PROTOCOL CIT-05

B-Lymphocyte Immunotherapy in Islet Transplantation: Toward Calcineurin Inhibitor Free Immunosuppression

Version 3.0 (September 3, 2008)

BB-IND 9336

[CIT-05]

This clinical study is sponsored by the National Institute of Allergy and Infectious Diseases (NIAID) and the National Institute of Diabetes & Digestive & Kidney Diseases (NIDDK).

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Short Title: **B-Lymphocyte Immunotherapy in Islet Transplantation**	
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INSTRUCTIONS: Please have the Principal Investigator print, sign, and date at the indicated location below. A copy should be kept for your records and the original signature page sent to the Data Coordinating Center.

After signature, please return the original of this form by surface mail to:

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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 United States Code of Federal Regulations (CFR) – 21 CFR Parts 45, 50, 56, and 312, and 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 Site Principal Investigator, I agree to conduct "CIT-05: B-Lymphocyte Immunotherapy in Islet Transplantation: toward calcineurin-inhibitor free immunosuppression" according to good clinical practices. 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 NIDDK.

_____
Site Principal Investigator (Print)

_____ _____
Site Principal Investigator (Signature) **Date**

Protocol Synopsis

Title	B-Lymphocyte Immunotherapy in Islet Transplantation: Toward Calcineurin-Inhibitor Free Immunosuppression
Short Title	B-Lymphocyte Immunotherapy in Islet Transplantation
Clinical Phase	Phase 2
IND Sponsor	DAIT/NIAID/NIH
IND Number	BB-IND 9336
Activation Date:	November 2006
Accrual Objective	12 subjects
Accrual Period	24 months
Study Duration	12 months after final transplant
Study Design	Open-label, single-center, prospective single arm study.
Treatment Description	Islet transplantation will be performed under an experimental immunosuppression arm. The experimental immunosuppression will consist of the anti-B lymphocyte agent rituximab in combination with Thymoglobulin® (daclizumab or basiliximab instead of Thymoglobulin® for the 2nd and 3rd transplants, if applicable) for induction and sirolimus alone for maintenance, a calcineurin-inhibitor free regimen.

Study Design	This is an open-label, prospective, single-arm, single-center trial. Objectives of this study include gaining experience with the experimental immunosuppression treatment regimen as well as to determine the proportion of subjects with insulin independence on this experimental regimen. Should these subjects demonstrate a very high rate of insulin independence at 75 ± 5 days post-transplant (a rate of 40% or higher would be considered high by current standards), then this treatment may be considered for further study. There is a larger parallel study with additional centers and more subjects (CIT-07) where subjects are treated with a standard Islet Transplant immunosuppression regimen. Subjects will be randomized to receive either the standard immunosuppression regimen under CIT-07 or the experimental immunosuppression in this protocol (CIT-05) at the time an islet preparation meets release criteria. The randomization will provide a mechanism for balance between the two studies and provide an objective selection of subjects to the two studies.
Primary Endpoint	The primary endpoint will be insulin-independence at 75± 5 days following the first islet transplant.
Primary Analysis	The primary analysis will be to provide an estimate of the proportion of subjects with insulin independence and a corresponding 95% confidence interval.

Secondary Endpoints

Efficacy Endpoints

At 75 ± 5 days following the first and final islet transplant:

- Proportion of insulin-independent subject
- The percent reduction in insulin requirements
- HbA1c
- Mean amplitude of glycemic excursions (MAGE)
- Glycemic lability index (LI)
- Ryan hypoglycemia severity (HYPO) score
- Basal (fasting) and 90-min glucose and c-peptide derived from the mixed-meal tolerance test (MMTT)
- β-score
- C-peptide: (glucose X creatinine) ratio
- Acute insulin response to glucose (AIR_{glu}), insulin sensitivity, and disposition index derived from the insulin-modified frequently-sampled intravenous glucose tolerance (FSIGT) test
- Glucose variability and hypoglycemia duration derived from the continuous glucose monitoring system®
- Quality of life measures

At 365 ± 14 days following the first islet transplant:

- The proportion of subjects with an HbA1c <7.0% at Day 365 AND free of severe hypoglycemic events from Day 28 to Day 365 inclusive
- The proportion of subjects receiving a second islet transplant
- The proportion of subjects receiving a third islet transplant

At 365 ± 14 days following the first and final islet transplant:

- The proportion of insulin-independent subjects
- The percent reduction in insulin requirements
- HbA1c
- MAGE
- LI
- Clarke hypoglycemia awareness score
- HYPO score
- Basal (fasting) and 90-min glucose and c-peptide (MMTT)
- β-score
- C-peptide: (glucose X creatinine) ratio
- Acute insulin response to glucose (AIR_{glu}), insulin sensitivity, and disposition index derived from the insulin-modified frequently-sampled intravenous glucose tolderance (FSIGT) test.
- QOL (DQOL, HSQ 2.0, HFS)
- The proportion of subjects receiving a second islet transplant
- The proportion of subjects receiving a third islet transplant
- The proportion of subjects with a HbA1c ≤7.0% and free from severe hypoglycemic events

Safety Endpoints

At 75 ± 5 and 365 ± 14 days following the first and final islet transplant:

- The incidence and severity of adverse events related to the islet transplant procedure including: bleeding (>2 g/dL decrease in hemoglobin concentration); segmental portal vein thrombosis; biliary puncture; wound complication (infection or subsequent hernia); and increased transaminase levels (>5 times upper limit of normal [ULN])
- The incidence and severity of adverse events related to the immunosuppression including: allergy; reduction in GFR; increase in urinary albumin excretion; addition or intensification of anti-hypertensive therapy; addition or intensification of anti-hyperlipidemic therapy; oral ulcers; lower extremity edema; gastrointestinal toxicity; neutropenia, anemia, or thrombocytopenia; viral, bacterial, or fungal infections; and benign or malignant neoplasms
- The incidence of a change in the immunosuppression drug regimen
- The incidence of immune sensitization defined by detecting anti-HLA antibodies not present prior to transplantation

At 365 ± 14 days following the first islet transplant:

- The incidence of worsening retinopathy as assessed by change in retinal photography

Insulin Independence Definition

Islet transplant recipients will be considered insulin-independent with full islet graft function if they are able to titrate off insulin therapy for at least 1 week and all of the following criteria are met:

- HbA1c ≤7.0% or a ≥2.5% decrease from baseline;
- fasting capillary glucose level should not exceed 140 mg/dL (7.8 mmol/L) more than three times in the past week (based on measuring capillary glucose levels a minimum of 7 times in a seven day period);
- 2-hour post-prandial capillary glucose should not exceed 180 mg/dL (10.0 mmol/L) more than three times in the past week (based on measuring capillary glucose levels a minimum of 21 times in a seven day period);
- fasting serum glucose level ≤126 mg/dL (7.0 mmol/L); if the fasting serum glucose level is >126 mg/dL (7.0 mmol/L), it must be confirmed in an additional one out of two measurements;
- evidence of endogenous insulin production defined as fasting or stimulated c-peptide levels ≥0.5 ng/mL (0.16 nmol/L).

Secondary Analyses

The secondary analyses will compare the primary and secondary endpoints in this study to the corresponding outcomes in the CIT-07 study. Estimates and 95% confidence intervals will also be calculated.

Severe Hypoglycemic Event Definition

A severe hypoglycemic event is defined as an event with one of the following symptoms: memory loss; confusion; uncontrollable behavior; irrational behavior; unusual difficulty in awakening; suspected seizure; seizure; loss of consciousness; or visual symptoms, in which the subject was unable to treat him/herself and which was associated with either a blood glucose level <54 mg/dL (3.0 mmol/L) or prompt recovery after oral carbohydrate, IV glucose, or glucagon administration.

Inclusion Criteria:

Patients who meet *all* of the following criteria are eligible for participation in the study:

1. Male and female patients age 18 to 65 years of age.

2. Ability to provide written informed consent.

3. Mentally stable and able to comply with the procedures of the study protocol.

4. Clinical history compatible with T1D with onset of disease at <40 years of age and insulin-dependence for ≥5 years at the time of enrollment, and a sum of patient age and insulin dependent diabetes duration of ≥28 years.

5. Absent stimulated c-peptide (<0.3ng/mL) in response to a mixed meal tolerance test (MMTT; Boost® 6 mL/kg body weight to a maximum of 360 mL; another product with equivalent caloric and nutrient content may be substituted for Boost) measured at 60 and 90 min after the start of consumption.

6. Involvement in intensive diabetes management defined as self monitoring of glucose values no less than a mean of three times each day averaged over each week and by the administration of three or more insulin injections each day or insulin pump therapy. Such management must be under the direction of an endocrinologist, diabetologist, or diabetes specialist with at least 3 clinical evaluations during the 12 months prior to study enrollment.

7. At least one episode of severe hypoglycemia, defined as an event with one of the following symptoms: memory loss; confusion; uncontrollable behavior; irrational behavior; unusual difficulty in awakening; suspected seizure; seizure; loss of consciousness; or visual symptoms, in which the subject was unable to treat him/herself and which was associated with either a blood glucose level <54 mg/dL [3.0 mmol/L] or prompt recovery after oral carbohydrate, IV glucose, or glucagon administration, in the 12 months prior to study enrollment.

8. Reduced awareness of hypoglycemia as defined by a Clarke score of 4 or more OR a HYPO score greater than or equal to the 90th percentile (1047) during the screening period and within the last 6 months prior to randomization;

OR

Marked glycemic lability characterized by wide swings in blood glucose despite optimal diabetes therapy and defined by an LI score greater than or equal to the 90th percentile (433 $mmol/L^2/h \cdot wk^{-1}$) during the screening period and within the last 6 months prior to randomization;

OR

A composite of a Clarke score of 4 or more and a HYPO score greater than or equal to the 75th percentile (423) and an LI greater than or equal to the 75th percentile (329) during the screening period and within the last 6 months prior to randomization.

Exclusion Criteria:

Patients who meet *any* of these criteria are *not* eligible for participation in the study:

1. Body mass index (BMI) >30 kg/m^2 or patient weight ≤50kg.

2. Insulin requirement of >1.0 IU/kg/day or <15 U/day.

3. HbA1c >10%.

4. Untreated proliferative diabetic retinopathy.

5. Blood Pressure: SBP >160 mmHg or DBP >100 mmHg.

6. Measured glomerular filtration rate (using iohexol) of <80 mL/min/1.73m^2 (or for subjects with an iodine allergy, calculated using the subject's measured serum creatinine and the Modification of Diet in Renal Disease [MDRD] study estimation formula). Strict vegetarians (vegans) with a calculated GFR <70 mL/min/1.73m^2 are excluded. The absolute (raw) GFR value will be used for subjects with body surface areas >1.73m^2.

7. Presence or history of macroalbuminuria (>300mg/g creatinine).

8. Presence or history of panel-reactive anti-HLA antibodies above background by flow cytometry.

9. For female subjects: Positive pregnancy test, presently breast-feeding, or unwillingness to use effective contraceptive measures for the duration of the study and 4 months after discontinuation. For male subjects: intent to procreate during the duration of the study or within 4 months after discontinuation or unwillingness to use effective measures of contraception. Oral contraceptives, Norplant®, Depo-Provera®, and barrier devices with spermicide are acceptable contraceptive methods; condoms used alone are not acceptable.

10. Active infection including hepatitis B, hepatitis C, HIV, or tuberculosis (TB) as determined by a positive skin test or clinical presentation, or under treatment for suspected TB. Positive tests are acceptable only if associated with a history of previous vaccination in the absence of any sign of active infection. Positive tests are otherwise not acceptable, even in the absence of any active infection at the time of evaluation.

11. Negative screen for Epstein-Barr Virus (EBV) by IgG determination.

12. Invasive aspergillus, histoplasmosis and coccidioidomycosis infection within one year prior to study enrollment.

13. Any history of malignancy except for completely resected squamous or basal cell carcinoma of the skin.

14. Known active alcohol or substance abuse.

15. Baseline Hb below the lower limits of normal at the local laboratory; lymphopenia (<1,000/μL), neutropenia (<1,500/μL), or thrombocytopenia (platelets <100,000/μL).

16. A history of Factor V deficiency.

17. Any coagulopathy or medical condition requiring long-term anticoagulant therapy (*e.g.,* warfarin) after transplantation (low-dose aspirin treatment is allowed) or patients with an international normalized ratio (INR) >1.5.

18. Severe co-existing cardiac disease, characterized by ***any one*** of these conditions:
    a) recent myocardial infarction (within past 6 months).
    b) evidence of ischemia on functional cardiac exam within the last year.
    c) left ventricular ejection fraction <30%.

19. Persistent elevation of liver function tests at the time of study entry. Persistent serum glutamic-oxaloacetic transaminase (SGOT [AST]), serum glutamate pyruvate transaminase (SGPT [ALT]), Alk Phos or total bilirubin, with values >1.5 times normal upper limits will exclude a patient.

20. Symptomatic cholecystolithiasis.

21. Acute or chronic pancreatitis.

22. Symptomatic peptic ulcer disease.

23. Severe unremitting diarrhea, vomiting or other gastrointestinal disorders potentially interfering with the ability to absorb oral medications.

24. Hyperlipidemia despite medical therapy (fasting low-density lipoprotein [LDL] cholesterol >130 mg/dL, treated or untreated; and/or fasting triglycerides >200 mg/dL).

25. Receiving treatment for a medical condition requiring chronic use of systemic steroids, except for the use of ≤5 mg prednisone daily, or an equivalent dose of hydrocortisone, for physiological replacement only.

26. Treatment with any anti-diabetic medication other than insulin within 4 weeks of enrollment.

27. Use of any investigational agents within 4 weeks of enrollment.

28. Administration of live attenuated vaccine(s) within 2 months of enrollment.

29. Any medical condition that, in the opinion of the investigator, will interfere with safe participation in the trial.

30. Treatment with any immunosuppressive regimen at the time of enrollment.

31. A previous islet transplant.

32. A previous pancreas transplant, unless the graft failed within the first week due to thrombosis, followed by pancreatectomy and the transplant occurred more than 6 months prior to enrollment.

