addition, insulin use and hypoglycemic events will be captured at each visit on the appropriate CRFs. Participants will be required to record the amount of insulin they have used during the 5-day period immediately preceding each study visit. Insulin use logs will be provided to participants at each study visit and collected at the next visit. These logs will serve as the source documents.

6.2 RANDOM ASSIGNMENT, BLINDING, AND UNBLINDING

6.2.1 Random Assignment

Participants who sign the informed consent and meet the eligibility criteria will be randomly assigned in a 2:1 ratio to either the experimental or control group. A central automated randomization system will be used for treatment assignment and to create a unique identifier for each new study participant. Random assignment will be stratified according to site.

6.2.2 Blinding

Blinding will be maintained throughout the study for all study participants and study personnel, except the pharmacists.

6.2.3 Unblinding

Unblinding before the study is completed will occur only if a participant's well-being is threatened and the investigator believes unblinding is necessary to protect the participant.

Before treatment assignment for an individual participant is unblinded, the investigator must confer with the study sponsor and the medical monitor. The site investigator will notify the protocol chairs of the unblinding event, and the medical monitor will notify the study management team (SMT).

The emergency unblinding will be recorded and reported to the DSMB. A full account of the event will be recorded, including the date and time of the emergency, the reason for the decision to unblind, and the names of the medical monitor and others who were notified of the emergency. During site visits, the site monitor must verify that the medical monitor was notified and that a written account was completed. The reasons for unblinding of a participant's treatment will be included in the final study report.

Sponsor approval is required for unblinding the treatment of an individual participant or subgroups of participants for unplanned interim analyses to support DSMB reviews and final analysis.

6.3 VISIT WINDOWS

6.3.1 Scheduled Visits

All scheduled study visits in Appendix 1 must occur within the time limits specified below. Visits that occur outside of the specified windows will be considered protocol deviations.

- **Visit -1** **Up to** 2-4 weeks before visit 0
- **Visit 0** No window
- **Visit 1** (± 3 days)
- **Visits 2-7** (± 7 days)
- **Visits 8-11** (± 14 days)

6.4 GENERAL ASSESSMENTS

- Medical history includes T1DM time of diagnosis and clinically significant diseases or medical procedures. Copies of growth velocity charts should be obtained if available
- Adverse events. Participants will be assessed for AEs
- Concomitant medications. All concomitant medications and their indications will be recorded
- Comprehensive physical examination and vital signs. Weight, temperature, blood pressure, respiration, and pulse

6.5 LABORATORY ASSESSMENTS

- Serum chemistries: Electrolytes (sodium, calcium, potassium, chloride, phosphate, total CO_2), blood urea nitrogen (BUN), creatinine, and liver panel (AST, ALT, alkaline phosphate, direct and total bilirubin)
- Hematology: Complete blood cell count with differential, and platelets
- Infectious disease serology, hepatitis B (HBsAg and anti-HBcAb) and C, HIV, toxoplasmosis, VZV, EBV (IgG, IgM, EBNA), CMV (IgG, IgM)
- PPD skin test or IGRA
- Urine hCG
- Urinalysis
- C-peptide levels (measured as part of MMTT)
- Plasma Glucose (measured as part of MMTT)
- HbA_{1c} levels

6.6 DISEASE-SPECIFIC ASSESSMENTS

- MMTT
- Glucose (glucometer readings)
- Insulin use (U/kg body weight/day)
- Hypoglycemia events
- Serum Adiponectin levels
- Plasma Glucagon levels
- Plasma Proinsulin levels

6.7 BONE AND MINERAL METABOLISM ASSESSMENTS

Serum calcium, serum phosphate, PTH, 25-OH Vitamin D and serum bone turnover markers (serum CTX, osteocalcin). Bone turnover markers are exploratory only and are not part of the secondary endpoint safety assessment, per section 3.3.2.

6.8 GROWTH RATE ASSESSMENTS FOR GROWING-AGE PARTICIPANTS (AS DEFINED IN SECTION 5.2.9)

- Plain radiograph of left hand to determine bone age using the FELS atlas (Assessing the skeletal maturity of the hand-wrist).[179]
- Height measured by Stadiometer.
- Secondary sexual characteristics.
- Arm span.
- Biochemical assessments. IGF-1, IGF-BP3, LH, FSH, estradiol (females), testosterone (males), TSH, and tissue transglutaminase.

7. MECHANISTIC ASSAYS

The study team will partner with the ITN for mechanistic studies, taking full advantage of their systems for sample collection and archiving (see letter from Nepom). Following results of the study, various immunological assays will be considered through the available ITN cores, and beyond. We recognize that this field is rapidly evolving, and thus novel assays that are particularly relevant to this study population, and to this drug, will be considered. Our mechanistic assays plan is outlined below.

7.1 RATIONALE

The rationale for treating T1DM patients with imatinib stems from results in animal studies where imatinib therapy led not only to reversal of T1DM but also to reversal of the loss of tolerance, a process ultimately responsible for the pathology of the disease.

Treatment with imatinib potentially leads to a change from a pro-inflammatory to an anti-inflammatory state. In the NOD mouse model it was shown that both adaptive immune and inflammatory mechanisms directly trigger insulitis, insulin resistance, faulty insulin signaling and islet cell destruction, all leading to the loss of tolerance to islets.

It is anticipated that drug treatment is likely to affect T cells, B cells and mast cells directly, resulting in decreased numbers and function of islet antigen-specific T cells and perhaps changes in autoantibodies. In T1DM, there is a growing belief that generalized inflammation and adaptive immunity are major contributing factors to the induction and progression of this autoimmune process. Thus, a decrease in these cells or their function might help to eliminate activated autoreactive T cells resulting in tolerance to islet antigens in T1DM patients. In addition, we hypothesize that imatinib alters PDGF signaling that alters macrophage differentiation, affecting the balance of pathogenic versus alternatively activated macrophages and inflamed endothelial cells altering cell trafficking.