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Glossary of Abbreviations

ACE	American College of Endocrinology
ACR_{arg}	Acute c-peptide Response to Arginine
ACR_{glu}	Acute c-peptide Response to Glucose
ACR_{max}	Maximal Acute c-peptide Response to Glucose-Potentiated Arginine
AE	Adverse Event
AIDS	Acquired Immunodeficiency Syndrome
AIR_{arg}	Acute Insulin Response to Arginine
AIR_{glu}	Acute Insulin Response to Glucose
AIR_{max}	Maximal Acute Insulin Response to Glucose-Potentiated Arginine
ALS	Antilymphocyte Serum
APC	Antigen Presenting Cell
ATG	Anti-thymocyte Globulin
AST	Arginine Stimulation Test
BG	Blood Glucose
Boost	Liquid meal replacement drink
BMI	Body Mass Index
BW	Body Weight
CBC	Complete Blood Count
CFR	Code of Federal Regulations
cGCP	Current Good Clinical Practice
cGMP	Current Good Manufacturing Practices
CGMS	Continuous Glucose Monitoring System®
CIT	Clinical Islet Transplantation Consortium
CITR	Collaborative Islet Transplant Registry
CIT-TCAE	CIT Terminology Criteria for Adverse Events
CMV	Cytomegalovirus

CNI	Calcineurin Inhibitor
CRF	Case Report Form
CRO	Clinical Research Organization
CTCAE	Common Terminology Criteria for Adverse Events
DAIT	Division of Allergy, Immunology, and Transplantation
DCC	Data Coordinating Center
DCCT	Diabetes Control and Complications Trial
DI	Disposition Index
DIC	Disseminated Intravascular Coagulation
DSMB	Data Safety Monitoring Board
EBV	Epstein-Barr Virus
EC	Ethics Committee
EDTA	Ethylenediaminetetraacetic Acid
EKG	Electrocardiogram
EU	European Union
FDA	Food and Drug Administration
FSIGT	Frequently Sampled Intravenous Glucose Tolerance
GAD	Glutamic Acid Decarboxylase
GCRC	General Clinical Research Center
G-CSF	Granulocyte Colony Stimulating Factor
GFR	Glomerular Filtration Rate
GPA	Glucose-Potentiated Arginine
HbA1c	Glycosylated hemoglobin
HFS	Hypoglycemic Fear Survey
HIV	Human Immunodeficiency Virus
HLA	Histocompatibility Antigen
HPLC	High Pressure Liquid Chromatography
HSA	Human Serum Albumin

HSV	Herpes Simplex Virus
HTLV1	Human T-cell Lymphotropic Virus Type 1
ICH	International Conference on Harmonization
IDS	Investigational Drug Service
IE	Islet Equivalents
IgG	Immune Globulin G
IMPDH	Inhbitor of Inosine Monophosphate Dehydrogenase
IND	Investigational New Drug
INR	International Normalized Ratio
IRB	Institutional Review Board
ITN	Immune Tolerance Network
IV	Intravenous
LDL	Low-density Lipoproteins
LFTs	Liver Function Tests
LI	Lability Index
MAGE	Mean Amplitude of Glycemic Excursions
MMF	Mycophenolate Mofetil
mTor	Mammalian Target of Rapamycin
MMTT	Mixed-Meal Tolerance Test
NCI	National Cancer Institute
NFAT	Nuclear Factor of Activated T-cells
NHP	Non-Human Primate
NIAID	National Institute of Allergy and Infectious Disease
NIDDK	National Institute of Diabetes & Digestive & Kidney Diseases
NIH	National Institutes of Health
NOD	Non-obese Diabetic
PAID	Problem Areas in Diabetes
PBMC	Peripheral Blood Mononuclear Cell

PCP	Pneumocystis Carinii Pneumonia
PCR	Polymerase Chain Reaction
PI	Principal Investigator
pit-hGH	Pituitary Growth Hormone
PLT	Platelet Count
PNF	Primary Non-Function
PPD	Protein Purified Derivative
PRA	Panel Reactive Antibodies
PT	Prothrombin Time
PTLD	Post-Transplant Lymphoproliferative Disorder
PTT	Partial Thromboplastin Time
PSA	Prostate Specific Antigen
QOL	Quality of Life
RDA	Recommended Daily Allowance
RNA	Ribonucleic Acid
SAE	Serious Adverse Event
SAEC	Safety Adverse Event Coordinator
SAP	Statistical Analysis Plan
SC	Subcutaneous
SGOT	Serum Glutamic-Oxaloacetic Transaminase
SGPT	Serum Glutamate Pyruvate Transaminase
SLE	Systemic Lupus Erythematosis
SOP	Standard Operating Procedure
SSL	Secure Socket Layer
T1D	Type 1 Diabetes
TAT	Thrombin-Antithrombin
TB	Tuberculosis
TCAE	Terminology Criteria for Adverse Events

TSH	Thyroid Stimulating Hormone
ULN	Upper Limit of Normal
UNOS	United Network for Organ Sharing
VAT	Videoscopic Assisted Thorascopy
WBC	White Blood Cell Count
WHO	World Health Organization
WOCBP	Women of Child Bearing Potential

1. BACKGROUND AND RATIONALE

1.1 Background

Type 1 Diabetes (T1D) is an autoimmune disease where destruction of the insulin producing pancreatic β cells occurs, leading to severely dysregulated glucose homeostasis. It afflicts nearly 2 million people in the United States, most of them children or young adults. Despite the effectiveness of insulin therapy in allowing these patients to survive, the imperfect control of blood glucose excursions common with insulin injections eventually results in vascular complications in many. In fact, in adults diabetic retinopathy is the most common cause of blindness and diabetic nephropathy is the most common indication for kidney transplantation. The Diabetes Control and Complications Trial (DCCT) established that these microvascular complications of diabetes can be prevented by maintaining near-normal glucose control in patients with T1D[1]. However, this degree of control can be impossible to achieve in many patients despite modern insulin analogs and delivery systems[2], and also leads to life threatening episodes of insulin-induced hypoglycemia[3].

The hope of achieving near-normal glucose control without hypoglycemia in T1D patients has provided the strong impetus for developing effective strategies for β-cell replacement via pancreas or isolated islet transplantation. When successful, pancreas transplantation can normalize blood glucose (BG) in diabetic recipients, with resultant stabilization and even reversal of microvascular complications[4]. However, despite the ability of whole organ pancreas transplantation to correct glucose homeostasis in T1D, the procedure is not without risk. According to UNOS pancreas registry data, almost 10% of whole organ pancreas grafts fail early due to technical complications. Other morbid complications such as anastomotic leak, bleeding, and infection are even more common. As a result of the magnitude of the operation and its potential complications (including death – usually from a postoperative myocardial infarction), this procedure is generally reserved in most centers, including our own, for diabetics who are less than 50 years of age, have minimal if any coronary artery disease, and because of the risks of chronic immunosuppression, already require a kidney transplant for the treatment of end-stage diabetic nephropathy. In fact, whole pancreas transplantation without concomitant kidney transplantation is associated with inferior long-term graft function and survival when compared to simultaneous pancreas-kidney transplantation, due primarily to immunologic graft loss[5, 6]. Thus, T1D patients in need of β-cell replacement to stabilize their metabolic control are often excluded from whole pancreas transplantation unless they also require a kidney graft.

Transplantation of isolated pancreatic islets offers the distinct advantage over whole organ pancreas transplantation that it can be accomplished without a major surgical operation. Consequently, isolated islet transplantation is a much safer treatment, and so may be considered as an option for patients before the development of irreversible diabetic complications. But until recently, <10% of islet transplant recipients experienced insulin-independence after one year, in contrast to the ~ 80% of pancreas transplant recipients. The lower rate of insulin-independence following islet transplantation was attributed to a low engrafted islet mass combined with a high metabolic demand imposed by the glucocorticoids used as part of the immunosuppression[7]. Accordingly, the islet transplant group from Edmonton initiated a protocol where islets isolated

from two or more donor pancreases were transplanted under a glucocorticoid-free immunotherapy regimen.

In the year 2000, the initial report of success with the "Edmonton protocol" represented a major advance in the field of clinical islet transplantation, where insulin-independent amelioration of hyper- and hypoglycemia occurred in seven consecutive T1D recipients with a median follow-up of 12 months[8]. The immunosuppression regimen consisted of a combination of novel T lymphocyte directed induction therapy with the interleukin-2 receptor monoclonal antibody daclizumab, and maintenance therapy with the potent calcineurin-inhibitor (CNI) tacrolimus and the more recently developed agent sirolimus. The efficacy of the Edmonton approach has now been confirmed by several other centers, including our own where even single donor transplant recipients have enjoyed a high rate of initial insulin-independence[9]. Unfortunately, we have found that despite early insulin-independent graft function, hyperglycemia gradually progresses and a second transplant is generally required to sustain an insulin-free state. Comparable loss of graft function over time has been reported from Edmonton, where insulin-independence rates have declined from 80% at one year to 10% by five years[10].

We have recently demonstrated a functionally low engrafted β-cell mass in insulin-independent transplant recipients under Edmonton immunosuppression that likely declines over time[11], suggesting that the eventual recurrence of diabetes and return to insulin therapy may result from both early (engraftment) and late (survival) immunologic graft loss. Thus, immunotherapy according to the "Edmonton protocol," which is directed against T lymphocytes, seems insufficient in protecting islet allografts from immunological rejection and recurrent anti-β-cell autoimmunity. Not withstanding the important effector role of activated T lymphocytes in these two processes, it is well established that a concomitant and specific B lymphocyte response against β-cell derived allo- and auto-antigenic epitopes also occurs, culminating in the generation of islet reactive allo- and autoantibodies. It is our premise that transient B lymphocyte depletion in the setting of islet transplantation may provide several tolerogenic effects: 1) abrogation of cognate T-B lymphocyte collaboration, 2) prevention of *de novo* anti-HLA antibody formation against donor alloantigens and 3) curtailment of the memory B lymphocyte response to islet autoantigens in T1D patients. Together, these effects should enhance the engraftment and survival of transplanted islets, thus improving and sustaining their function so that long-term metabolic control may be realized.

Another limitation of the "Edmonton protocol" is the use of the CNI tacrolimus, which is both nephrotoxic and β-cell toxic. The use of tacrolimus in Edmonton has been implicated in the deterioration of kidney function in islet transplant recipients who have early diabetic nephropathy. Two transplant recipients with pre-existing kidney disease both experienced a rise in serum creatinine while on tacrolimus, and all four subjects who had pre-existing microalbuminuria progressed to macroalbuminuria post-transplant[12]. Furthermore, microalbuminuria has developed and the glomerular filtration rate (GFR) has declined in the Edmonton cohort despite accomplishing near-normal glucose control, pointing to a potentially deleterious renal consequence of the tacrolimus containing immunosuppression[13]. These outcomes have precluded the further involvement of T1D patients with evidence of early diabetic nephropathy from participation in islet transplantation trials. The demonstration of efficacious islet transplantation using a CNI-free immunosuppression regimen would represent a major advance toward the future inclusion of T1D subjects with early diabetic nephropathy in islet

transplantation trials. Such trials would enable the determination of the impact of restoring normoglycemia on the progression of diabetic nephropathy.

In addition to nephrotoxicity, tacrolimus is β-cell toxic, and has been implicated in the pathogenesis of diabetes that occurs after kidney and liver transplantation by impairing insulin secretion[14, 15]. There is even greater concern for the effect of tacrolimus on β cells in islet transplantation, where islets are delivered intraportally and plasma concentrations of the drug reach two-fold higher peak concentrations in the portal versus systemic circulation[9, 16]. Thus, CNI-free immunotherapy should also result in improved functional graft outcomes and contribute to the maintenance of an insulin-independent state after transplantation. Because CNIs lead to a 50% reduction in β-cell secretory capacity[17], the elimination of tacrolimus from islet transplant immunosuppression may result in a 2-fold improvement in post-transplant β-cell function.

In the present protocol we propose to determine the safety and efficacy of B lymphocyte directed immunotherapy using rituximab for achieving insulin-independence (primary endpoint) 75 ±5 days following islet transplantation in a prospective single arm study. In a separate study (CIT-07) a control group will receive standard immunosuppression consisting of the anti-T lymphocyte agent Thymoglobulin® for induction and sirolimus in combination with tacrolimus for maintenance. Because the inclusion of the anti-B lymphocyte agent rituximab in the induction regimen may obviate the need for a CNI, rituximab will replace tacrolimus in this study. Thus, this group will receive rituximab in combination with Thymoglobulin® for induction and sirolimus alone for maintenance in a CNI free regimen. Secondary endpoints have been designed to measure the engraftment of the transplanted islets at 75 ±5 days after the first islet transplant and one year thereafter. Survival of the transplanted islets between the two arms at 365 days after the last islet transplant will also be measured. These endpoints will be complimented by a series of exploratory metabolic and immunologic studies aimed at elucidating the metabolic and immunologic consequences of the islet transplantation procedure and the accompanying immunosuppressive regimens that will hopefully provide new insights into the mechanisms leading to graft success or failure.

1.2 Preclinical and Clinical Experience

1.2.1 Preclinical Studies

The first report of a systematic analysis of the efficacy of anti-lymphocyte serum (ALS) to prevent rejection of islet allograft was reported by Barker, *et. al*. in 1973, demonstrating prolonged survival of allogeneic rat islets in recipients treated with rabbit anti-rat lymphocyte serum[18]. Further confirmation of the tolerogenic efficacy of ALS was confirmed by the permanent survival of rat islet allografts implanted into the thymus or the intraportal site following treatment of the recipients with a single injection of ALS.[19, 20] The efficacy of ALS in large animal models was reported by Hirschberg, *et.al*[21], in a cynomolgus monkey model of islet transplantation including induction with high dose (20mg/kg) of Thymoglobulin® followed by sirolimus monotherapy as a maintenance immunosuppression. Thymoglobulin® resulted in marked lymphocyte depletion that gradually recovered in approximately one month after initiation of the treatment. In this study the majority of the cynomolgus monkeys suffered from

toxicities that were attributed to rapamycin; however, the surviving animals remained insulin independent for 169 days after reduction of rapamycin dose.

1.2.2 Clinical Studies

The rationale for utilization of Thymoglobulin® as an induction immunosuppression has been based on a number of basic studies demonstrating the beneficial effect of this agent on prevention of the recurrent autoimmune disease in transplanted islets, mediated by deletion of autoreactive memory cells or induction of regulatory T cells. As indicated below (please see section 1.4.3.1) two polyclonal anti-thymocyte antibody preparation are available in the US, Thymoglobulin® and ATGAM®. In randomized double blind clinical trials Thymoglobulin® was noted to be more efficacious than ATGAM® for induction immunosuppression for the treatment of acute renal allograft rejection in adult renal transplant recipients. The repertoire of antibodies present in Thymoglobulin® includes a variety of anti-adhesion molecules that have been reported to interfere with leukocyte responses to chemotactic signals inhibiting expression of integrins required for cellular adhesions and mobility. This later effect may be the basis for the effectiveness of Thymoglobulin® in reducing the non specific inflammation during the reperfusion injury of the renal allografts. The clinical experience utilizing Thymoglobulin® as a component of induction therapy in renal transplantation or islet after kidney transplantation has been reviewed in section 1.4.3.1 (please see below). The most relevant experience utilizing induction with Thymoglobulin® in conjunction with maintenance with Rapamune® and Tacrolimus has been reported by Hering, *et.al*. in eight type 1 diabetic recipients of islet transplants[22]. The objective of the study was to assess the efficacy and safety of a single donor islet transplant utilizing induction immunotherapy with Thymoglobulin® with the secondary objective of assessing the proportion of islet transplant recipients who achieve insulin independence in the first year after islet transplantation. There were no serious, unexpected procedure or immunosuppression related adverse events (AEs) and all recipients achieved insulin independence and freedom from hypoglycemia. This clinical experience may be related to improved islet engraftment secondary to pretransplant induction therapy with Thymoglobulin® and use of the anti-inflammatory agent etanercept.

1.2.3 B-Lymphocyte Immunotherapy Preclinical and Clinical Studies

In this trial we will use induction therapy that targets both B and T lymphocytes to prevent allosensitization and recurrent autoimmunity in T1D patients receiving islet transplants. Our prediction is that rituximab will induce B lymphocyte tolerance in transplant recipients. Based on work in transgenic mouse models, central B lymphocyte tolerance can arise through clonal deletion[23] or by receptor editing[24]. Clonal deletion can occur when an immature B lymphocyte encounters a high-affinity self-antigen[25]. Receptor editing, or the continued rearrangement of antibody genes when a B lymphocyte harbors an autoreactive receptor, can rescue autoreactive cells from deletion by changing its specificity[26]. Peripherally, the engagement of a B lymphocyte receptor in the absence of a second signal can result in anergy and/or clonal elimination[27, 28]. Rituximab may promote B lymphocyte tolerance by decreasing the number, diversity or altering the phenotype of B lymphocytes that serve as specific antigen-presenting cells (APCs).

Rituximab therapy has resulted in clinical improvement for autoimmune diseases such as systemic lupus erythematosus[29] and rheumatoid arthritis[30], and can help reverse refractory allograft rejection[31]. This is best explained by the important role for B lymphocyte antigen presentation function in the development and amplification of autoimmune responses[32], and by the deleterious effects of B lymphocyte derived alloantibodies in allograft rejection[33, 34]. Our studies in rodent models[33] have further established that T cell mediated responses to transplanted islets are substantially reduced when cognate T/B collaboration is blocked. When immature B cells begin to re-emerge following rituximab depletion in this protocol, they will do so in the presence of the islet allograft. These immature cells may be more susceptible to tolerance induction[35, 36], and receptor editing may also occur in transitional B lymphocytes exposed to a soluble self-antigen[37]. Collectively, it is proposed that "resetting the clock" for the B lymphocyte repertoire ontogeny opens a window of tolerance susceptibility wherein auto- and allo-reactive B cells are purged from the developing repertoire.

As an essential step toward clinical implementation of this proposition, we initiated a preclinical study to determine whether transient B lymphocyte depletion during the induction phase of immunotherapy protects islet allografts from rejection in cynomolgus monkey (*Macaca fascicularis*) recipients rendered diabetic utilizing 150 mg/kg of streptozotocin. It is generally accepted that induction of immunosuppression with Thymoglobulin® alone does not promote immunological tolerance to allografts[21, 38]; however, this agent is utilized extensively as an induction agent for clinical transplantation. Notwithstanding, as an initial control group, we transplanted three diabetic cynomolgus monkeys with an intraportal infusion of 5000 IEq/kg allogeneic islets, and the recipient monkeys receive an induction immunotherapy regimen consisting of 5 mg/kg Thymoglobulin® on days 0, 2, 5, and 10 post-transplantation. Rapamycin maintenance immunosuppression at 0.1mg/kg was administered daily. Despite the use of Thymoglobulin® at induction and rapamycin maintenance, these recipients rejected the transplanted islets within 14-20 days following transplantation.