The goal of the mechanistic studies will be to determine if the biological effects of imatinib correlate with therapeutic efficacy in T1DM patients. The proposed immunologic mechanistic studies are designed to determine how imatinib therapy may arrest ongoing autoimmune pancreatic β-cell destruction and preserve insulin secretion in patients with T1DM. These will be conducted in the existing Immune Tolerance Network core laboratories. We recognize that this field is rapidly evolving, and thus novel assays that are particularly relevant to this population and this agent may be incorporated into the trial at a later date. Whenever possible, samples will be frozen and archived for batch analysis at a later date. Finally, since this trial has a safety component, emphasis will be placed on the study of immune responses to pathogens and overall immune competence.

7.2 RETENTION OF SAMPLES

Biological specimens collected in this trial may be used to re-evaluate biologic responses as new research tools become available. The specimens will be stored at the ITN sample repository until the end of the ITN contract. Residual specimens may be used by the investigators for development of new immunologic assays or for cross-trial comparisons. Specimens for mechanistic studies will be obtained throughout the study. While specimens are described in this protocol in the context of assays to be performed, it should be noted that not necessarily all assays will be performed for all participants at each time point. Decisions to perform assays will be made according to statistical and scientific planning, questions being asked, and current technologies to be utilized. Finally, clinical outcomes will be taken into account to determine the potential value of the assays. For example, if a clinical effect fails to occur, the assays performed by the ITN may be minimal.

7.3 FROZEN PBMCS

Peripheral blood will be collected at the sites, processed at an ITN core laboratory, and frozen cells stored in a central ITN repository.

7.3.1 Functional Cell-Based Assays

The functional status of lymphocyte subsets, specifically T/ B cells, mast cells and monocytes, can be assessed in various cell-based assays. Inflammatory and TCR-driven autoantigen-specific stimulations need to be examined to fully evaluate the effect of imatinib on immunological responses. To address the participants' overall inflammatory response potential during imatinib treatment, PBMCs or whole blood can be stimulated with anti-CD3 plus anti-CD28 and/or lipopolysaccharide (LPS) to determine the array of secreted cytokines and chemokines made by T and B cells, respectively. Since inflammatory cytokines direct the commitment of antigen-activated CD4 T cells to specific effector or FoxP3 regulatory cells, the overall pattern of secreted cytokines might indicate a preference towards specific CD4 T-cell commitment. T-cell assessments will include those measuring both the number and function of T cells. These measures may include the use of class I and class II tetramers to enumerate antigen-specific T cells. Additionally, *in vitro* cell culture techniques may be utilized to study T cell functionality. Readouts will include ELISPOT and intracellular staining technology for cytokine determination. Additional studies may be done to study regulatory T cells in these treated participants. Both carboxyfluorescein diacetate succinimidyl ester (CSFE) and ^{3}[H]-thymidine assays could be employed depending on cell numbers. Harvested blood cells will be separated by flow-based sorting using CD4, CD25 and CD127 antibodies and assayed for their ability to suppress polyclonal T cell responses. Follow-up experiments can include examination of Treg cell function as well as Foxp3 methylation. Several assays can address responses to autoantigens implicated in T1DM. Secretion of various cytokines can be determined, thus enabling characterization of overall phenotype (Th1/Th17/Th2/Treg) of T-cell responses, which might indicate skewing towards a more regulatory phenotype during imatinib treatment.

Imatinib can alter the differentiation of human and mouse DCs into mature APCs. The treated cells could not be induced to mature with IFNγ or CD40 agonists and were unable to stimulate T cells. In fact, the DCs look very much like the "tolerogenic" DCs defined in a number of settings. Other cell types may be analyzed that have been shown to play important roles in diabetes development and progression. These include B cells, NK cells, NKT cells and macrophages. We may perform functional analyses of these cells during and after therapy.

PBMCs will be isolated from blood, frozen by established ITN protocols, and studied in batches for quality control and standardization. These assays will be used in an attempt to test the hypothesis that imatinib treatment in humans, as in the NOD model, will tip the balance of inflammation toward an anti-inflammatory state, thus triggering

beneficial changes in T cell-directed autoimmunity.

7.3.2 FLOW CYTOMETRY PANEL STAINING

It is essential that we examine both *in vivo* and *in vitro* the effects of imatinib on DC maturation and function. The ITN core will perform multi-parameter flow cytometry on frozen cells. Changes in the following sub-sets of cells during treatment may be analyzed using various cell surface markers: B-cells (IgM, CD21, CD23, MHC class II, B7, etc); NK and NKT cells; macrophages, and mast cells. Myeloid DC and lymphoid DC-specific markers may also be examined to determine if DC number and subsets are altered. In addition, expression of activation markers MHC class II, CD40 and CD80, and CD86 molecules may be assessed to determine the state of DC maturation. Of special interest would be examination of various T cell subsets based on phosphoprotein and transcription factor expression, after activation. Flow cytometric evaluations of T cell and antigen-presenting cell subsets might complement the functional studies, enabling a better understanding of adaptive and innate immune compartments. Flow cytometric evaluations may also include the use of class I tetramers for detection of CD8 T cells implicated in the pathogenesis of T1DM. Specifically, HLA-A2 tetramers that enable detection of CD8 cells specific for the following antigenic peptides could be used: GAD 65 114-123, preproinsulin 2-10, IGRP 228-236, insulin B chain 10-18, and insulin A chain 1-10. As shown in preliminary studies, these cells can be visualized directly *ex vivo* without a need for *in vitro* auto-antigen induced expansion. When possible other validated class I tetramers may be included as they become available that utilize other autoantigen specificities and HLA restriction elements. Alternatively, other tools for visualization of autoantigen specific T cells might be used, such as engineered antibodies containing dimeric MHC/peptide complexes or other MHC/peptide multimers. Finally, we will work with Drs. Eisenbarth and Kappler in Denver to use newly developed class II MHC tetramers that take advantage of mutations that fix autoantigenic peptides in specific registers to maximize TCR binding. Identification of autoantigen-specific CD4 or CD8 T cells directly *ex vivo* would provide a unique opportunity to reveal the function of these cells without manipulation. In combination with *ex vivo* polyclonal activation with agents like 4-phorbol 12-myristate 13-acetate (PMA) and ionomycin, it would be possible to also address the cytokine production profile of these cells along with expression of co-stimulatory and activation molecules.