The objective of our preclinical studies was to determine whether induction immunotherapy, targeting B-lymphocytes, could promote islet allograft survival in cynomolgus monkeys. To meet this experimental objective we utilized the B lymphocyte specific mAb, rituximab. We modeled our dosage regimen to parallel that used in human lymphoma patients (*i.e.*, 375 mg/m^2) with the modification of administering only two boluses on days 0 and 2[39]. A cohort of four recipient monkeys was transplanted using an induction regimen consisting of combined rituximab and Thymoglobulin® (5mg/kg). As expected, this regimen effectively induces a transient state of B lymphocyte depletion lasting for at least 6-8 weeks. The maintenance immunosuppression included monotherapy with low-dose rapamycin. Rapamycin maintenance therapy was discontinued in all 4 recipients at 200 days following transplantation. The survival time of islet allografts in these four recipients has been: 230, > 310, > 340, and > 400 days. Only one out of the four recipients experienced diabetes recurrence, presumably as a result of rapamycin discontinuation at day 200. The long-term graft survival achieved by including rituximab as an induction agent is in contrast to that seen in control recipients treated with Thymoglobulin®, which even in conjunction with rapamycin maintenance led to rejection of the transplanted islets. These results strongly suggest that inclusion of a B lymphocyte-specific agent into the induction regimen may promote long-term islet allograft tolerance in T1D recipients without the need for a CNI agent[39].

1.3 Rationale for Selection of Study Population

Iatrogenic hypoglycemia is a major unresolved problem for many patients with T1D. It is the limiting factor in the management of T1D, causing some deaths as well as recurrent physical, and recurrent (or even persistent) psychosocial, morbidity[40]. Iatrogenic hypoglycemia is a consequence of 3 compromised defense mechanisms, whose pathophysiology was thoroughly reviewed by Cryer[40-43].

First and perhaps most important, glucose-regulated insulin levels are not present in c-peptide-negative type 1 diabetic patients. The second defense mechanism, glucagon secretion in response to developing hypoglycemia, is lost in virtually all patients with T1D within 5 to 10 years after its onset[44]. Third, epinephrine response to falling glucose levels is compromised, in terms of the magnitude of the response and the threshold for the response[45],, in a subgroup of patients with T1D. Epinephrine is not normally critical, but becomes so when the insulin and glucagon responses are deficient or absent. Those type 1 diabetic patients with an absent insulin response and combined deficiencies of their glucagon and epinephrine responses to falling plasma glucose levels have the clinical syndrome of defective glucose counterregulation; their risk of severe hypoglycemia is 25-fold or more higher than that of those with absent glucagon but intact epinephrine responses[46, 47]. Type 1 diabetic patients with impaired epinephrine responses also have the clinical syndrome of hypoglycemia unawareness, which refers to the absence of adequate autonomic warning symptoms of developing hypoglycemia.

Hypoglycemia unawareness and the associated inability to respond adequately to falling glucose levels explain the frequent episodes of neuroglycopenia in such patients. Moderate hypoglycemia refers to a hypoglycemic episode complicated by neuroglycopenia in which the patient is still able to overcome the situation without assistance. Severe hypoglycemia refers to a situation in which neurologic impairment is severe enough to prevent self-treatment, placing patients at risk for injury to themselves or others. Accordingly, the DCCT Research Group defined severe hypoglycemia as an event with symptoms consistent with hypoglycemia in which the patient requires the assistance of another person; it is associated with a BG level below 50 mg/dL and with prompt recovery after oral carbohydrate, intravenous (IV) glucose, or glucagon administration[48]. The DCCT Research Group definition replaced the more stringent 1980s definition of severe hypoglycemia based on loss of consciousness[49-51].

Cryer suggested viewing the 3 clinical syndromes (defective glucose counterregulation, hypoglycemia unawareness, and elevated glycemic thresholds) during effective intensive insulin therapy as manifestations of hypoglycemia-associated autonomic failure. All 3 syndromes segregate together and are associated with a high frequency of iatrogenic hypoglycemia. Parenthetically, they do not segregate with classical diabetic autonomic neuropathy[46, 52, 53]. Hypoglycemia-associated autonomic failure is triggered by recurrent episodes of hypoglycemia, which reduce the magnitude of hormonal counterregulation and reduce symptomatic responses to a given degree of subsequent hypoglycemia[43, 54], thereby initiating and perpetuating a vicious cycle.

Hypoglycemia-associated autonomic failure is an important risk factor for severe hypoglycemia, which is associated with significant morbidity and mortality. Patients with hypoglycemia unawareness have a nearly 7-fold increased risk of severe hypoglycemia[55]. Those with combined deficiencies of their glucagon and epinephrine responses to falling plasma glucose levels have a 25-fold or more greater risk of subsequent severe hypoglycemia, as compared with those with absent glucagon but intact epinephrine responses[46, 47]. The patient characteristic that most strongly predicted severe hypoglycemia in the DCCT was a history of prior severe hypoglycemic events[56].

Hypoglycemia is said to be a major concern of prospective employers[57]. Neuroglycopenia can cause social embarrassment, and even lead to ostracism or be mistaken for disorderly or unlawful behavior[40]. The more distressing the severe hypoglycemic episode, the greater the psychological fear of hypoglycemia[58]. The threat and fear of severe hypoglycemia can significantly discourage patients and health care providers from pursuing intensive insulin therapy and can therefore can be a major but unrecognized impediment to achieving euglycemia[40, 58]. Pramming *et al*. found that their patients were as concerned about the development of severe hypoglycemia as they were about the development of blindness or renal failure[59].

In patients with long-term (*i.e.*,>15 years) T1D, scrupulous avoidance of hypoglycemia fails to restore normal glycemic thresholds or magnitudes of hormonal counterregulation to hypoglycemia. Avoidance of iatrogenic hypoglycemia sufficient to reverse the clinical syndrome of hypoglycemia unawareness does not normalize the key components of the clinical syndrome of defective glucose counterregulation (*i.e*., deficient glucagon and epinephrine responses to hypoglycemia)[60-64]. In one recent report on patients with autonomic neuropathy and longstanding diabetes, Fanelli *et al*. demonstrated that, after meticulous prevention of hypoglycemia, only the threshold – not the magnitude – of responses of autonomic symptoms was normalized. In addition, the recovery of epinephrine responses to hypoglycemia was barely appreciable[65]. Thus, it appears that, while hypoglycemia unawareness is reversible by meticulous prevention of hypoglycemia, defective glucose counterregulation may not be reversible[66].

A successful pancreas transplant restores epinephrine responses and symptom recognition during hypoglycemia in patients with longstanding T1D and autonomic neuropathy[67]. In type 1 diabetic islet transplant recipients with documented pretransplant hypoglycemia unawareness and defective hormonal counterregulatory responses during hypoglycemia, Meyer *et al*. demonstrated, at 1 month post-transplant, improved glycemic thresholds and/or peak incremental responses of epinephrine, norepinephrine, and cortisol, as well as restoration of autonomic warning symptoms during hypoglycemia[68]. In a more recent study by Paty *et al*., intrahepatic islet transplantation did not restore hypoglycemic hormonal counterregulation or symptom recognition in type 1 diabetic recipients[69]. Ryan *et al*. documented the absence of episodes of severe hypoglycemia in 12 successful islet transplant recipients (median follow-up, 10.2 months)[70] whose diabetes was complicated by recurrent episodes of severe hypoglycemia pretransplant. This would suggest that hypoglycemia associated autonomic failure associated with defective counter-regulation and impaired sympathodrenal responses is not just due to recurrent hypoglycemia. After a sustained period without any hypoglycemia, most patients post-

islet transplant still had defective responses to hypoglycemia. The absence of clinically significant hypoglycemia post-islet transplant despite the persistent defect in counterregulation in most subjects demonstrates the dominance of the absence of glucose regulated insulin secretion in the pathogenesis of severe hypoglycemia. Correction of this can only currently be attained with transplantation of beta cell tissue.

Given the above reasons, the risk of an islet transplant and of the associated immunosuppressive treatments is particularly justifiable in the subgroup of patients whose T1D is complicated by hypoglycemia-associated autonomic failure (as clinically manifested by hypoglycemia unawareness and a history of recurrent severe hypoglycemia). For the subgroup of patients unable to continue intensive insulin therapy because of recurrent severe hypoglycemia, an islet transplant may currently be the only approach to achieving the benefits of euglycemia, without the risks associated with hypoglycemia and without the extensive surgery required for a vascularized pancreas transplant. Thus, the potential direct benefits to this subgroup are sufficient to offset the risks of participation in islet transplant trials.

1.3.1 Glycemic Lability

Defining labile diabetes is a challenge but a working definition of labile diabetes may be: "Very variable glucose control associated with unpredictable responses to insulin". Labile diabetes is akin to the more extreme brittle diabetes which has been defined as describing the patient "whose life is constantly being disrupted by episodes of hypo- or hyperglycemia whatever their cause[71, 72]. Brittle diabetes in addition to lability has the added connotation that there may be associated frequent admissions to hospital [73, 74]. Given the rationing of health care over the last decade, use of such parameters such as admission to hospital has become problematic. Early publications postulated that brittle diabetes was related to subcutaneous (SC) insulin degradation[75] but now the most severe cases are recognized to usually have a factitious origin[76]. While the most extreme cases of labile diabetes, whether associated with recurrent hypoglycemia or diabetic ketoacidosis, may fall into the traditional brittle definitions, there are many patients with T1D who have very labile glucose control that is a source of frustration for them and their caregivers.

When faced with labile diabetes the first consideration is that of diabetes management. It is prudent to assess the insulin regimen, the appropriateness of the insulin dose, the timing of the insulin relative to meals, the meal plan and use of carbohydrate counting. Comorbid conditions that should be sought are coeliac disease, Addison's disease and hyperthyroidism in addition to a history of gastrointestinal surgery. Particular attention has to be paid to any psychological issues or stresses having an impact on diabetes management. The erratic response of glucose to exogenous insulin in some patients, despite optimization of diet intake, modulation of exercise, use of all the newer insulin analogues or insulin pumps leaves some patients totally frustrated and unable to trust what response they will have to any given amount of insulin. It is also testimony to the intrinsic merit of a glucose sensing insulin delivery system.

The HbA1c is the standard measure of glucose control and is used in all major studies as an endpoint of glycemic control. It has been valuable as a risk predictor of diabetes complications. Yet the HbA1c may be misleading. Patients with erratic glucose control, especially if having

hypoglycemic unawareness, can have an HbA1c below 7%, yet the most chaotic and difficult glycemic control. Mean glucose values suffer the same problem in that swings in glucose values between 2 and 20 and back to 2 mmol/L may give a mean glucose of 8 mmol/L, a poor reflection of the real state of affairs.

Previous efforts at measuring glucose lability have ranged from qualitative to quantitative. Earlier definitions of brittle diabetes have incorporated visits to the hospital[73, 74] but these are subject to the vagaries of local practice. More quantitative measures have been the mean amplitude of glycemic excursions (MAGE)[77] and the M value of Schlichtkrull[78]. The MAGE relies on capillary glucose readings over two days (a minimum of seven readings a day) and an amplitude is an excursion of glucose in excess of the standard deviation of the mean values for the day. If the mean of these amplitudes is ≥11.1 mmol/L the subject is considered to have labile diabetes. Where the MAGE fails as a measure of lability is with the subject who has a gradual decline in glucose over the day from 22 to 2 mmol/L. Such a profile will give a MAGE of 20 but such a gradual decline need not be considered truly labile.

Also used in the past has been the M value of Schlichtkrull[78] but this logarithmic expression of the glucose deviation from a standard glucose level has not been validated. More recently[79] the advent of continuous glucose monitoring system® (CGMS) has allowed insight into the patterns of glucose. The CGMS® profiles give exquisite details that have been quantified in terms of mean and standard deviation. Determining lability with this process has been more difficult and the suggested method has been the determination of the absolute value of measured glucose minus 5.5 mmol/L. This has the drawback that sustained high glucose levels will result in a high value but the profile may not necessarily be labile. In addition, the technique is currently limited to three days of monitoring and may be less accurate at low glucose levels[80].

Any measure needs to be robust enough to handle a variety of glucose monitoring patterns used in day-to-day diabetes practice, intuitive in that it measured glucose swings, mathematically rigorous and finally easy to use. A newer measure of lability based on the change in glucose over time has been the Lability Index (LI)[81]. A typical range for a diabetes population was calculated in 100 subjects with T1D who were not selected because of any particular problems. Most subjects have scores 300 $mmol/L^2/h \cdot wk^{-1}$ with a median of 223 ($25 - 75^{th}$ percentiles 130 – 329 $mmol/L^2/h \cdot wk^{-1}$). An LI ≥ 433 $mmol/L^2/h \cdot wk^{-1}$ (90^{th} percentile) indicated serious problems with glycemic lability. The LI correlated well with a clinical scoring of lability by diabetologists and showed improvement after successful islet transplantation and rose when graft function was lost.

The LI has proven useful in the assessment of subjects being considered for an islet transplant. Many patients have been referred with labile diabetes based on the subjective impressions of their caregivers. The LI helps place the difficulty of their glucose control in perspective. The LI has also been useful in the follow-up of subjects after transplantation. The LI after the first transplant improved dramatically once endogenous insulin was provided to smooth insulin delivery and with insulin independence, the LI was superb. It should be clear that the LI is simply a measure of the glucose lability and not an indication for an islet transplant. Rather it

indicates that there is a problem, and islet transplantation is only an option when other avenues of diabetes management have been exhausted.

Severe glycemic lability is of great importance to a minority of patients that experience it and consumes a disproportionate amount of clinic resources. In the long term, the lability of glucose control in addition to the elevation of the HbA1c may be important in terms of diabetes complications. Quantifying lability as outlined here is a first step to help studying it and the effects of various interventions such as continuous SC insulin infusion, carbohydrate counting, insulin analogues, etc. If these avenues have been exhausted and comorbid disease excluded in a patient with labile diabetes, then beta cell replacement therapy, either as an islet or pancreas transplant, may be the only way to correct the erratic glucose levels and give back to the patient a sense of normality and control over his/her life. For this select group of subjects with very disruptive labile diabetes, islet transplantation and its concomitant risks is a reasonable alternative to be considered.

1.4 Rationale for Selection of Study Treatment Regimen

1.4.1 Primary Investigational Product: Allogeneic Islets

Islet transplantation is an experimental mode of β-cell replacement therapy, of which whole pancreas transplantation is the only currently available alternative. As discussed above, the potential morbidity associated with whole pancreas transplantation limits its application to patients already undergoing major abdominal surgery for the placement of a kidney graft to treat end-stage diabetic nephropathy. Because islet transplantation is a much simpler procedure, its use is being advocated before the development of irreversible diabetic complications where it should allow for determination of the long-term effect of β-cell replacement therapy on diabetes complications. Until any hoped for long-term benefits of islet transplantation are demonstrated, its experimental application has been in T1D patients with hypoglycemia unawareness or glycemic lability who can benefit from islet transplantation in the short-term through achieving near-normal glycemia without life-threatening hypoglycemia. Also being studied in other protocols are T1D patients already receiving immunosuppression for a kidney graft, but who did not receive a simultaneous whole pancreas transplant (Protocol CIT-06). In this study, an initial kidney transplant is performed alone because of either the availability of a living kidney donor or the unavailability of a suitable cadaveric pancreas. Instead of undergoing another major abdominal operation to receive a pancreas, islets are transplanted for β-cell replacement therapy – a much less risky alternative. Finally, because islets are isolated from deceased donor pancreata not utilized for whole organ transplantation, their use extends the availability of β-cell replacement therapy to patients with severe diabetes, who would otherwise have no therapeutic options.

1.4.2 Other Investigational Agent: Rituximab

Islet transplantation under the Edmonton immunosuppression regimen results in a functionally low engrafted β-cell mass[11], and a progressive loss of graft function over time[10]. These observations, made by our and the Edmonton group, respectively, suggest that both early (engraftment) and late (survival) immunologic graft loss currently preclude long-term insulin-

independent success from islet transplantation. Because immunotherapy according to the "Edmonton protocol" is directed only against T lymphocytes, and it is well established that a concomitant and specific B lymphocyte response against β-cell-derived allo- and auto-antigenic epitopes also occurs after transplantation, we believe that the addition of transient B lymphocyte depletion may provide several tolerogenic effects: 1) abrogation of the cognate T-B lymphocyte interatctions, 2) prevention of *de novo* anti-HLA antibody response against donor alloantigens and 3) modulation of the islet autoantigen memory B lymphocyte reportoir. Together, these effects should enhance the engraftment and survival of transplanted islets, thus improving and sustaining their function so that long-term insulin-independent success may be realized. Protocol CIT-05 will add the B lymphocyte anti-CD20 monoclonal antibody rituximab to the T lymphocyte anti-thymoglobulin polyclonal antibody used in the induction regimen.