7.3.3 GENOMICS AND PROTEOMICS

Imatinib has been shown to have several downstream targets. For instance, imatinib treatment of DCs resulted in decreased activation-induced upregulation of nuclear-localized RelB, RelA, c-Rel, NFkB, p50 and reduced phosphorylation of AKT, suggesting that imatinib mediates its effects in part via the NFkB pathway (AKT is upstream of NFkB). A reduction of NFkB and phosphorylation of Lck and ERK1/2 were observed in PHA-stimulated T cells treated with imatinib. These biochemical results suggest a series of pathways to be investigated in cells isolated from treated

individuals. We may analyze the biochemical effect of imatinib in T, B and dendritic cells, including the examination of the global effect of imatinib on tyrosine phosphorylation in T cells *in vitro* and in cells recovered from imatinib-treated patients. Studies will be designed to determine if imatinib affects phosphorylation of TCR, ZAP-70 and LAT equally, differentially or not. More precise studies on important protein kinases in the TCR signaling pathway, notably ZAP-70, ERK1/2 and AKT, could be performed by immunoprecipitation followed by western blotting in order to track their phosphorylation state (intra-cellular phospho-flow cytometry may also be used). The active form of c-abl may also be assessed in these cells. Similar studies will be performed in B cells if data suggests that B cells are a major target for imatinib activity in T1DM. The ITN may also examine the effect of imatinib treatment on DCs. Purified splenic $CD11c^+$ DCs could be cultured with LPS or CD40L and in the presence or absence of imatinib for 24 hours, then nuclear extracts prepared to assess translocation of NFkB.

7.4 SERUM ASSAYS

7.4.1 SERUM-AUTOANTIBODY ANALYSES

Key markers for the presence of the autoimmune processes directed against pancreatic islets include assessing the presence and titers of anti-GAD65, anti-insulin, anti-ICA512/IA-2, and anti-ICA autoantibodies. Detection of these autoantibody combinations has proven to be an accurate predictor of T1DM in several natural history studies. In the DPT-1 prevention study, over half of the individuals who were positive for two of these antibodies progressed to full disease. Shifts in the titers of Ig isotypes may indicate a change in the type of T-helper cell responses to autoantigen. For example, increases in titers of anti-GAD IgE, IgG2, or IgG4 antibodies could indicate a shift to a more regulatory-type cytokine profile following drug administration. In summary, this study will test the hypothesis that successful treatment will be associated with a reduction in the titer or isotype of diabetes-related autoantibodies.

7.4.2 SERUM ARCHIVE

Patient serum will be archived for future studies. These studies might include measurements of cytokines and subsequent correlation with induction of clinical tolerance. The archived plasma samples could potentially be used for analysis of immune and inflammatory molecules at the proteosome and transcriptosome levels. In addition, expression of pro-inflammatory cytokines (PDGF, IL-1ß, IL-6, IL-12, TNFα), molecules that are induced by inflammation (SOCS1, 2, 3 and acute phase reactants, e.g., ceruloplasmin, SAA, CRP), and anti-inflammatory cytokines (TGF-ß, IL-10) could also be assessed to determine the effect of imatinib treatment. We anticipate that novel assays relating to beta cell death may also be run from archived samples using differentially methylated circulating DNA[180].

7.5 WHOLE BLOOD-GENE-EXPRESSION PROFILING

To further elucidate possible changes in cytokine and cellular profiles, gene-expression profiling analysis may be performed on RNA isolated from peripheral blood using microarray or high-throughput real-time polymerase chain reaction (RT-PCR). RT-PCR can be used to compare the expression of several genes reported to play a role in T1DM, which might include IFNγ, TGFβ, IL-4, Tbet, RORγt, FOXP3, STAT molecules, IL-2, IL-5, IL-13, IL-15, IL-21, IL-23 and IL-25. The goal of these assays is to identify differences between a tolerant versus non-tolerant state and to find new genes that could serve as potential markers of disease. These types of analyses may also explain why some individuals respond better to this treatment or elucidate mechanisms resulting in adverse responses to treatment. This assay has proven informative in characterizing unique genes that determine the clinical course in systemic lupus erythematosus, and preliminary studies with this technology have enabled us to determine a distinct subset of genes that are either up- or down-regulated in those with new-onset T1DM as opposed to controls in other studies.

7.6 WHOLE BLOOD DNA-HLA GENOTYPES

DNA collected from participants will be used to perform sequence-based HLA typing. A complete class I and class II haplotype will be performed, including fine typing of the DQB and DRB regions. Genotyping for single nucleotide polymorphisms (SNPs) in selected immune-response genes may also be performed. The results of genotype analyses might be used to correlate with disease progression and therapeutic responses (tolerance induction). In addition, collected DNA may be used for epigenetic analysis that includes histone modification and DNA methylation studies.