Because CNIs such as tacrolimus are both nephrotoxic and β-cell toxic, there is a need to develop immunosuppressive regimens for islet transplantation. Such regimens promise to extend the eligibility of islet transplantation to T1D subjects with early diabetic nephropathy, and permit determination of the impact of restoration of normoglycemia on nephropathy progression. Importantly, a cohort of successful islet (after kidney) transplant recipients experienced significantly improved kidney graft survival and stable urinary albumin excretion when compared to a cohort of unsuccessful islet recipients who had progressive albuminuria, suggesting that islet transplantation may retard the progression of diabetic nephropathy[82]. Furthermore, the elimination of CNIs should result in improved β-cell function and improved functional graft outcomes after transplantation. Protocol CIT-05 will determine the feasibility of a CNI free regimen in human islet transplantation by eliminating the tacrolimus used in the control arm (CIT-07) with the addition of rituximab during the initial induction of immunotherapy in the experimental arm (CIT-05) based on the efficacy of this approach in our NHP model of islet transplantation (please see below).

1.4.3 Immunosuppressive Medications for Initial Islet Transplant

1.4.3.1 Anti-Thymocyte Globulin

The rationale for anti-thymocyte globulin (ATG) induction immunosuppression includes prevention of autoimmune recurrence in transplanted islets via deletion of autoreactive memory cells, prophylaxis of islet allorejection, avoidance of the use of calcineurin inhibitors (CNIs) in the immediate post-transplant period, induction of regulatory T cells with reduced requirements for maintenance immunosuppression, and attenuation of nonspecific inflammatory responses to transplanted islets, thereby maximizing engraftment and functional survival of transplanted islets and the success rate of single-donor islet transplants.

Two polyclonal anti-thymocyte antibody preparations have been marketed in the United States, Thymoglobulin® and ATGAM®. Two randomized double-blind clinical trials indicated that Thymoglobulin® is more efficacious than ATGAM® for induction immunosuppressive therapy and for the treatment of acute graft rejection episodes in adult renal transplant recipients[83, 84]. Thymoglobulin® induction therapy achieved rejection-free allograft survival in 96% of the patients. The incidence of cytomegalovirus (CMV) disease in the first year was 12.5%, and no patient developed post-transplant lymphoproliferative disease (PTLD). ATG is known to

contain a variety of anti-adhesion molecule antibodies.[85]. It interferes with leukocyte responses to chemotactic signals and inhibits the expression of integrins required for firm cellular adhesion. Such mechanisms of action may account for the effect of ATG on nonspecific inflammation and reperfusion injury and may explain the 1% incidence of delayed graft function in kidney recipients[38, 83, 85-87]. Recent studies have shown that early administration of a variety of antibodies directed at adhesion molecules reduces graft dysfunction, and acute and chronic rejection associated with ischemia-reperfusion injury and brain death[88].

The resistance of islet-directed autoimmune responses to conventional immunosuppressive drugs[89-93] and the immediate exposure of intraportally transplanted islets to primed autoreactive, islet beta cell-directed T cells provide a strong rationale for pretransplant initiation of ATG, which is known to cause selective depletion of activated T cells and dose-dependent depletion of resting T cells[86]. Experimental data suggest that the protection of whole pancreas transplants from recurrent autoimmunity is functionally related to the inclusion of a significant quantity of lymphoid tissue (possibly containing an immunoregulatory T cell subset) as part of the pancreas graft and not to immunosuppression alone[94]. Clinical evidence also indicates that destructive anti-islet autoimmunity persists for decades after manifestation of T1D[90, 95, 96] and that type 1 diabetic individuals with long disease duration do not spontaneously anergize their autoreactive effector Th1 cells and/or restore Th2 or other regulatory T cell function. Accordingly, reprogramming the recipient's immune system seems to be of paramount importance if autoimmune recurrence in transplanted islets is to be prevented.

Maki *et al*. demonstrated that immunotherapy of non-obese diabetic (NOD) mice with ALS after development of overt autoimmune diabetes leads to long-lasting abrogation of autoimmunity[97]. ALS given within 14 days of disease onset gradually reversed hyperglycemia with a 76% cumulative incidence of remission. Diabetic NOD mice that failed to respond to ALS treatment accepted subsequent islet isografts for a prolonged period (mostly >100 days), indicating that autoimmunity was abrogated in the latter animals in which extensive irreversible beta cell destruction had already occurred by the time of ALS treatment. These experimental findings are corroborated by clinical observations reported by the Brussels group[98]. Of 7 islet-after-kidney recipients treated at Brussels, only the 3 patients who had received ATG as induction immunosuppressive therapy during the first 10 days following their previous kidney transplant showed long-term islet graft survival. Furthermore, according to an analysis performed by the International Islet Transplant Registry on all 50 insulin-independent, type 1 diabetic islet allograft recipients transplanted through 1999, 23 had received single-donor islet transplants, and 19 of those 23 had received anti-thymocyte or anti-lymphocyte globulin for induction immunosuppression and 1 had received ATG at the time of a previous pancreas transplant[99]. It is conceivable that the need for 2-3 donor pancreata as a source of islets in the Edmonton experience reflects the inability of the induction immunotherapy to completely abrogate the anti-islet autoimmune response. Even a low level of persistent autoimmunity may interfere with the function of transplanted islets via pro-inflammatory cytokine mediated inhibition of insulin secretion. The ATG immunotherapy as proposed in this trial may be advantageous due to the deletion/inhibition of anti-islet directed autoreactive T cells.

There are two published reports of steroid-free transplantation with Thymoglobulin®. Birkeland reported on 68 kidney transplant recipients treated with steroid-free immunosuppression using an initial 10-day ATG induction and maintenance therapy with cyclosporine and mycophenolate mofetil. No steroids were given at any time. After an observation for up to 2.5 years (median 488 days, range 127-945 days), 66 patients (one died from sepsis after six months and one died from peritonitis after returning to dialysis) were alive and well, 64 grafts were functioning well, hemolytic-uremic syndrome recurred in one graft, one graft had to be removed for non-compliance, and two patients returned to dialysis after chronic rejection. These investigators observed only 10 acute rejections (15%)[100]. Cantarovich reported on 28 consecutive type 1 diabetic patients who underwent simultaneous kidney-pancreas transplantation. All patients received ATG, cyclosporine, and mycophenolate mofetil. Steroids were not administered at any time. Only two patients required anti-rejection treatment. Patient, kidney, and pancreas survival has been reported to be 96.4%, 96.4% and 75%, respectively. CMV infection was diagnosed in eight patients. All but one patient tolerated the ATG course well[101]. These two studies indicate that ATG can be used safely and effectively without concomitant steroid administration.

The total ATG dose to be administered is 6 mg/kg. This dose is based on studies performed at Washington University in St. Louis[102]. This reduced total dose of ATG has been found to be equally effective for induction immunosuppression in kidney transplantation when compared to historical controls that had received 1.5 mg/kg per day for at least seven days[83]. The proposed ATG dose escalation strategy has been pioneered by James Russell in Calgary, Alberta, in more than 70 bone marrow transplant recipients (presented at the European Bone Marrow Transplant Meeting in Innsbruck, Austria, April, 2000). The University of Minnesota has reported their preliminary experience with this regimen of ATG administration in 8 type 1 diabetic islet transplant recipients[99]. ATG was found to be effective in preventing rejection and autoimmune recurrence. All eight recipients have achieved insulin independence. The medication was well tolerated in all subjects; unexpected acute complications were not encountered. Serious adverse events (SAEs) were not encountered secondary to ATG.

In the event that a second or third transplant is required to achieve or maintain insulin independence, a monoclonal anti-interleukin-2 receptor antibody (daclizumab or basiliximab) will be used to limit the total dose of ATG administered to any one recipient.

Induction immunotherapy with anti-interleukin-2 receptor antibody is a critical component of the steroid-free immunosuppressive protocol recently developed for islet transplantation by the Edmonton group[8]. The safety and efficacy of daclizumab and basiliximab have previously been documented in multi-center trials in renal transplantation. When added to therapy with cyclosporine, azathioprine, and prednisone, daclizumab reduced the frequency of acute rejection and improved short-term graft survival in renal transplant recipients, and basiliximab reduced the frequency of acute rejection and did not affect graft or patient survival.At six months, there were no significant differences between the daclizumab or basiliximab and the placebo group with respect to infectious complications or cancers[103].

1.4.3.2 Maintenance Immunosuppression with Sirolimus

Diabetogenic side effects of immunosuppressive therapy are particularly deleterious in the situation of a reduced beta cell mass (like in islet transplantation), contributing to the historically poor success rate of human islet allografts. The combination of CNIs and prednisone is associated with the development of an insulin-dependent diabetic state in up to 25% of non-diabetic kidney transplant recipients[15]. To maintain normoglycemia, immunosuppressed non-diabetic kidney transplant recipients must increase insulin secretion 2.5 times[104]. Even when systemic drug levels are carefully controlled, intraportally transplanted islets bathed in portal blood are exposed to higher and probably toxic local concentrations of orally administered immunosuppressive drugs[16]. This may not matter when there is a normal beta cell mass, as with a whole pancreas transplant. The limited mass of engrafted islet beta cells however, is inadequate to restore insulin independence in the presence of impaired insulin secretion and action mediated by CNIs in combination with steroids[8, 104-106].

Sirolimus is a promising agent for maintenance immunosuppression of islet allograft recipients, mainly because of its efficacy in the absence of diabetogenic side effects[107]. Sirolimus is as effective as cyclosporine A in preventing renal graft loss due to rejection while maintaining superior graft function[108]. Sirolimus combined with the concentration-controlled regimen of cyclosporine presents a promising synergistic regimen which reduces the incidence of acute rejection episodes among recipients of kidney grafts markedly, permits profound cyclosporine dose reduction, and facilitates corticosteroid avoidance or withdrawal[109]. A recent pilot study in 32 organ transplant recipients (liver, kidney, and pancreas) demonstrated the safety and efficacy of a regimen combining sirolimus with a low dose (33% of the recommendation), of tacrolimus and steroids[110]. The almost complete absence of renal dysfunction, hypertension, and diabetes in these patients is explained by the low blood levels of tacrolimus (5.7 ± 3.2 ng/mL). Extremely low rejection rates are an essential prerequisite for islet transplantation, since without access to reliable diagnostic markers or early rejection, irreversible islet destruction may occur before the onset of hyperglycemia. The low rate of opportunistic infections suggests that the patients were not excessively immunosuppressed.

1.4.4 Induction Immunosuppression for Subsequent Islet Transplants

The immunosuppressive regimen for subsequent islet transplants will be identical to the regimen for the initial islet transplant with the exception of Thymoglobulin®. Daclizumab or basiliximab will be used instead of Thymoglobulin® for all subsequent islet transplants.

1.5 Known and Potential Risks and Benefits to Human Subjects

1.5.1 Risks of Use of Investigational Agent: Transplant of Allogeneic Islets

Transplantation of islets is associated with several potential risks. These risks may be categorized in terms of: a) transmission of disease from donor to recipient, b) risk of microbial contamination of islet preparations, c) sensitization of the recipient to donor antigens, d) acceleration of retinopathy with acute correction in glycemic control, and e) psychological

impact of successful or failed islet transplantation. Other risks including portal thrombosis, portal hypertension, bleeding or hepatic steatosis are discussed separately in section 1.5.3.

1.5.1.1 Transmission of disease from donor to recipient

Selection of potential donors for islet isolation must follow stringent guidelines. The aim of this process is to avoid use of any potential donor that might harbor transmissible viral disease or malignancy.

A potential donor must have a favorable medical, sexual and social history, and clear all standard laboratory tests for low-risk of transmission of donor disease. Donor families are therefore questioned about high risk lifestyle and detailed medical history. Donor blood samples are screened for conditions including (but not limited to) Human Immunodeficiency Virus (HIV)1, HIV2, Human T-cell Lymphotropic Virus Type 1 (HTLVI) or HTLVII, hepatitis B, hepatitis C, CMV, Epstein Barr Virus (EBV) disease and syphilis.

Donors are excluded if: a) there is known pre-existing metabolic disease including T1 or Type 2 diabetes, or if the HbA1c is elevated above 6.1% in the absence of transfusions in the week prior to death, b) if there is malignancy other than primary brain tumors, c) septicemia is present or suspected at the time of death, d) there is evidence of clinical or active viral hepatitis (A, B or C), acquired immunodeficiency syndrome (AIDS), syphilis, active viral encephalitis of unknown origin, Creutzfeldt-Jacob disease, rabies, treated or active tuberculosis, septicemia, dementia, individuals that have received pituitary growth hormone (pit-hGH), or serious illness of unknown etiology.

Therefore islets will only be isolated from donors who have undergone the same screening process used by the UNOS or similar procedures as required by competent organ procurement organizations in the country performing solid organ transplants. With careful donor selection as summarized above, the risk of transmission of disease from donor to recipient is regarded as low.

The administration of valganciclovir routinely post-transplant may minimize risk for certain viral pathogens. The risk of transmission of CMV disease from donor to recipient has been surprisingly low in recipients of islet allografts to date, particularly in the most recent era with routine use of purified islet preparations. For instance, there have been no episodes of CMV disease in 77 consecutive islet recipients transplanted at the University of Alberta. In the international Immune Tolerance Network (ITN)/NIAID multi-center islet trial, there was no CMV disease in any of the 36 patients transplanted at the nine different sites. Sixteen of 36 (44%) subjects were CMV positive initially. Two initially negative subjects became CMV IgG positive without any apparent clinical sequelae. The University of Miami recently presented data on three islet recipients that became CMV positive and one did develop CMV disease occurring late, after discontinuation of anti-viral prophylactic therapy.

Therefore while CMV transmission from donor to recipient may occur in islet transplantation, the fact that islet preparations are purified and are contaminated with only a low number of passenger lymphocytes may explain why the risk of CMV transmission from donor to recipient is much less in islet transplantation than in other solid organ transplant grafts.

With respect to EBV transmission, only recipients who are EBV positive are acceptable for the current trial. EBV polymerase chain reactions (PCR) monitoring will be carried out routinely after transplantation at defined intervals throughout the trial. EBV disease and the risk of PTLD have not been reported in the recent era of clinical islet transplantation, suggesting that the risk of this complication may be less than 2%.

1.5.1.2 Risk of microbial contamination of islet preparations

As isolated islets have gone through an extensive processing technique, the potential risk of bacterial contamination of the islet product exists. The processed islets must fulfill stringent in-process and lot release criteria before use in transplantation. A Gram stain is obtained (and must be negative), and an endotoxin determination is completed (less than 5 EU/kg based on the recipient weight), prior to product release for transplantation. A sample of the final islet product is obtained prior to the addition of antibiotics and the absence of adventitious microbial and fungal contaminants is confirmed. Broad-spectrum antibiotics are added to the released final product prior to transplant to further diminish the subjects' risk of infection.

In 152 islet preparations transplanted consecutively at the University of Alberta since 1999, there have been no cases of transmission of bacterial or fungal disease through islet transplantation when islets are prepared under cGMP conditions. One recipient of an islet autograft received an infected islet preparation as the autograft pancreas contained a chronic embedded pancreatic stent that likely led to bacterial colonization and contamination. This recipient developed transient complete thrombosis of the portal vein with subsequent recanalization.

In 74 islet preparations transplanted consecutively at the University of Miami since 1999, there have been no cases of transmission of bacterial or fungal disease through islet transplantation, when islets are prepared under cGMP conditions.

There have been previous reports of two cases of islet transplantation-related septicemia (Enterobacter cloacae) due to transplantation of contaminated cryopreserved pancreatic islets[111]. Additionally, the University of Minnesota investigators have previously reported on the incidence and significance of contaminated islet preparations in clinical islet auto- and allotransplantation[112]. Positive cultures from islet tissue preparations were identified in 11 of 29 patients (38%) receiving autologous islets. The occurrence of serious infection morbidity (as defined as positive blood cultures, abscesses, or intra-abdominal infections) did not differ significantly between the positive and negative culture groups (p=0.99). In the allogeneic islet transplant group, 7 of 33 patients (21%) received tissue that retrospectively were determined to be contaminated. None of these patients developed serious infectious complications (despite broad-spectrum immunosuppression). Despite the occurrence of contaminated grafts, there was no serious increase in infectious morbidity. Presumably the inocula were kept low by the multiple washing steps allowing the recipients to clear the organisms without serious sequelae.

Of the islet allotransplants performed at the University of Minnesota between 1993 and 1999, 3 of 20 patients (15%) received tissue that was retrospectively determined to be contaminated. The species isolated included *Candida krusei*, *Enterococcus faecium*, and two strains of

coagulase-negative Staphylococcus. None of these patients have had SAEs related to the contamination of the transplanted islet tissue.

Additional steps have been taken to decrease the incidence of contamination. First, since 2000, pancreatectomy specimens for clinical islet allotransplantation have exclusively been processed under cGMP regulations. Overall, the risk of islet transplantation-related septicemia is considered very low in view of the precautions detailed in the islet manufacturing protocol.

1.5.1.3 Sensitization of the recipient to donor antigens

As with any allogeneic transplant, islet transplant recipients may become sensitized to islet-donor histocompatibility antigens (HLA), leading to development of panel reactive alloantibodies (PRA). These alloantibodies may develop while the recipients demonstrate full or partial islet function on maintenance immunosuppression. Furthermore, donor specific alloantibodies may develop after loss of the islet transplant function and discontinuation of the immunosuppressant drug. Data on the development of cytotoxic antibodies against donor HLA in islet allotransplant recipients with failing grafts have been reported from several islet transplant centers[20, 113, 114]. In the ITN-sponsored trial of islet transplantation using the Edomonton protocol of steroid-free immunosuppression, 5 of 36 subjects had evidence of elevated PRA post-transplant when measured by flow cytometry. Two of these 5 subjects experienced primary islet non-function. Moreover, data from five participating centers in the current CIT consortium indicate that approximately 25% of the islet alone transplant recipients developed a PRA >20% while on maintenance immunosuppression. These results are comparable to those reported for recipients of kidney transplant with stable serum creatinine and on maintenance immunosuppression[115-117]. Importantly, the incidence of elevated PRA (>20 %) in recipients who had lost their islet transplant function and discontinued their immunosuppression rose to approximately 84%.

The available information suggests that there is a strong correlation between islet allograft failure and a rise in anti-donor HLA sensitization as detected by PRA testing. A potential consequence of high PRA levels in type 1 diabetic recipients with failed islet transplants is that if these individuals develop diabetic nephropathy in the future, it may increase their time waiting on a transplant list to qualify for a suitable kidney[118].

1.5.1.4 Acceleration of retinopathy with acute correction in glycemic control

In the DCCT study[48], about 10% of patients with pre-existing retinopathy receiving intensive treatment experienced a transient worsening of their retinopathy during the first year, but nonetheless had a lower cumulative incidence of sustained progression when compared to the conventional group after the third year. A transient worsening of retinopathy has not been formally documented in islet transplantation trials, but it is assumed that a similar process might occur. Exclusion of patients with unstable retinopathy and careful post-transplant follow-up will help to minimize the incidence of such occurrences and their morbidity should they occur.

When type 1 diabetic recipients of successful and unsuccessful pancreas transplants were compared for the end point of an increase of two or more grades in the retinopathy score, they did not differ significantly in the rate of progression whether retinopathy was mild (Grade P0 to P5) or advanced (Grade P6 to P14) at baseline [119]. Long-term follow-up of both groups suggested that successful pancreas transplantation may have a late beneficial effect that becomes evident only after 36 months.

1.5.1.5 Psychological impact of successful or failed islet transplantation

Clinical islet transplantation, as a potential therapy for T1D, has been discussed in the media and diabetes lay publications with an excessive degree of optimism not justified on the basis of clinical results to date. Therefore, failure of the procedure to reverse hyperglycemia and maintain insulin independence could be associated with a level of psychological disappointment that might progress to clinical depression. The informed consent process has been carefully organized to minimize unrealistic expectations or legal ramifications. Patients who appear to be incapable of understanding and/or coping with the possibility of failure will not be transplanted.

1.5.2 Risks of Induction and Maintenance Immunosuppressive Therapies

Administration of all immunosuppressive and immunomodulatory therapies used presently to prevent rejection of transplanted tissues carry general risks of opportunistic infection and malignancy, including lymphoma (~1%), and skin cancers. These agents are not recommended for nursing mothers, and it is recommended (and mandated in the current protocol) that women of childbearing potential use effective contraception before, during and for at least 4 months following administration of these agents.

1.5.2.1 Monoclonal Antibody IL-2 Receptor Blocker

All subjects will receive one of the following monoclonal antibody IL-2 receptor blockers (daclizumab or basiliximab):

1.5.2.1.1 Daclizumab (Zenapax®)

Daclizumab is a humanized anti-CD25 monoclonal antibody approved by the Food and Drug Administration (FDA) since 1997 for prophylaxis against acute organ rejection in adult recipients of renal allografts. It is generally well-tolerated without substantial side effects, and is usually given at a dose of 1-2mg/kg IV either as a two-dose regimen (on Day 0 and 4), or as five doses given at bi-weekly intervals. In four kidney transplant trials including 336 patients receiving daclizumab compared to 293 receiving placebo, there was no difference in the rates of reported AEs or incidence of infections (13 vs. 16% for CMV) or malignancies (1.5 vs. 2.7%, with <1% lymphoma in both groups, see product monograph for details). The most frequently reported AEs were gastrointestinal complaints (constipation, nausea, diarrhea, vomiting) occurring equally in 67 vs. 68% of subjects. There may be an increase in cellulitis and wound infections (8.4 vs. 4.1%), but infectious mortality was lower (<1 vs. 2%). As with any protein product, anaphylaxis can occur, particularly with repeated administration, but this has been reported only rarely.

1.5.2.1.2 Basiliximab (Simulect®)

Basiliximab is a chimeric (murine/human) monoclonal antibody (IgG1k) approved by the Food and Drug Administration (FDA) for prophylaxis against acute organ rejection in adult recipients of renal allografts. It is usually given at a dose of 20 mg IV on Days 0 and 4. Basiliximab is associated with constipation, nausea, abdominal pain, vomiting, diarrhea, dyspepsia, peripheral edema, fever, viral infections, hyperkalemia, hypokalemia, hyperglycemia, hypercholesterolemia, hypophosphatemia, hyperuricemia, urinary tract infections, upper respiratory infections, surgical wound complications, acne, hypertension, headache, tremor, insomnia, and anemia. In the four placebo-controlled studides, the pattern of adverse events in 590 patients treated with the recommended dose of basiliximab was similar to that in 594 patients treated with placebo (see product monograph for details). Basiliximab did not increase the incidence of serious adverse events observed compared with placebo. As with any protein product, anaphylaxis can occur, particularly with repeated administration, but this has been reported only rarely.

1.5.2.2 Rabbit Antithymocyte Globulin (Thymoglobulin®)

Rabbit Thymoglobulin® was approved by the FDA in 1999 for the treatment for acute renal graft rejection in conjunction with concomitant immunosuppression (see product monograph for details). It is a polyclonal IgG antibody obtained by immunization of rabbit with human thymocytes and contains cytotoxic antibodies directed against antigens expressed on human T lymphocytes. Thymoglobulin® has shown a consistent safety profile with most AEs being manageable and reversible; the most common events are fever, chills and leukopenia. While rare, the most severe events include allergic or anaphylactoid reactions and serum sickness. As with all immunosuppression, administration of Thymoglobulin® may be associated with an increased risk of infection and development of malignancy (especially of the skin and lymphoid system).

In 82 kidney transplant recipients receiving 1.5 mg/kg/day for 7 – 14 days, the principal AEs were fever (52%) and chills (47%) associated with the infusions, leucopenia (47%), and thrombocytopenia (30%). CMV infection (13%) and PTLD (2%). Neutropenia has been described; anaphylaxis has been reported rarely.

Published results of the use of Thymoglobulin® in clinical and experimental islet transplantation are limited to relative small cohorts. Hirshberg *et al* described the successful role of rabbit ATG and sirolimus in reducing rejection of islet allografts in primates, with no evidence of direct islet toxicity from Thymoglobulin®[120]. Hering *et al* described a beneficial role of Thymoglobulin® induction (6mg/kg) in 8 patients with T1D receiving single donor islet grafts, all of whom achieved insulin independence and were protected against recurrence of hypoglycemia[22]. Acute islet rejection was described in patients receiving calcineurin-free immunosuppression when sirolimus levels fell below 9ng/mL. The use of higher doses of sirolimus exacerbated the neutropenic side effects of Thymoglobulin®, but these could be managed safely without risk of opportunistic infections when appropriate dose reduction and/or administration of Granulocyte Colony Stimulating Factor (G-CSF; Neupogen®) if required[22].

1.5.2.3 Rituximab (Rituxan®)

In 121 rheumatoid arthritis patients receiving 1 gram of rituximab twice in combination with cyclophosphamide, methotrexate, or nothing compared to 40 patients receiving methotrexate alone, there was an increase in AEs associated with the first infusion more than the second that included transient hypotension (26 vs. 18%), cough (7 vs. 0%), pruritus (7 vs. 0%), rash (7 vs. 3%), and dyspnea (3 vs. 0%)[48]. There was no increase in serious infections (3.3 vs. 2.5%); however, fatal bronchopneumonia developed in one patient receiving rituximab who had underlying ischemic heart disease.[48] Of 27 kidney transplant recipients who received rituximab as part of treatment for refractory graft rejection, no post-transplant lymphoproliferative disease was identified after 605 ± 335 days of follow-up[47]. Recently, a clinical picture resembling serum sickness has been described in 2 out of 6 patients with Sjogren's syndrome who received rituximab[121]. However, serum sickness is not a common side effect of rituximab therapy in other diseases in which this therapy has been attempted to date. Rare severe infusion and mucocutaneous reactions, some with fatal outcomes, have been reported in patients treated with rituximab for malignant diseases such as leukemia and lymphoma.

In December 2006 the FDA posted an alert concerning the safety of rituximab therapy for systemic lupus erythematosus (SLE). This alert was based on the occurrence of two deaths caused by progressive multifocal leukoencephalopathy (PML) in SLE patients who had received repeated courses of rituximab in addition to past cytotoxic medications, including cyclophosphamide. PML is a rare but often fatal disease caused by reactivation of latent JC virus in immunocompromised patients, such as those with advanced HIV and those who have received chemotherapy with or without bone marrow transplantation. PML has been reported in 24 other cases of SLE with no exposure to rituximab. PML has occurred in 23 patients treated with rituximab for hematologic malignancies who also received chemotherapy and sometimes hematopoietic stem cell transplantation. The manufacturer of rituximab, Genentech, estimates that ~ 10,000 patients with SLE have been treated with rituximab, and that ~ 250,000 – 1,000,000 patients all together have received rituximab. An expert review committee compiled by the NIDDK/Trial Net that oversees the use of rituximab in studies of recent-onset type 1 diabetes concluded that the present data do not support a causal relationship between rituximab and PML.

1.5.2.4 Sirolimus (Rapamune®)

The FDA approved sirolimus (rapamycin, Rapamune®) as an immunosuppressive agent in 1999 (see product monograph for details). In 208 kidney transplant recipients receiving 5 mg of sirolimus daily compared to 124 receiving placebo, there was an increased incidence of hypercholesterolemia (46 vs. 23%), hyperlipemia (57 vs. 23%), rash (20 vs. 6%), arthralgia (31 vs. 18%), diarrhea (35 vs. 27%), anemia (33 vs. 21%), leucopenia (13 vs. 8%), thrombocytopenia (30 vs. 9%), and hypokalemia (17 vs. 9%). Side effects are related to drug concentration and are improved with maintenance of the sirolimus 24-hour trough level between 10–20 ng/mL.

Of infections, only mucosal herpes simplex virus (HSV) occurred at a greater rate with sirolimus. There was no increase in rate of malignancy (3.4 vs. 3.1%). While sirolimus was originally proposed as a non-nephrotoxic agent, it is becoming apparent that sirolimus-associated

nephrotoxicity does occur in clinical practice. Crew *et al* described two patients with thrombotic microangiopathy secondary to sirolimus exposure[122]. Sirolimus alters the pharmacokinetic profiles of other CNIs (*e.g*. tacrolimus) and may thereby potentiate nephrotoxicity[123]. Fervenza *et al* described nephrotoxicity from sirolimus in patients with chronic glomerulopathies that was non-reversable on cessation of therapy[124]. Nephrotoxicity from combined sirolimus and tacrolimus has been described in patients with T1D undergoing islet transplantation, particularly where there is underlying pre-existing renal damage from diabetes[12, 125].

The majority of islet transplant recipients receiving sirolimus in conjunction with tacrolimus have experienced transient mouth ulceration, lower extremity edema[8, 125]; perinephric edema and a high incidence of benign ovarian cysts have also been described in islet recipients in association with sirolimus[126]. Pneumonitis and colitis have also occurred[127].

Concerns have been raised by the FDA regarding trials of combined sirolimus/tacrolimus in liver transplant recipients, where there has been a statistically increased risk of hepatic artery thrombosis and late death in sirolimus-treated recipients. A careful analysis of these events does not establish causative association between sirolimus/tacrolimus and thrombosis or death events. There was no increased association with portal venous thrombosis in the liver transplant trials. While sirolimus continues to be used off-label in islet recipients, there is not presently felt to be an association between portal thrombus formation in islet recipients and the use of sirolimus or tacrolimus.

1.5.2.5 Tacrolimus (Prograf®)

Tacrolimus (Prograf®, FK506) has been in wide clinical use for the prevention of allograft rejection since 1994 when the FDA approved it after several years of testing. Tacrolimus is a macrolide antibiotic which inhibits calcineurin after binding intracellularly to FKBP12 within T cells, inhibiting IL-2 transcription. Tacrolimus is invariably administered with other immunosuppressive agents but is known to be associated with several side effects including hypertension, diabetes, nephrotoxicity, hyperkalemia, dyslipidemia, pruritis, and neurologic sequelae (including tremor, ataxia, and extremely rarely central pontine myelinolysis), nausea, vomiting and diarrhea (see product monograph for details). In 205 kidney transplant recipients receiving tacrolimus, the principal AEs were neurologic (tremor [54%], headache [44%], insomnia [32%], parathesia [23%]) and gastrointestinal (diarrhea [44%], nausea [38%], constipation [35%]) complaints, hypertension (50%), and kidney dysfunction (52%); hyperkalemia (31%) and hyperglycemia (22% in previous non-diabetics) also occurred. The severity of these events appears to be dose dependent, with very high plasma levels also producing delirium, seizures, and coma. Complications can be minimized with the relatively low dose long-term therapy typically used in islet transplant trials.

1.5.2.6 Cyclosporine (Neoral®)

Cyclosporine is associated with renal dysfunction, tremors, hirsutism, hypertension, and gum hyperplasia.

1.5.2.7 Mycophenolate Mofetil (CellCept®) And Mycophenolate Sodium (Myfortic®)

CellCept® and Myfortic® are associated with diarrhea, leucopenia, vomiting, and evidence of higher frequency of certain types of infections. CellCept® and Myfortic® may increase the risk of developing lymphomas and other malignancies, particularly of the skin, and have been known to cause fetal harm when administered to a pregnant woman. Cases of progressive multifocal leukoencephalopathy, sometimes fatal, have been reported in patients treated with CellCept® or Myfortic®.

1.5.3 Risks of Study Procedures

The procedures involved with the care of research subjects undergoing clinical islet transplantation include risks pertaining to: a) blood draw testing, b) metabolic stimulation testing, c) the procedural risks of islet implantation (using either the percutaneous transhepatic or direct surgical cannulation of tributaries of the portal vein approach), and d) specific follow-up testing.

1.5.3.1 Blood Draw Testing

Peripheral blood draws performed during these research studies will not exceed 450 mL per six-week period. The subject may experience some discomfort at the site of the needle entry, and there is risk of bruising at the site. There is a remote risk of fainting or local infection.

1.5.3.2 Metabolic Stimulation Testing

The risks associated with metabolic testing are generally regarded as minor. Placement of IV cannulae may be associated with pain and discomfort at the puncture site, bruising, bleeding, displacement, interstitial infusion of fluids; rarely local vein thrombosis, infection or thrombophlebitis may develop.

The administration of bolus glucose or insulin by mouth or intravenously may lead to acute hypoglycemia or hyperglycemia; or rarely ketoacidosis may occur. The administration of bolus arginine intravenously may cause a transient metallic taste in the mouth; allergy is rare. The administration of insulin during the FSIGT, euglycemic clamp, or hypoglycemic clamp studies could lead to a greater degree of hypoglycemia than expected, but would be rapidly corrected with intravenous glucose. The stable isotope of glucose infused during the glycemic clamps carries no additional risks.