7.7 CHANGE IN BETA CELL FUNCTION

Many of the measures that we will utilize in assessing changes in beta cell function are now standard assessments in new-onset T1DM trials (see Section 3.3). However, these studies have ignored changes in insulin sensitivity. Imatinib may have novel effects on beta cell function and insulin sensitivity, as noted by the pre-clinical studies and effects in subjects with type 2 diabetes. We recognize that euglycemic hyperinsulinemic clamps are the gold standard for assessment of insulin sensitivity, but pose significant additional subject and investigative team burden and expense. We will assess changes in adiponectin levels in study patients, as these have shown dramatic increases in patients with type 2 DM on imatinib[126]. We will also use data derived from MMTTs to model beta cell function and insulin sensitivity, as has been employed by others[181,182](see letter of collaboration from Ferrannini). In addition to glucose and c-peptide levels, plasma glucagon, and proinsulin levels will be measured to further explore the effects of Imatinib on insulin sensitivity.

8. ADVERSE EVENTS

8.1 ADVERSE EVENT DEFINITION

8.1.1 Adverse Event

In this clinical trial, an adverse event is any occurrence or worsening of an undesirable or unintended sign, symptom or disease whether or not associated with the treatment and study procedures.

Throughout the study, the investigator must record all adverse events on source documents. Events not related to hypo or hyperglycemia that are Grade 2 or greater per the NCI CTCAE 4.0 (see Section 8.1.4. Grading Event Severity below) must be reported to the Coordinating Center on the appropriate adverse event form. 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.

An abnormal value or result from a clinical or laboratory evaluation (e.g., a radiograph, an ultrasound, or an electrocardiogram) can also indicate an AE if it is determined by the investigator to be clinically significant. If this is the case, it must be recorded in the source document and as an AE on the appropriate AE form(s). The evaluation that produced the value or result should be repeated until that value or result returns to normal or can be explained and the participant's safety is not at risk.

8.1.2 Serious Adverse Event

For this trial, an adverse event associated with the treatment or study procedure that suggests a significant hazard, contraindication, side effect or precaution (as described below) is to be reported as a serious adverse event (SAE). A serious adverse event (experience) or reaction is any untoward medical occurrence that:

• results in death,

• is life-threatening,

• requires inpatient hospitalization or prolongation of existing hospitalization,

• results in persistent or significant disability/incapacity, or

• is a congenital anomaly/birth defect.

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

8.1.3 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.

8.1.4 Grading Event Severity

This study has adopted usage of the National Cancer Institute (NCI) Common Technology Criteria for Adverse Events (CTCAE) version 4.0 and/or study-specific criteria for classification to describe the severity of adverse events with the exception of hyper and hypoglycemia. For this study, a reportable hypoglycemic event is defined as those resulting in loss of consciousness, seizure, or requiring assistance of others due to altered state of consciousness and hyperglycemic event is one resulting in DKA.

Adverse events will be graded on a scale from 1 to 5 according to the following standards in the NCI-CTCAE manual:

- Grade 1 = mild adverse event.
- Grade 2 = moderate adverse event.
- Grade 3 = severe and undesirable adverse event.
- Grade 4 = life-threatening or disabling adverse event.
- Grade 5 = death.

For additional information and a printable version of the NCI-CTCAE manual, go to http://ctep.cancer.gov/reporting/ctc.html

8.1.5 Attribution Definitions

Adverse events will be categorized for their relation to imatinib. The investigator will determine the relation, or attribution, of an AE to study participation and will record the determination on the appropriate CRF and/or SAE reporting form. The relation of an AE to study participation will be determined using definitions in Table 1.

Table 1. Attribution of adverse events

Code	Descriptor	Definition
Unrelated Category		
1	Unrelated	The adverse event is *clearly* not related.
Related Categories		
2	Unlikely	The adverse event is *doubtfully* related.
3	Possible	The adverse event *may be* related.
4	Probable	The adverse event is *likely* related.
5	Definite	The adverse event is *clearly* related.

8.2 ADVERSE EVENT REPORTING AND MONITORING

Study personnel will assess adverse events and the use of concomitant medications throughout the study. Adverse events will be reported to the Coordinating Center as described below. They will be graded as to severity according to common toxicity criteria or study-specific criteria and the investigator will make a determination as to the relation to therapy. Events will be assessed and reported in accordance with the ICH Guideline For Good Clinical Practice and per the guidance of the DHHS Office for Human Research Protections (OHRP). The adverse event case report form for the protocol must be completed for all adverse events (AE) of Grade 2 or greater severity regardless of relationship to therapy. For reporting serious adverse events (SAE), the MedWatch Form should also be completed and faxed to the Coordinating Center *within 24 hours of when the site was notified of the event.* This will be reviewed by the study Safety Monitoring Committee, and the Data and Safety Monitoring Board (DSMB) as appropriate. Deaths must be reported immediately. Event outcome and other follow-up information regarding the treatment and resolution of the event will be obtained and reported when available, if not known at the time the event is reported. The follow-up information should contain sufficient detail to allow for a complete medical assessment of the case and an independent determination of possible causality.

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

8.2.1 Reporting Pregnancy

The investigator should be informed immediately of any pregnancy. At each visit the investigator will determine the pregnancy status of female participants and that the sexual partners of male participants. Pregnancy information should be entered into the electronic data capture (EDC) system within 24 hours of becoming aware of the event. The investigator should counsel the participant and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the participant should continue until the conclusion of the pregnancy. Follow-up information detailing the outcome of the pregnancy should be entered into the EDC system as it becomes available. Any premature termination of the pregnancy will be reported.

While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be recorded as an AE or SAE as described in sections 8.2.1 and 8.2.2.

9. 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.