1.5.3.3 The Procedural Risks of Islet Transplantation

Islets may be infused into the hepatic portal vein either by an open surgical approach or by a percutaneous transhepatic approach.

Open Surgical Approach

This procedure is usually carried out under general anesthesia, but can be performed occasionally under local anesthesia if required. The potential risk of acute bleeding is anticipated to be less with a controlled operative approach as opposed to a percutaneous approach, especially where a transplant site does not have access to local expertise in advanced interventional radiological procedures. Access to a tributary of the portal vein using the open technique requires a surgical incision for exposure, and direct cannulation of a branch of the middle colic vein, the inferior mesenteric vein, a tributary of the superior mesenteric vein or direct cannulation of a small omental vein. Potential acute surgical risks include bleeding at the surgical site, portal thrombosis, hepatic abscess, hepatic infarction, mesenteric ischemia and mesenteric thrombosis. The general risks of surgery include wound infection, wound hernia, adhesional bowel obstruction, deep vein thrombosis and pulmonary embolism. Risks associated with anesthesia include difficulties with airway management, cardiac arrhythmias and drug-related anaphylactic reactions. Pain and discomfort at the surgical site is expected in the early period following surgery, and may be reduced by administration of opiate, opioid or non-steroidal analgesic medications. If an ileus develops, a prolonged hospital stay may be anticipated.

Percutaneous Transhepatic Approach

Transhepatic portal vein catheterization may have complications and morbidity similar to those associated with transhepatic cholangiography and percutaneous core needle biopsies of the liver. The most common morbidity of transhepatic portal vein catheterization (percutaneous approach) is abdominal or right shoulder tip referred pain. In addition, liver hemorrhage and intra-abdominal bleeding have been known to occur as well as pneumothorax, hemothorax, damage to the gall bladder, or pleural effusion. If a percutaneous approach is used, ablative techniques are employed to reduce the risk of acute bleeding after catheter withdrawal. This procedure is usually carried out in interventional radiology using a combination of ultrasound and fluoroscopic guidance with administration of radio-opaque contrast media to assure proper localization of the infusion. Though the use of contrast media will be minimized, some subjects can develop local or systemic reactions to such products.

Risk of Bleeding after Percutaneous Islet Transplantation

In the 158 islet transplant procedures submitted to the Collaborative Islet Transplant Registry (CITR), the reported SAEs associated with bleeding include hemoperitoneum (n=1), intraabdominal bleed (n=2), low hemoglobin (n=1), right hemothorax (n=1), and subcapsular hematoma of the liver.[128] Subcapsular hematoma of the liver following percutaneous transhepatic injection of islets into the portal vein in two cases has also been reported to the International Islet Transplant Registry. No surgical intervention was necessary[7]. One instance of injury to hepatic artery leading to death during percutaneous transhepatic catheterization of

the portal vein has been reported previously to the Islet Transplant Registry[7]. Reports on intra-abdominal (n=1)[127] and intrathoracic bleeding (n=1)[129] have been published. The risk of significant hemorrhage after percutaneous islet transplantation defined as a drop in hemoglobin of more than 25 g/L or the need for transfusion or surgery was 9% in the Edmonton series[12]. Subsequently, a further increase in risk of bleeding has been observed by the Edmonton program and has been attributed in part to concomitant aspirin therapy[125]. The risk has since been ameliorated by avoidance of pre-transplant aspirin and more effective measures to seal the catheter tract in the liver[125]. When effective methods are used to ablate the transhepatic portal catheter tract, bleeding can be avoided completely; at the University of Miami D-Stat thrombostatic agent has been used to seal the catheter tract and has avoided risk of bleeding[130]. At the University of Minnesota, no bleed-related complications occurred in 20 consecutive subjects when the catheter tract was sealed with combined coils and gelfoam[22].

Hypoglycemia

Severe hypoglycemia is a risk associated with the infusion of islets. Iatrogenic hypoglycemia in the immediate post-transplant period is a rare event. Frequent blood glucose monitoring immediately following islet transplantation is recommended to avoid severe unrecognized hypoglycemia in the early post-transplant period, In longer-term follow-up, life-threatening hypoglycemia (Grade 4) occurred in six of the 236 SAEs reported to CITR[131]. For these six occurrences, the events occurred at the following time intervals; 59 days post the third infusion, 230 days post the second infusion, 296 days post the second infusion, 360 days post the third infusion, 673 days post third infusion, and 318 days post the second infusion. The local CITR investigators did not attribute any of the six events to the infusion procedure or to the immunosuppression medication..

Hypotension

Hypotension induced by infusion of islets into the portal vein is a rare complication of islet transplantation. Severe, grade 3 hypotension (*i.e.*, sustained hypotension persisting for more than 24 hrs requiring therapy) has not been experienced by any subject participating in a 36 subject international multicenter ITN islet trial, nor was it a recognized complication in 151 islet transplant procedures carried out consecutively at the University of Alberta. Frequent blood pressure monitoring in the post-transplant period is part of the protocol-regulated safety assessments.

In the era of non-purified islet preparations and high endotoxin collagenase preparations (before the availability of Liberase®), post-islet transplant hypotension requiring transient use of vasopressors was noted in 15% of the islet autograft recipients, of whom 50% required inotropic support with dopamine following injection until the end of surgery[132].

Disseminated Intravascular Coagulation (DIC)

DIC has been documented after autologous islet transplantation of dispersed pancreatic islet tissue in 3 out of about 400 patients expected to have undergone this procedure[133-135].

Consumption of clotting factors from the extensive pancreatectomy surgery as well as the preparation of non-purified islet tissue from a chronic pancreatitis specimen may have contributed to the coagulopathy. DIC following islet allotransplantation has neither been reported in the literature nor communicated to the CITR. Frequent monitoring of coagulation parameters in the post-transplant period will be part of the protocol-regulated safety assessments.

Hepatic Dysfunction and Steatosis

Transient abnormalities in liver enzyme tests have been observed immediately following intraportal islet transplantation[136, 137]. Three of the 86 islet transplant recipients reported to CITR have experienced transient elevations of liver enzymes requiring prolongation of post-transplant hospitalization or admission[128]. Persistence of laboratory abnormalities indicative of liver dysfunction and likely or definitely induced by intraportal islet transplantation is a rare event; abnormalities in liver function tests (LFTs) usually resolved within 4 weeks[136]. No correlation between the increase in LFTs and graft characteristics or graft function was found. Periportal hepatic steatosis has been described following intraportal islet allotransplantation in 20% of the studied subjects[138, 139] and appears to be due to a paracrine action of insulin secreted from intrahepatic islets. More subjects with steatosis required supplementary exogenous insulin than not[138], suggesting that steatosis may be associated with insulin resistance and graft dysfunction. The clinical relevance of steatosis associated with intrahepatic islet transplantation remains questionable. To the best of our knowledge, there is no evidence of clinically significant, persistent liver dysfunction following intraportal islet transplantation.

Portal Hypertension

Portal hypertension following intraportal infusion of unpurified allogeneic islet tissue resulted in a tear of the splenic capsule requiring splenectomy in one case[7]. The elevation in portal pressure following intraportal islet transplantation is temporary in most instances. In 1981, Cameron *et al*. reported on 4 patients with chronic pancreatitis who developed portal hypertension during intraportal infusion of only partially purified auto islet preparations, and in whom direct or indirect measurements of portal pressure were performed 3 to 12 months later[140]. In all patients, the portal pressure had returned to normal and portal venograms were normal. Casey *et al* reported on changes in portal pressure following sequential islet transplants at the University of Alberta, and found that third islet transplants were associated with significantly greater final portal pressures (18 mmHg) than first or second transplants (12 mmHg)[141]. The baseline pressures were normal in all cases, suggesting absence of chronic portal hypertension[141]

Portal Vein Thrombosis

Transplanted islets release tissue factor and exhibit prothrombotic properties when infused to an intravascular site such as the portal vein[142]. A partial portal vein thrombosis has been reported in one of six patients transplanted at the intramural National Institutes of Health (NIH) program[127]. In the Edmonton single-center experience, the risk of partial vein thrombosis was 3% in more than 100 intraportal islet transplants[125]. The management of partial vein thrombosis includes

anticoagulation therapy which may lead to intra-abdominal hemorrhage requiring transfusion and surgical intervention[127]. There is one published report of complete thrombosis of the portal vein thrombosis after transplantation of partially purified pancreatic islets in a combined islet/liver allograft, which necessitated emergency re-transplantation of the liver[143]. This complication probably related to the transplantation of partially purified islet tissue derived from 4 donors into a freshly transplanted liver. A right upper quadrant ultrasound including Doppler examination of the portal vein is performed on islet transplant recipients on days 1 and 7 post-transplant. Early diagnosis and prompt management of branch vein portal occlusion with systemic heparinization may prevent clot propagation. Repeated intraportal islet transplants are generally contraindicated in patients that have experienced prior portal thrombus.

Injuries to Other Structures

One instance of gall bladder perforation during percutaneous transhepatic catheterization of the portal vein requiring laparoscopic cholecystectomy has been reported to the Islet Transplant Registry[7]. Acute cholecystitis, possibly related to percutaneous transhepatic catheterization of the portal vein, has been noted in 2 of the 86 islet allograft recipients reported to CITR[128]. Gall bladder hematoma (n=1) and gall bladder opacification (n=2) have been observed as well.

1.5.3.4 Follow-up Procedures

Glomerular Filtration Rate (GFR)

Risks associated with the GFR procedure are minimal and are related to the blood draw process. Rarely, the following will occur: excessive bleeding at blood draw site, syncope, extravasation of injection, hematoma, or infection. Iohexol has been widely used and has an excellent safety record. Very occasionally, allergic reactions to iohexol may occur[144].

1.5.4 Benefits

Successful islet transplantation alleviates T1D patients from life-threatening hypoglycemia and psychosocially debilitating glycemic lability[125]. While the long-term durability of these responses is at present uncertain, they persist for as long as some graft function is maintained, despite the eventual return to insulin therapy in the majority of recipients. This partial function, as indicated by continued c-peptide production, may be present in as many as 80% of recipients after 5 years[10]. Furthermore, as long as graft function is maintained, fear of hypoglycemia and anxiety are significantly lower after islet transplantation[145, 146]. Indeed, T1D subjects in the DCCT who had persistent c-peptide production had a significantly reduced risk of severe hypoglycemia despite intensive insulin therapy[146]. Additionally, while most transplant recipients experience only a temporary reprieve from exogenous insulin therapy, a few have maintained insulin-independent graft function for more than 3 years. Novel strategies aimed at promoting the engraftment or survival of transplanted islets may lead to improved long-term graft function and further the duration of insulin-independence after transplantation, and hopefully lead to reductions in the secondary complications of T1D.

2. OBJECTIVES

2.1 Primary Objective

The primary objective is to determine the proportion of subjects who are insulin independent at 75 ± 5 days following the first islet transplant among subjects treated with an experimental islet transplant immunosuppression regimen which includes rituximab and excludes tacrolimus.

This group will be compared, as a secondary analysis, to subjects in a concurrent protocol, CIT-07, which uses a standard immunosuppressive regimen.

2.2 Secondary Objectives

The primary and secondary endpoints will be evaluated in this trial. Endpoints will be compared to corresponding endpoints in the CIT07 study.

In addition to achieving insulin independence, we hope to demonstrate that islet transplantation can result in near-normal glycemic control with amelioration from excessive glycemic lability and severe hypoglycemic episodes, and in an improved quality-of-life (QOL). Then the degree and durability of these responses will be determined.

Assessment for progression or development of secondary diabetes complications will include retinal photography, urinary albumin excretion (UAE), and GFR. Finally, we will monitor for procedural complications and AEs related to the immunosuppression regimen.

As previously mentioned, a drawback of the Edmonton approach is the use of CNI agents with known nephrotoxic and diabetogenic side effects. In fact, it's been recognized that some patients transplanted according to the "Edmonton protocol," who have early nephropathy, will experience a deterioration of renal function, mandating discontinuation of tacrolimus. Therefore, the large subset of T1D patients with early nephropathy are currently excluded from clinical islet transplantation trials due to the use of tacrolimus. In this regard, our pre-clinical results in NHP recipients (please see Preclinical Studies) have indicated that combined B- and T- lymphocyte immunotherapy (using a combination of rituximab and Thymoglobulin®), may obviate the need for tacrolimus maintenance therapy. Thus, we will determine the feasibility of a CNI free regimen in human islet transplantation by eliminating the tacrolimus used in the control arm (CIT-07) with the addition of rituximab during the initial induction of immunotherapy in the experimental arm (CIT-05). The benefit or harm of eliminating tacrolimus will be assessed by determining the proportion of subjects who remain insulin-independent at 365 days following the last islet transplant.

Further exploratory metabolic and immunologic studies will evaluate the mechanisms of graft function and dysfunction following islet transplantation. Metabolic studies will include: CGMS®; a frequently-sampled intravenous glucose tolerance (FSIGT) test that provides measures of first-phase insulin secretion (AIR_{glu}), insulin sensitivity (S_I), and their composite disposition index (DI); a glucose-potentiated arginine (GPA) test that provides estimates of functional β-cell mass (AIR_{arg} and AIR_{max} [β-cell secretory capacity]); and paired hypoglycemic-euglycemic clamps that provide an evaluation of glucose counter-regulation. Immunologic

studies will include: evaluations of innate immunity; autoantibody responses; alloantibody responses; immunophenotyping and immunogenetic analysis of lymphocytes; and analysis of protective immunity and antigen-specific immune responses.

Together with other CIT data this will help to establish islet release criteria that accurately characterize the islet product and are predictive of transplant outcomes.

3. STUDY DESIGN

This is an open-label, prospective, single-arm, single-center trial of islet transplantation under a novel experimental regimen that replaces the CNI tacrolimus with the anti-B lymphocyte agent rituximab. We will use rituximab at a dose of 375 mg/m^2 per injection administered twice, one week apart. This is a modification of the dose used for non-Hodgkin's lymphoma in humans, which is 375 mg/m^2 per injection administered four times at weekly intervals,[147] and is less than the recommended dose use for the treatment of rheumatoid arthritis, which is 1000 mg twice 15 days apart.[30] In addition, this dose lead to sustained (>6 months) B lymphocyte depletion, and caused a low (4.3%) incidence of human antichimeric antibody (HACA) formation[31]. Previous studies demonstrated that this dose is safe and depletes B lymphocyte in patients with high-titer panel reactive antibodies experiencing kidney allograft rejection and awaiting kidney transplantation[148, 149].

Objectives of this trial include gaining experience with the experimental immunosuppression treatment regimen as well as to determine the proportion of subjects with insulin independence on this experimental regimen. Should these subjects demonstrate a very high rate of insulin independence at 75 ± 5 days post-transplant (a rate of 40% or higher would be considered high by current standards), then this treatment may be considered for further study.

There is a larger parallel trial with additional centers and more subjects (CIT-07) where subjects are treated with the standard Islet Transplant immunosuppression regimen. Subjects will be randomized to receive immunosuppression under either CIT-07 or this study (CIT-05) at the time a suitable islet preparation is available for transplantation. The randomization will provide a mechanism for balance between the two studies and to provide an objective selection of subjects to the two studies.

All subjects will be non-obese adults aged 18 – 65 years who have a more than 5 year history of T1D, absent stimulated c-peptide (<0.3 ng/mL), and either frequent moderate-to-severe hypoglycemic episodes or marked glycemic lability despite intensive insulin therapy and glucose monitoring under the direction of a diabetologist for at least 12 months. Additionally, all subjects will have a normal GFR and no macroalbuminuria while receiving ACE-inhibitors or angiotensin receptor antagonists in addition to other anti-hypertensive agents as needed in order to maintain their blood pressure <130/85 mmHg.

T1D islet transplant subjects will receive rituximab (375 mg/m^2 on days -2, and +6) and Thymoglobulin® (0.5 mg/kg on day -2, 1.0 mg/kg on day -1, and 1.5 mg/kg on days 0, 1, and 2 for a total of 6 mg/kg) for the induction of immunosuppression. One dose of methylprednisolone (2 mg/kg) will be administered with the Thymoglobulin® on day -2 to mitigate the transient cytokine-release associated with initial doses of Thymoglobulin® and rituximab. The first dose of rituximab will follow the first dose of Thymoglobulin®. Additional medications administered before each dose of Thymoglobulin® and rituximab include acetaminophen and diphenhydramine. During the immediate post-transplant period pentoxyfilline 400 mg po TID for 4 weeks will be administered as anti-inflammatory therapy. The maintenance regimen will include only sirolimus at a loading dose of 0.2 mg/kg of body weight on day -2, followed by 0.1 mg/kg per day adjusted to maintain a therapeutic trough level of 12 - 15 ng/mL.