9.1 PRIMARY OUTCOME AND ANALYSES

The primary outcome of each participant is the area under the stimulated C-peptide curve (AUC) over the first 2 hours of a 4-hour mixed meal glucose tolerance test conducted at the one-year visit. The AUC is computed using the trapezoidal rule that is a weighted sum of the C-peptide values over the 120 minutes. By the mean value theorem of integral calculus, the weighted mean C-peptide in pmol/mL is simply AUC/120.

The primary statistical hypothesis to be assessed in the study is whether:

- The mean C-peptide value for study subjects receiving imatinib is significantly higher than the mean value for placebo subjects.

The primary analysis will employ the weighted mean derived from the 2 hour AUC for each participant transformed as log(mean C-peptide+1). The comparison between the two treatment arms will be based on a t-test of treatment effect in an ANCOVA model adjusting for gender, baseline age and baseline log(C-peptide+1)[190].

9.2 SECONDARY OUTCOMES AND ANALYSES

Additional analyses of the primary outcome will include:

- A log rank test of the difference in the hazard function between groups in the incidence of the loss of the 2 hour peak C-peptide < 0.2 pmol/ml on a semi-annual MMTT [191], and
- Longitudinal analyses using mixed effects models with a random intercept and slope of the C-peptide values over the post-treatment period, adjusted for the baseline level of C-peptide. The average intercept and slope will be compared between groups adjusting for age, gender and the baseline *log(C-peptide+1)*.

Additional secondary objectives are to examine how imatinib affects the following:

- Mean area under the stimulated C-peptide curve (AUC) curve at 12 months
- Mean area under the stimulated C-peptide curve (AUC) over 4 hours at 24 months
- HbA1c levels over time
- Insulin dose (units/kg) over time
- Number of severe hypoglycemic events
- Number and severity of adverse events

The mean levels of quantitative variables (e.g. HbA1c and insulin dose) over all follow-up values will be compared between groups using a normal errors longitudinal analysis.

The prevalence of a binary characteristic (e.g. yes/no or positive/negative) at a single visit (e.g. a history of hypoglycemia during follow-up) will be assessed using a logistic regression model. The prevalence of a binary characteristic over time will be assessed using generalized estimating equations.

The rates of severe hypoglycemic events and severe adverse events will be computed (total number of events divided by total patient years of follow-up) and the rates compared using a Poisson regression model, allowing for over-dispersion using a quasi-likelihood model as appropriate. Tests of significance will employ a robust estimate of the variance.

The above analyses will also be conducted to adjust for age, gender, baseline *log(C-peptide+1)* and baseline HbA1c; and by race/ethnicity, as appropriate. Analyses will also be conducted to examine the effect of HLA or other genotype.

Analyses will also be conducted to assess heterogeneity of the effect of treatment group (the group difference) as a function of age, gender, baseline *log(C-peptide+1)* and baseline HbA1c; and by race/ethnicity and HLA or other genotype. Heterogeneity will

be assessed using a test of treatment group by covariate interaction in an appropriate regression model.

9.3 ADDITIONAL OUTCOMES AND ANALYSES

Additional outcomes of interest include

- Change in autoantibody levels and/or B cell function
- Antigen specific and non-specific T and B cell subset enumeration and function
- Responses to vaccination (tetanus and killed flu)

The analyses of each outcome will be conducted using the methods for a quantitative outcome, binary outcome, or rate as described in Section 9.2 above.

9.4 SAMPLE SIZE AND POWER CALCULATIONS

The primary analysis will compare the difference between the treated group versus the placebo group in the levels of the 2 hour AUC-mean using the log(mean C-peptide+1) in an ANCOVA model adjusting for gender, baseline age, and baseline log(C-peptide+1). Estimates of log(mean C-peptide+1) and root mean square error (RMSE) in the placebo group were obtained from prior studies. Among subjects with baseline C-peptide > 0.2 pmol/ml and age <=21 years, the mean log(C-peptide + 1) values is 0.306 with RMSE = 0.185.

The planning parameters for the protocol were based upon the inclusion of adults and children. Unfortunately, the FDA has declined the request to lower the age and, therefore, the study will only enroll adults at this time.

Hence, it is appropriate to revisit the design of the study in light of restricting the eligible population to be age 18 and over. In previous studies, it has shown the older a participant is, the lower the rate of C-peptide loss. This means that the current design of detecting a one-year C-peptide projected level 45% greater than expected on the control arm corresponds to an increase in C-peptide over the baseline value at enrollment, which is not practical. However, analyses completed after this study was initially designed indicate that the appropriate variance estimate to use for the sample size calculation should be smaller than what was used in the current design. This has the net effect of increasing the sensitivity of the trial to detecting an effect size smaller than the advertised value in the current design.

Using standard equations for the comparison of two means, a sample size of 40 Imatinib treated and 20 placebo treated subjects with complete data needed for the primary analysis, would provide power of 85% to detect a 0.35 increase in the mean log(C-peptide + 1) (0.306 vs. 0.445, which corresponds to 75% of the RMSE) in the experimental treatment group using a two-sample T- test at the 0.05 level (one-sided).

Assuming that 10% of the subjects will have missing data (one-year MMTT was not done or subject withdrew prior to the one-year assessment), the sample size goal for

this study is 66 subjects (46 + 22). Should the number with missing data exceed 10%, the study will enroll additional replacement subjects as needed.