Intraportal delivery of islets isolated from cadaveric donors will be achieved using a transperitoneal approach through a mini-laparotomy[84]. Islets will be ABO compatible and have a negative serum crossmatch for T lymphocytes, but HLA matching will not be required. It is estimated that >600,000 IEq will be required per recipient at an average of 9,000 IEq/kg of recipient body weight to achieve insulin independence. To optimize the chance of achieving insulin-independence with no more than two procedures, islet preparations ≥5,000 IEq/kg will be used for the first infusion, and islet preparations ≥4,000 IE/kg will be used for subsequent infusions[8, 11]. The quantity of tissue transplanted will be < 7 mL per infusion[8, 150]. Quality control testing will include assessment of islet purity (> 30%), viability (> 70%), sterility (negative gram stain), and endotoxin content (< 5 IU/kg recipient) prior to release[150]. Functional assessment of islets will also be performed.

Islets will be infused together with heparin and ciproflaxin (70 U/kg) with periodic portal pressure monitoring. Heparin will be continued for 48 hours after the procedure as an intravenous drip with target PPT levels of 50-60 seconds. Following completion of heparin, subjects will be treated with enoxaprin 30 mg subcutaneously bid through day 7.

Subjects who do not meet criteria for insulin-independence at any time after 75 days following the initial transplantation may be eligible for a second transplantation of islets. If the development of panel-reactive anti-HLA antibodies has occurred, only subjects with a PRA ≤ 20% will remain eligible, and both antibody specific and prior mismatched antigens will be avoided. To avoid problems related to Thymoglobulin® sensitization, we will administer daclizumab (2 mg/kg on day 0 followed every two weeks with 1 mg/kg for 4 additional doses) in order to sustain an anti-T lymphocyte induction effect. Should a third infusion of islets occur to achieve insulin-independence, only if the interval between the second and third islet infusions is >10 weeks will an additional course of daclizumab be administered. Rituximab administration will not be repeated with either a second or third infusion of islets.

The therapeutic trough level for sirolimus will be lowered to 10 – 12 ng/mL three months following the last islet infusion, or earlier if intolerable mouth ulcers or myelosuppression develops. If therapeutic trough levels of sirolimus cannot be maintained, mycophenolate mofetil (MMF, 500-1500 mg twice daily) may be added to ensure adequate immunosuppression: alternatively, sirolimus may be replaced by tacrolimus at a dose of 0.015 mg/kg PO BID adjusted to maintain trough levels of 6 - 10 ng/mL with the addition of mycophenolate mofetil (MMF, 500 - 1500 mg twice daily).

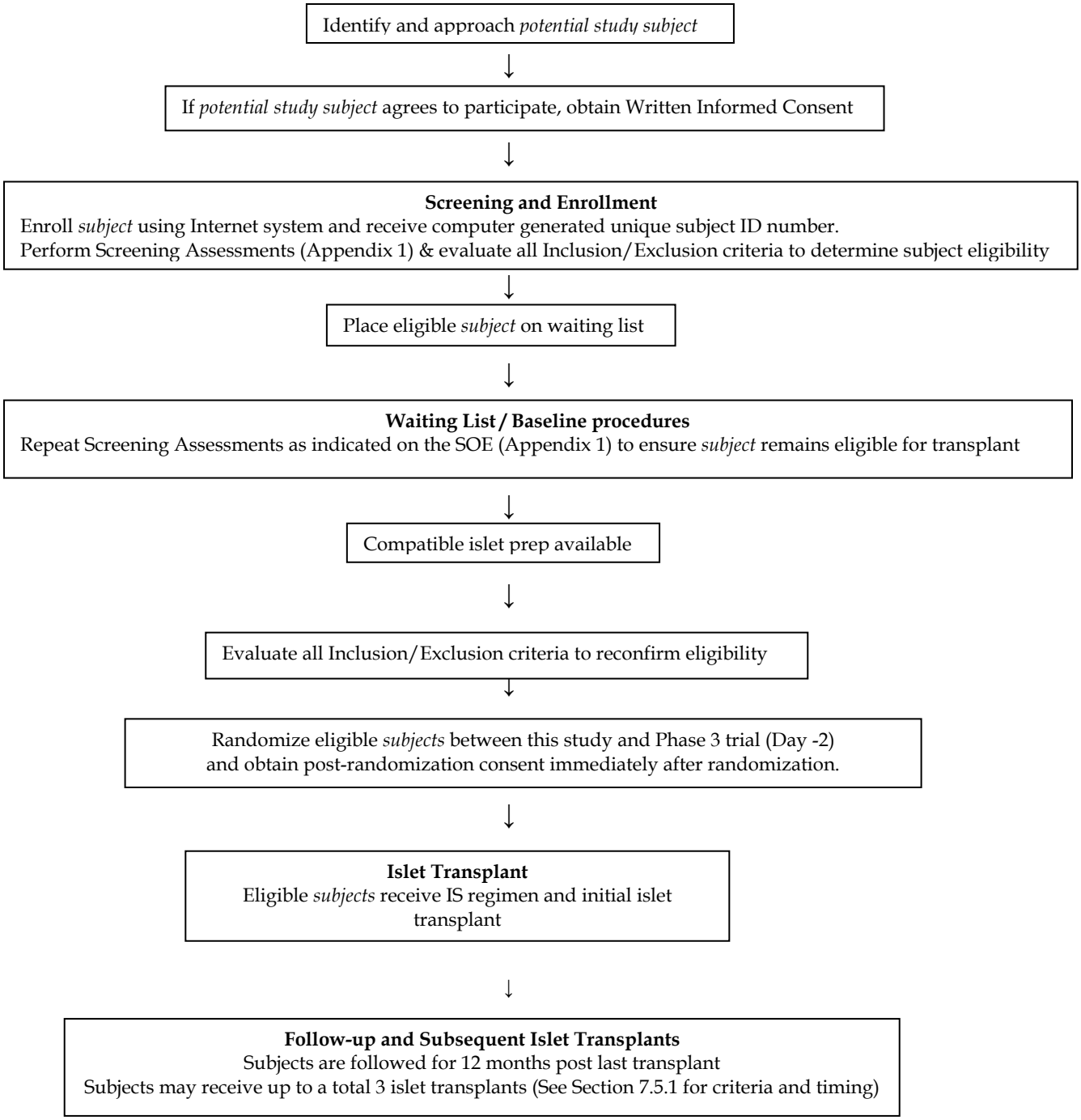

Figure 1: Study design diagram

Study Endpoints

3.1 Primary Endpoint

The primary endpoint will be insulin-independence at 75± 5 days following the first islet transplant.

Insulin Independence Definition:

Islet transplant recipients will be considered insulin-independent with full islet graft function if they are able to titrate off insulin therapy for at least 1 week and all of the following criteria are met:

- HbA1c ≤7.0% or a ≥2.5% decrease from baseline.
- fasting capillary glucose level should not exceed 140 mg/dL (7.8 mmol/L) more than three times in the past week (based on measuring capillary glucose levels a minimum of 7 times in a seven day period);
- 2-hour post-prandial capillary glucose should not exceed 180 mg/dL (10.0 mmol/L) more than three times in the past week (based on measuring capillary glucose levels a minimum of 21 times in a seven day period);
- fasting serum glucose level ≤126 mg/dL (7.0 mmol/L); if the fasting serum glucose level is >126 mg/dL (7.0 mmol/L), it must be confirmed in an additional one out of two measurements;
- evidence of endogenous insulin production defined as fasting or stimulated c-peptide levels ≥0.5 ng/mL (0.16 nmol/L).

3.1.1 Secondary Endpoints

Efficacy Endpoints

At 75 ± 5 days following the first and final transplant(s):

- The proportion of insulin-independent subjects
- The percent reduction in insulin requirements
- HbA1c
- MAGE[77]
- LI[81]
- Ryan hypoglycemia severity (HYPO) score[81]
- Basal (fasting) and 90-min glucose and c-peptide derived from the mixed-meal tolerance test (MMTT)
- β-score[151]
- C-peptide: (glucose X creatinine) ratio
- Acute insulin response to glucose (AIR_{glu}), insulin sensitivity, and disposition index derived from the insulin-modified frequently-sampled IV glucose tolerance (FSIGT) test[152, 153]
- Glucose variability6[79] and hypoglycemia duration[119] derived from the CGMS

- Quality of life (QOL) measures

If a third transplant occurs less than 75 days after the second transplant, the 75 day endpoint data for the second transplant will not be collected.

At 365 ± 14 days following the first transplant

- The proportion of subjects with an HbA1c <7.0% at Day 365 AND free of severe hypoglycemic events from Day 28 to Day 365 inclusive
- The proportion of subjects receiving a second islet transplant
- The proportion of subjects receiving a third islet transplant

A severe hypoglycemic event is defined as an event with one of the following symptoms: memory loss; confusion; uncontrollable behavior; irrational behavior; unusual difficulty in awakening; suspected seizure; seizure; loss of consciousness; or visual symptoms, in which the subject was unable to treat him/herself and which was associated with either a blood glucose level <54 mg/dL [3.0 mmol/L] or prompt recovery after oral carbohydrate, IV glucose, or glucagon administration.

At 365 ± 14 days following the first and final islet transplant:

- The proportion of insulin-independent subjects
- The percent reduction in insulin requirements
- HbA1c
- MAGE
- LI
- Clarke score
- HYPO score
- Basal (fasting) and 90-min glucose and c-peptide (MMTT)
- β-score
- C-peptide: (glucose X creatinine) ratio
- AIR_{glu} insulin sensitivity, and disposition index derived from the FSIGT test[152, 153]
- CGMS
- QOL
- The proportion of subjects receiving a second islet transplant
- The proportion of subjects receiving a third islet transplant
- The rate of subjects with an HbA1c ≤7.0% and free of severe hypoglycemic events)

Safety Endpoints

At 75 ± 5 days following each transplant and 365 ± 14 days following the first and final islet transplant:

- The incidence and severity of AEs related to the islet transplant procedure including: bleeding (>2 g/dL decrease in hemoglobin concentration); segmental

portal vein thrombosis; biliary puncture; wound complication (infection or subsequent hernia); and increased transaminase levels (>5 times upper limit normal [ULN])
- The incidence and severity of AEs related to the immunosuppression including: allergy; reduction in GFR; increase in urinary albumin excretion; addition or intensification of anti-hypertensive therapy; addition or intensification of anti-hyperlipidemic therapy; oral ulcers; lower extremity edema; gastrointestinal toxicity; neutropenia, anemia, or thrombocytopenia; viral, bacterial, or fungal infections; and benign or malignant neoplasms
- The incidence of a change in the immunosuppression drug regimen
- The incidence of immune sensitization defined by presence of anti-HLA antibodies absent prior to transplantation

At 75 ± 5 and 365 ± 14 days following the first islet transplant:

- The incidence of worsening retinopathy as assessed by change in retinal photography

3.1.2 Additional Hypothesis Generating Endpoints:

At 75± 5 days following the second and third transplants, the following will be reported for the subset who receive a second transplant who also have data collected and also for the subset who receive a third transplant.

- the proportion of insulin-independent subjects
- the percent reduction in insulin requirements
- HbA1c
- MAGE
- LI
- HYPO score
- Basal (fasting) and 90-min glucose and c-peptide (MMTT)
- β-score
- C-peptide: (glucose X creatinine) ratio
- Acute insulin response to glucose (AIR_{glu}), insulin sensitivity, and disposition index derived from the insulin-modified frequently-sampled intravenous glucose tolerance (FSIGT) test[153]
- Glucose variability[79] and hypoglycemia duration[119] derived from the continuous glucose monitoring system® (CGMS)
- QOL

At 365 ± 14 days following the last islet transplant will include the change in the following measures from the results obtained at 75 ± 5 days following the last islet infusion:

- the proportion of insulin-independent subjects
- the percent reduction in insulin requirements
- HbA1c
- MAGE
- LI
- Clarke score
- HYPO score
- Basal (fasting) and 90-min glucose and c-peptide (MMTT)
- β-score
- C-peptide: (glucose X creatinine) ratio
- Glucose variability[79] and hypoglycemia duration[119] derived from the continuous glucose monitoring system® (CGMS)
- QOL

3.1.3 Additional Exploratory Endpoints

Section 9 describes the mechanistic assays that will be done. Additional exploratory analyses will generate metabolic and mechanistic hypotheses for further investigation. Each of the following tests allows for the quantification of several metabolic parameters explained in Section 9. End points from subjects in CIT-07 will be compared to subjects from University of Pennsylvania in CIT-05 in Section 10.

- A Glucose-potentiated arginine (GPA) test [11] will be conducted at 75 ± 15 days after each transplant and at 365 ± 14 days after the last transplant and the endpoints of acute c-peptide and insulin responses to arginine computed and analyzed.

- Among subjects who are insulin independent, the glucose potentiation slope and β-cell secretory capacity will also be calculated and analyzed at 75 ± 15 and 365 ± 14 days after last transplant.

- Glucose counter-regulation (paired eu- and hypoglycemic clamp studies)[68, 69,154, 155] will be conducted pre-transplant and at 180 ± 30 days and 365 ± 60 days after the last transplant.

- Immunophenotyping of lymphocyte subsets will be performed at baseline, days 7 and 28, and months 2.5, 6, 9 and 12.

- HiD (high-resolution multiparameter (flow cytometry) of B lymphocyte subsets will be performed at baseline and on days 7 and 28, and months 5 and 6. These time points were chosen because they are likely to be most informative for analyzing B cell depletion and reconstitution following treatment with anti-CD20.

- B lymphocyte repertoire analysis (CDR3 spectratyping) will be performed on CD19+ cells at baseline, and months 6, 9, and 12. Samples will be collected for clone

tracking analysis at baseline and at all subsequent study time points.

- ELISpot analysis of islet-reactive peripheral blood T Lymphocytes will be performed at baseline, days 0, 7, and 28, and months 2.5, 6, 9, and 12. If the ELISpot is positive, the specificity and cytokine profile of reacting T cells will be characterized in father detail.

- The memory B cell response to influenza will be analyzed at baseline and at month 6.

4. SELECTION OF SUBJECTS

4.1 Inclusion Criteria

Patients who meet *all* of the following criteria are eligible for participation in the study:

1. Male and female patients age 18 to 65 years of age.
2. Ability to provide written informed consent.
3. Mentally stable and able to comply with the procedures of the study protocol.
4. Clinical history compatible with T1D with onset of disease at <40 years of age and insulin-dependence for ≥5 years at the time of enrollment, and a sum of patient age and insulin dependent diabetes duration of ≥28 years.
5. Absent stimulated c-peptide (<0.3ng/mL) in response to a mixed meal tolerance test (MMTT; Boost® 6 mL/kg body weight to a maximum of 360 mL; another product with equivalent caloric and nutrient content may be substituted for Boost) measured at 60 and 90 min after the start of consumption.
6. Involvement in intensive diabetes management defined as self monitoring of glucose values no less than a mean of three times each day averaged over each week and by the administration of three or more insulin injections each day or insulin pump therapy. Such management must be under the direction of an endocrinologist, diabetologist, or diabetes specialist with at least 3 clinical evaluations during the 12 months prior to study enrollment.
7. At least one episode of severe hypoglycemia defined as an event with one of the following symptoms: memory loss; confusion; uncontrollable behavior; irrational behavior; unusual difficulty in awakening; suspected seizure; seizure; loss of consciousness; or visual symptoms in which the subject was unable to treat him/herself and which was associated with either a blood glucose level <54 mg/dL [3.0 mmol/L] or prompt recovery after oral carbohydrate, IV glucose, or glucagon administration), in the 12 months prior to study enrollment.
8. Reduced awareness of hypoglycemia as defined by a Clarke score of 4 or more OR a HYPO score greater than or equal to the 90th percentile (1047) during the screening period and within the last 6 months prior to randomization;

OR

Marked glycemic lability characterized by wide swings in blood glucose despite optimal diabetes therapy and defined by an LI score greater than or equal to the 90th percentile (433 $mmol/L^2/h \cdot wk^{-1}$) during the screening period and within the last 6 months prior to randomization;

OR

A composite of a Clarke score of 4 or more and a HYPO score greater than or equal to the 75th percentile (423) and an LI greater than or equal to the 75th percentile (329) during the screening period and within the last 6 months prior to randomization.