9.5 INTERIM MONITORING PLAN

For purposes of the 6-month analysis, a trend for benefit would be an outcome that does not exclude a significant result being achieved at the end of Phase 2 with a twelve month outcome as specified by the protocol. Changes from baseline in stimulated C-peptide mean AUC in T1D patients on placebo have been reported as approximately 30% at 6 months (Orban et al 2011[39], Raz et al 2007[194]). Sample size calculations were thus based on the assumption of a ~30% treatment effect at around 6 months of treatment and a standard deviation for baseline- and placebo-adjusted stimulated C-peptide mean AUC of 0.39 nmol/liter/2h (Greenbaum et al 2012). In this case, an interim analysis will calculate the conditional power after 21 participants have been evaluated. Based upon the table that follows, should the calculated interim Z value be greater than or equal to 0.8, the conditional power would be estimated to be at least .26 and .74 if the true effect is as assumed under the alternative hypothesis at study conclusion. Therefore, a threshold will be set on the conditional power as the level-of-proof that the difference in stimulated C-peptide mean AUC between gleevec and placebo is of presumed benefit to allow the study to move forward to phase two.

Table of Conditional Power Assuming an Alternate Effect Size (defined as (mu1-mu0)/2(sd) = 0.37, an interim analysis at 21 patients and a final analysis at 66 patients. Two-tailed alpha = 0.05

Interim Z Value	Conditional Power Under Observed Trend	Conditional Power Under Protocol Assumed Effect
0.0	<.01	.54
0.4	.06	.65
0.8	.26	.74
1.2	.58	.82
1.6	.86	.89

Additional interim analyses will be conducted when 50% of the targeted number of study subjects have reached the planned end point (i.e., one year of follow up) and will be reviewed by the Data and Safety Monitoring Board (DSMB) for assessment of effectiveness and safety. If a group sequential stopping boundary is crossed, the DSMB may recommend termination of the trial early. The Lan-DeMets[195] 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

periodically during the trial. The spending function that approximates the O'Brien-Fleming boundaries is:

$$\alpha_1(t^*) = 2 - 2\Phi\left[\frac{Z_{\alpha/2}}{\sqrt{t^*}}\right]$$

where t^* is the information fraction ($0 < t^* \leq 1$), α_1 is the $\alpha\text{-}level$ of the interim (one-sided) test and α is the over-all type I error.

The DSMB will also consider early termination due to absence of a treatment effect (i.e. futility) based on the method suggested by Ellenberg et al[196]. The stopping rule is: if the t-test (as described in the primary analysis and positive values reflect a higher values among the experimental group) is less than or equal to 0 at $t^* \geq 0.5$, the study should be stopped based on the futility of rejecting the null hypothesis at the completion of the trial. Simulation studies conducted confirmed that this rule combined with the Lan-DeMets stopping rules have negligible effect on the type I and II error probabilities. Additional analysis will assess potential adverse outcomes of treatment and will assess the incidence of all severe adverse events.

10. ACCESS TO SOURCE DATA/DOCUMENTS

The investigational sites participating in this study will maintain the highest degree of confidentiality permitted for the clinical and research information obtained from participants in this clinical trial. 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 sites must permit authorized representatives of the sponsor, and health authorities to examine (and to copy when required by applicable law) clinical 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 (and any personally identifying information must be obscured). Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that may be linked to identified individuals. The investigational sites will normally be notified in advance of auditing visits.

11. QUALITY CONTROL AND QUALITY ASSURANCE

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

The sponsor is responsible for regular inspection of the conduct of the trial, for verifying adherence to the protocol, and for confirming the completeness, consistency, and accuracy of all documented data.

The CRFs will be completed online via a web-based electronic data capture (EDC) system that has been validated and is compliant with Part 11 Title 21 of the Code of Federal Regulations. Study staff at the site will enter information into the electronic CRFs, and the data will be stored remotely at a central database. All elements of data entry (i.e., time, date, verbatim text, and the name of the person performing the data entry) will be recorded in an electronic audit trail to allow all changes in the database to be monitored and maintained in accordance with federal regulations.

Study staff will enter data from a study visit on the relevant eCRFs within 14 days following the visit or the time when data becomes available.

12. ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE

12.1 STATEMENT OF COMPLIANCE

This trial will be conducted in compliance with the protocol, current Good Clinical Practice (GCP) guidelines—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 the sponsor and an appropriate ethics review committee or institutional review board (IRB). Any amendments to the protocol or consent materials must also be approved by the Sponsor, the IRB and submitted to FDA before they are implemented.

12.2 INFORMED CONSENT

The informed consent form is a means of providing information about the trial to a prospective participant and allows for an informed decision about participation in the study. All participants (or their legally acceptable representative) must read, sign, and date a consent form before participating in the study, taking the study drug, and/or undergoing any study-specific procedures.

The informed consent form must be updated or revised whenever important new safety information is available, whenever the protocol is amended, and/or whenever any new information becomes available that may affect participation in the trial.

A copy of the informed consent will be given to a prospective participant for review. The study investigator will review the consent and answer questions. The participant will be informed that participation is voluntary and that he/she may withdraw from the study at any time, for any reason.

12.3 PRIVACY AND CONFIDENTIALITY

A participant's privacy and confidentiality will be respected throughout the study. Each participant will be assigned a sequential identification number. This number, rather than the participant's name, will be used to collect, store, and report participant information.

13. PUBLICATION POLICY

The JDRF policy on publication of study results will apply to this study.

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Appendix 1. Schedule of Events

Week	-3 to -4	0	2	4	9	13	17	22	26	39	52	78	104
Visit	**-1**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
GENERAL ASSESSMENTS													
Informed consent	X												
Eligibility criteria	X												
Medical history	X												
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical examination[1]	X	X		X	X	X			X		X	X	X
Secondary sexual characteristics[2]		X									(X)		(X)
Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy Monitoring (if applicable)	X	X		X	X	X	X	X	X		X	X	X
ECG	X		X										
LABORATORY ASSESSMENTS													
Serum chemistries and liver panel[3]	X	X	X	X	X	X	X	X	X	X	X	X	X
Autoantibodies	X												
Hematology[4]	X	X	X	X	X	X	X	X	X	X	X	X	X
Infectious disease serology[5]	X												
PPD skin test or IGRA	X												
Urine hCG	X	X		X	X	X	X	X	X		X	X	X
Urinalysis	X								X		X		X
Prostate Specific Antigen (males only)		X							X		X		X
C-peptide levels (tested from MMTT)[6]	X					X			X		X	X	X

[1]Excluding genitalia unless clinically indicated

[2] The Tanner stages will be assessed at the baseline visit for every participant under 18 years of age. After the baseline visit, Tanner stages will be assessed annually on all participants who are < stage 3. If the Tanner stage is ≥3 at the baseline visit or any subsequent visit, Tanner stages will not need to be assessed at any future visit.

[3] To include sodium, calcium, potassium, chloride, phosphate, total CO_2, BUN, creatinine, AST, ALT, alkaline phosphate, direct and total bilirubin.

[4] Performed locally; to include CBC with differential and platelets.

[5] Hepatitis B and C, HIV, toxoplasmosis, VZV, EBV, and CMV serology. CMV/EBV PCR testing may be obtained (centrally) or locally, if necessary to confirm active infection.

[6] 4-hour MMTT at Visits -1, 9, 11 and 2-hour MMTT at Visits 4, 7, and 10.

Week	-3 to -4	0	2	4	9	13	17	22	26	39	52	78	104
Visit	**-1**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Plasma Glucose (tested from MMTT)[5]	X					X			X		X	X	X
HbA_{1C} levels	X					X			X	X	X	X	X
STUDY DRUG ADMINISTRATION													
Study drug administration		X	X	X	X	X	X	X					
Study drug compliance			X	X	X	X	X	X	X				
DISEASE SPECIFIC ASSESSMENTS													
Glucose (Glucometer Reading)		X	X	X	X	X	X	X	X	X	X	X	X
Insulin use		X	X	X	X	X	X	X	X	X	X	X	X
Hypoglycemia assessment		X	X	X	X	X	X	X	X	X	X	X	X
Serum-Adiponectin[7]		X		X					X		X		X
Plasma-Glucagon[6]	X					X			X		X	X	X
Plasma-Proinsulin[6]	X					X			X		X	X	X
BONE AND MINERAL METABOLISM													
Serum Calcium[8]		X		X		X			X		X		
Serum Phosphate[7]		X		X		X			X		X		
PTH		X		X		X			X		X		
25-OH Vitamin D		X									X		
Serum CTX[9]		X				X			X		X		
Osteocalcin[8]		X				X			X		X		
GROWTH RATE ASSESSMENTS[10]													
Plain Radiograph of Left Hand		X									X[11]		X[10]
Height by Stadiometer[12]	X	X	X	X	X	X	X	X	X	X	X	X	X
Arm span		X							X		X		X

[7] Samples will be archived for potential future testing.

[8] Tested as part of serum chemistry panel.

[9] Samples will be archived and tested if clinically indicated.

[10] To be performed on growing-age participants per Section 5.2.9.

[11] Bone age will be assessed annually until epiphyses are near complete closure (98% of mature height at bone age of 15 yrs 9 months for boys, 14 yrs for girls).

[12] Subjects will need to be measured with a wall mounted stadiometer to insure accurate height measurements.

Week	-3 to -4	0	2	4	9	13	17	22	26	39	52	78	104
Visit	**-1**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
IGF-1, IGF-BP3, LH, FSH, Estradiol (females), Testosterone (males)[13]		X							X		X		X
Total IgA		X[14]											
TSH and T4		X[13]									X[15]		X[14]
Tissue transglutaminase (IgA)[16]		X[13]									X[14]		X[14]
MECHANISTIC ASSAYS													
Serum-Autoantibody Analysis		X		X		X			X		X		X
PBMC-Flow Cytometry Panel Staining		X		X		X			X		X		X
PBMC-Cell Based Assays		X		X		X			X		X		X
PBMC-Genomics, Proteomics		X		X		X			X		X		X
PBMC- FOXP3 Methylation Assay		X		X		X			X		X		X
Whole Blood-Gene Expression Profiling		X		X		X			X		X		X
Whole Blood DNA-HLA Genotypes		X				X					X		
Plasma-Archive		X		X		X			X		X		X
Serum- Beta Cell Death Assay		X	X	X	X				X		X		X
Serum-Cytokines		X	X	X	X				X		X		

[13] Samples will be archived and tested if clinically indicated.

[14] Tested at Baseline only if not tested by referring physician within 3 months prior to Baseline.

[15] Samples will be collected at Visits 9 and 11 only if clinically indicated in growing age participants. If required, testing will be performed real-time.

[16] Performed locally.

Appendix 2. Procedures for Performing the Mixed-Meal Tolerance Test

The mixed-meal tolerance test (MMTT) is performed in the morning (between 7:00 a.m. and 10:00 a.m.), which means that administration must begin within this time. It is recommended that the tests be scheduled early in the morning (7:00–7:30 am) because blood glucose will be more likely to be within the target range at that time.

The mixed meal used in this protocol will be the Boost® High Protein Nutritional Drink (Nestlé Nutrition). If a participant has a known food allergy to one or more components of Boost, an equivalent substitution may be used. The MMTT should take 250 minutes to perform.

Dietary Guidelines and Pretest Instructions

Carbohydrates (CHO) should not be restricted from the diet before the test. A general guideline is that preadolescent participants should consume at least 25 kcal (6.25 g) CHO/kg/day and adolescent and adult participants should consume at least 15 kcal (3.75 g) CHO/kg/day for 3 days before the test. These are minimum amounts of CHO; most diets will include greater amounts of CHO. There is no need to alter the participant's diet unless he or she has been on a CHO-restricted diet.

In preparation for the visit, each participant should:

- Fast for at least 10 hours (but not more than 16 hours) before the test. Fasting should start the night before the test, and should continue up until the start of the test. Participants should not eat or drink anything except water. This means no coffee, tea, soda, cigarettes, alcohol, or chewing gum during the fasting period.
- Refrain from vigorous exercise during the fasting period.
- Refrain from working the night before the morning of the test.
- Discontinue taking any prescription medications that must be taken daily.

Glucose and Insulin Before the Test

- Short-acting insulin analogues (such as lispro or l-aspart) may be administered up to 2 hours before the test.
- Regular insulin may be administered up to 6 hours before the test.
- Intermediate-acting insulin (such as NPH) may be administered on the evening before the MMTT, but not on the morning of the test. Participants managed with intermediate-acting insulin (NPH or Lente) should administer their usual dose on the evening before the MMTT, but not on the morning of the test.
- Long-acting basal (such as glargine) insulin or continuous subcutaneous insulin infusion may be administered before, during and after the test as usual. Participants on glargine may take their usual injection at the appropriate time, and those on

continuous subcutaneous insulin infusion may continue with their usual basal settings.

Target Glucose Level at the Start of Test

The target glucose level at the start of the test is between 70 and 200 mg/dL. Regular insulin or short acting insulin analogues may be used up to 6 and 2 hours before the test, respectively, to achieve the desired glucose level. The investigator and the study participant should discuss the individual situation for insulin administration to attain the goal of meter capillary glucose values within the range of 70–200 mg/dL at the start of the test. For example, as a practical matter, participants may be instructed to check their blood glucose by meter at home 2 hours before the start of the test so that marked hyperglycemia can be treated with a short-acting insulin analogue. Alternatively, participants who arrive at the research unit with elevated blood glucose can receive additional short-acting insulin analogues at the time of their arrival, if the test itself does not start until at least 2 hours after insulin administration and occurs before 10 a.m.

IV Placement During the Test

- The IV should be in place for the duration of the test and must be flushed after each draw with saline solution or heparin flush.
- The participant should remain sitting or resting in bed quietly throughout the test and until the test is completed. However, he or she may engage in quiet, non-strenuous activities, such as reading, playing cards, or watching TV. The participant may walk to the bathroom between blood draws if necessary.

Testing Instructions

Time Point −10 minutes

- The first sample should be taken at least 10 minutes after establishing the line(s) and when the participant is calm and relaxed (if possible, depending on age) - this is the "−10 minute" sample.
- Draw one 1.2 mL sample into the lavender top K_2 ETDA tube for C-peptide. After each sample is collected, the sample tube must be inverted gently at least 8 to 10 times. Chill sample in a bucket of crushed ice or in a refrigerator set at 4˚C for 20 to 30 minutes. At the laboratory, spin the tube in a refrigerated centrifuge (1000–1300 g, ~3000RPM) for 10 minutes. Tubes must be spun within 60 minutes of blood draw. Freeze the sample at -80°C.
- Draw one 1.2 mL sample into the gray top K Oxalate / Na Fluoride tube for glucose. Invert tube gently 8 to 10 times. If it is not possible to centrifuge the sample immediately post collection, chill sample in a bucket of crushed ice or in a refrigerator set at 4˚C for 20 to 30 minutes. Refrigerate the sample no longer than one hour prior to centrifugation.

- Draw sample into the provided K2EDTA tube for glucagon/proinsulin. Invert tube gently 8 to 10 times. If it is not possible to centrifuge the sample immediately post collection, chill sample in a bucket of crushed ice or in a refrigerator set at 4˚C for 20 to 30 minutes. Refrigerate the sample no longer than one hour prior to centrifugation.

Time Point 0 minutes

- The second sample should be taken just before the participant drinks the Boost® High Protein; this is the "0- minute" sample.
- Then the MMTT dose should be given with 6 kcal/kg @ 1 kcal/mL of mixed meal, to a maximum of 360 mL. The participant should consume the MMTT dose in no more than 5 minutes.

Time Points 15, 30, 60, 90, 120, 150, 180, 210, and 240 minutes

- Draw one 1.2 mL sample into the lavender-top K_2 EDTA tube for C-peptide levels. Refrigerate the sample
- Draw one 1.2 mL sample into the gray-top K Oxalate / Na Fluoride tube for glucose at each of the time points specified.
- Draw one 1.2 mL samples into the lavender-top K2EDTA tube for glucagon/proinsulin at each of the time points specified.
- Invert all tubes gently 8 to 10 times after collection. If it is not possible to centrifuge the sample immediately post collection, chill sample in a bucket of crushed ice or in a refrigerator set at 4˚C for 20 to 30 minutes. Refrigerate the sample no longer than one hour prior to centrifugation.
- At the conclusion of the test, please check blood glucose by glucometer, and administer insulin as per participant's standard insulin plan.

Tube-Processing Instructions

- Spin the collection tubes. Then transfer the plasma into individual vials. Please make sure that each vial is properly identified with a label that indicates the time point.
- Freeze the samples for glucose, C-peptide levels, and glucagon/proinsulin.
- Ship the glucose and C-peptide specimens to the laboratory where the assays will be performed.
- Ship the glucagon/proinsulin specimens to the laboratory where the samples will be stored until testing is initiated at the end of the study.

A clogged line, missed sample, or other deviation from the protocol must be noted on the Comments section of the MMTT specimen transmittal form.

Time (minutes)	Glucose Sample Taken	C-peptide / Sample Taken
–10	X	X
0	X	X
Participant drinks Boost®		
15	X	X
30	X	X
60	X	X
90	X	X
120	X	X
150	X	X
180	X	X
210	X	X
240	X	X