4.2 Exclusion Criteria

Patients who meet *any* of these criteria are *not* eligible for participation in the study:

1. Body mass index (BMI) >30 kg/m^2 or patient weight ≤50kg.
2. Insulin requirement of >1.0 IU/kg/day or <15 U/day.
3. HbA1c >10%.
4. Untreated proliferative diabetic retinopathy.
5. Blood Pressure: SBP >160 mmHg or DBP >100 mmHg.
6. Measured glomerular filtration rate (using iohexol) of <80 mL/min/1.73 m^2 (or for subjects with an iodine allergy, calculated using the subject's measured serum creatinine and the Modification of Diet in Renal Disease [MDRD] study estimation formula). Strict vegetarians (vegans) with a calculated GFR <70 mL/min/1.73 m^2 are excluded. The absolute (raw) GFR value will be used for subjects with body surface areas >1.73 m^2.
7. Presence or history of macroalbuminuria (>300mg/g creatinine).
8. Presence or history of panel-reactive anti-HLA antibodies above background by flow cytometry.
9. For female subjects: Positive pregnancy test, presently breast-feeding, or unwillingness to use effective contraceptive measures for the duration of the study and 4 months after discontinuation. For male subjects: intent to procreate during the duration of the study or within 4 months after discontinuation or unwillingness to use effective measures of contraception. Oral contraceptives, Norplant®, Depo-Provera®, and barrier devices with spermicide are acceptable contraceptive methods; condoms used alone are not acceptable.
10. Active infection including hepatitis B, hepatitis C, HIV, or tuberculosis (TB) as determined by a positive skin test or clinical presentation, or under treatment for suspected TB. Positive tests are acceptable only if associated with a history of previous vaccination in the absence of any sign of active infection. Positive tests are otherwise not acceptable, even in the absence of any active infection at the time of evaluation.
11. Negative screen for Epstein - Barr virus (EBV) by IgG determination.

12. Invasive aspergillus infection, histoplasmosis, and coccidioidomycosis infection within one year prior to study enrollment.

13. Any history of malignancy except for completely resected squamous or basal cell carcinoma of the skin.

14. Known active alcohol or substance abuse.

15. Baseline Hb below the lower limits of normal at the local laboratory; lymphopenia (<1,000/μL), neutropenia (<1,500/μL), or thrombocytopenia (platelets <100,000/μL).

16. A history of Factor V deficiency.

17. Any coagulopathy or medical condition requiring long-term anticoagulant therapy (*e.g.*, warfarin) after transplantation (low-dose aspirin treatment is allowed) or patients with an international normalized ratio (INR) >1.5.

18. Severe co-existing cardiac disease, characterized by ***any one*** of these conditions:

    a) recent myocardial infarction (within past 6 months).

    b) evidence of ischemia on functional cardiac exam within the last year.

    c) left ventricular ejection fraction <30%.

19. Persistent elevation of liver function tests at the time of study entry. Persistent serum glutamic-oxaloacetic (SGOT [AST]), serum glutamate pyruvate transaminase (SGPT [ALT]), Alk Phos or total bilirubin, with values >1.5 times normal upper limits will exclude a patient.

20. Symptomatic cholecystolithiasis.

21. Acute or chronic pancreatitis.

22. Symptomatic peptic ulcer disease.

23. Severe unremitting diarrhea, vomiting or other gastrointestinal disorders potentially interfering with the ability to absorb oral medications.

24. Hyperlipidemia despite medical therapy (fasting low density lipoprotein [LDL]) cholesterol >130 mg/dL, treated or untreated; and/or fasting triglycerides >200 mg/dL).

25. Receiving treatment for a medical condition requiring chronic use of systemic steroids, except for the use of ≤5 mg prednisone daily, or an equivalent dose of hydrocortisone, for physiological replacement only.

26. Treatment with any anti-diabetic medication other than insulin within 4 weeks of enrollment.

27. Use of any investigational agents within 4 weeks of enrollment.

28. Administration of live attenuated vaccine(s) within 2 months of enrollment.
29. Any medical condition that, in the opinion of the investigator, will interfere with safe participation in the trial.
30. Treatment with any immunosuppressive regimen at the time of enrollment.
31. A previous islet transplant.
32. A previous pancreas transplant, unless the graft failed within the first week due to thrombosis, followed by pancreatectomy and the transplant occurred more than 6 months prior to enrollment.

4.3 Subject Withdrawal Criteria

4.3.1 Premature Discontinuation of Study Treatment

The criteria for premature discontinuation of study treatment with immunosuppressive medication are detailed in section 6. Subjects who prematurely discontinue treatment with study drug will remain in the study until normal termination. They will be followed to monitor safety and efficacy parameters. Data from these subjects will be used in the intent-to-treat analysis.

5. STUDY TREATMENT REGIMEN

Please refer to section 1.5, and to applicable product labeling for known and potential risks to human subjects associated with the study treatment regimen.

All immunosuppressant medications described in this protocol are FDA approved for use in solid organ transplantation except for rituximab (Rituxan®, IDEC Pharmaceuticals Corp., San Diego, CA and Genentech, Inc., San Francisco, CA), which is licensed for use in B-cell non-Hodgkin's lymphoma and rheumatoid arthritis. Please refer to sections 1.14 for discussions of the mechanism of action and associated risks, respectively, of each proposed immunotherapy agent. Each immunosuppressant medication will be prepared and administered as licensed, and the dosage will follow that detailed in section 3.0.

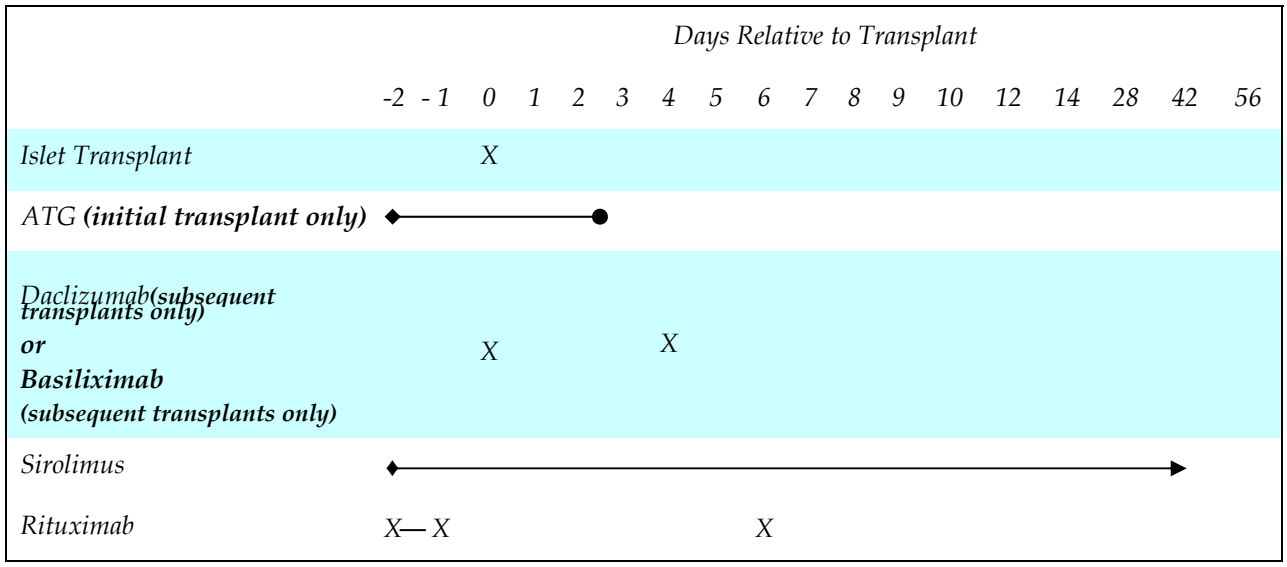

Figure 2: Islet transplant and immunosuppression regimen

5.1 Investigational Agent: Allogeneic Islets

5.1.1 Formulation, Dosage, and Administration

The final product is a 200 mL sterile suspension of ≥70% viable, ≥30% pure, allogeneic human purified islets in 2.5% human serum albumin (HSA), 25mM Hepes for administration by intraportal infusion. The final product is supplied in 600 mL Ricordi® bags, containing a dose of ≥5,000 IEQ/kg recipient body weight (BW) for the first infusion, and ≥4,000 IE/kg recipient BW for subsequent infusions.

Table 1: Composition of final drug product [Product Code: PHPI-A-01]

Component	Quantity per Batch
Purified Human Pancreatic Islets	≥4.0 x 10^3 IEQ[1]/kg recipient BW[2] (total IEQ/infusion)
CIT Transplant Media[3]	≤600 mL[3]
Human Serum Albumin (HSA), USP	2.5%
HEPES Buffer, USP	25 mM

[1] IEQ = Islet Equivalents

[2] BW = Body Weight

[3] 600 mL maximum total volume in Ricordi® infusion bags containing 200 mL each

Administration:

The islet mixture is delivered slowly via gravity drainage from a bag attached to the catheter in the portal vein or port vein tributary. Access to the portal vein is achieved by percutaneous transhepatic access under fluoroscopic, ultrasonographic, or real-time CT guidance. Alternatively, access to a mesenteric or omental venous tributary of the portal vein can be obtained by mini-laparotomy under general anesthesia (transplant site preference or in the extremely rare circumstance that percutaneous access cannot be achieved).

At a minimum, portal pressure will be monitored before and after infusion of each bag of the islet product, as well as after the final wash. Portal pressure measurements will be documented in the medical record.

Additional guidelines for islet administration and portal pressure measurements are located in the Manual of Procedures; however each participating site should follow its site-specific standards to ensure compliance with institutional and subject safety.

5.1.2 Drug Accountability

Under Title 21 of the Code of Federal Regulations (21CFR §312.62) the investigator is required to maintain adequate records of the disposition of the investigational agent, including the date and quantity of the drug received, to whom the drug was dispensed (subject-by-subject accounting), and a detailed accounting of any drug accidentally or deliberately destroyed.

Records for receipt, storage, use, and disposition will be maintained by the study site. A drug-dispensing log will be kept current for each subject. This log will contain the identification of each subject and the date and quantity of drug dispensed.

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

5.2 Immunosuppression Medications

5.2.1 Initial Allogeneic Islet Transplant

Please refer to applicable product labeling and Package Inserts for known and potential risks to human subjects associated with the consensus immunosuppressive medications.

5.2.1.1 Rituximab

Rituximab will be used at a dose of 375 mg/m^2 per injection administered twice, one week apart. This is less than the recommended dose used for non-Hodgkin's lymphoma in humans, which is 375 mg/m^2 per injection administered four times at weekly intervals[147], and is less than the recommended dose used for the treatment of rheumatoid arthritis, which is 1000 mg twice, 15 days apart[31]. In addition, this dose lead to sustained (> 6 months) B lymphocyte depletion, and caused a low (4.3%) incidence of human antichimeric antibody formation[31]. The proposed rituximab dose for CIT-05 is based on the pre-clinical non-human primate trial in allo-islet transplantation that utilized two doses of 375 mg/m^2 and achieved long-term islet allo-graft survival associated with B lymphocyte depletion that persisted for over 3 months[156]. Similarly in solid organ transplantation, rituximab has been used off-label for the prevention and treatment of rejection with rituximab 375 mg/m^2 given as one to four doses. It has been established that the use of one or two doses of 375 mg/m^2 leads to B lymphocyte depletion and assures safety in patients with high-titer PRA experiencing kidney allograft rejection and awaiting kidney transplantation[148, 153].

The first dose of rituximab will follow the first ATG infusion between day -2 and day -1 relative to transplant (please see below); the second doses of rituximab will occur on day +6 (±1). Acetaminophen 650 mg and diphenhydramine 50 mg will both be administered orally 30 minutes before the start of each rituximab infusion. Because infusion reactions generally occur only with the first dose of rituximab, the first dose will follow the initial dose of Thymoglobulin® where 2 mg/kg of intravenous methylprednisolone is given concurrently. Administration of this high dose of glucocorticoid should mitigate the incidence and severity of infusion reactions. Should a severe infusion reaction occur with the initial dose of rituximab, the subsequent dose will be administered with 0.5 mg/kg of methylprednisolone intravenously 30 minutes before infusion. In rheumatoid arthritis, only 36% of patients experience an infusion reaction with the initial dose of rituximab, and the majority of these were classified as mild-to-moderate, consisting of transient hypo- or hypertension, cough, pruritis, and rash[31, 147].

5.2.1.2 Rabbit Anti-thymocyte globulin (ATG, Thymoglobulin®)

A total of 6 mg/kg will be given as an IV infusion over 8 hours on days –2, -1, 0, +1, and +2. The dose will be 0.5 mg/kg on day –2, 1.0 mg/kg on day –1, and 1.5 mg/kg on days 0, +1, and +2. The first dose will be administered over 8 hours and subsequent doses will be administered over 6 hours. The dose of thymoglobulin may be reduced or eliminated on any day at the discretion of the treating physician for any subject safety concerns that may arise. Premedications will be used as follows:

#1: Acetaminophen (Tylenol®) 650 mg PO/PR ½ hr before and midway through ATG infusion

#2: Diphenhydramine (Benadryl®) 50 mg PO ½ hr before and midway through ATG infusion

#3: Methylprednisolone (Solu-Medrol®) 1 mg/kg IV one hour prior to and midway through the first ATG infusion only (*i.e.* on day –2)

#4: Pentoxifylline (Trental®) 400 mg PO TID to be initiated one hour prior to the first ATG infusion and to be continued through day +7

If the subject is admitted when the vascular access team is not available or at a time when the placement of a Peripherally Inserted Central Catheter could delay the first Thymoglobulin® dose, it may be administered IV via a peripheral line as follows:

- Dilute the Thymoglobulin® in 500 cc Normal Saline (not D5W)
- Combine with Heparin 1000 units and Hydrocortisone 20 mg.

5.2.1.3 Sirolimus (Rapamune®)

Sirolimus will be administered at an initial dose of 0.2 mg/kg PO on day -2 relative to islet transplant, followed by 0.1 mg/kg QD. The daily dose will be adjusted to the whole blood 24-hr trough to target, as tolerated, 12-15 ng/mL for the first 3 months and 10-12 ng/mL thereafter. If a subject develops intolerable or clinically undesirable side-effects related to sirolimus therapy, his/her therapy may be converted to maintenance mycophenolate mofetil (MMF) at the discretion of the principal investigator.

5.2.1.4 Tacrolimus (Prograf®)

Tacrolimus may be used as a replacement for sirolimus if sirolimus is not tolerated. Tacrolimus will be administered at an initial dose 0.015 mg/kg PO BID with the whole blood 12-hr trough adjusted to 6-10 ng/mL. Should subjects experience a decrease in their GFR of ≥33% compared with baseline, a nephrology consult will be obtained, and tacrolimus target trough levels will be reduced by 25% should CNI toxicity be suspected as the primary cause for the decline in renal function.

5.2.1.5 Cyclosporine, USP (Neoral®)

Cyclosporine may be used as a replacement for tacrolimus if clinically indicated. Cyclosporine will be administered at an initial dose of 6 mg/kg/d in 2 divided doses, with target levels of 150-200 ng/mL.

5.2.1.6 Mycophenolate Mofetil (CellCept®)

Mycophenolte mofetil may be used if sirolimus levels cannot be maintained ≥10 ng/mL. Mycophenolate mofetil will be administered at a dose of 500 to 1500 mg PO BID in addition to either tacrolimus or sirolimus. Subjects must practice two methods of contraception while taking MMF. If a subject experiences severe neutropenia (absolute neutrophil count <1x10^9/L) while taking mycophenolate mofetil, mycophenolate mofetil exposure will be reviewed and

mycophenolate mofetil administration will be adjusted as part of the study protocol's neutropenia management plan.

5.2.1.7 Mycophenolate sodium (Myfortic®)

Mycophenolate sodium may be used as a replacement for tacrolimus, sirolimus, or mycophenolate mofetil. Mycophenolate sodium will be dosed at 360 to 720 mg PO BID. Subjects must practice two methods of contraception while taking Myfortic®.

5.2.2 Subsequent Allogeneic Islet Transplants

The immunosuppressive regimen for subsequent islet transplants will be identical to the regimen for the initial islet transplant with the following exceptions.

5.2.2.1 Monoclonal Antibody IL-2 Receptor Blocker

All subjects will receive one of the following monoclonal antibody IL-2 receptor blockers: daclizumab or basiliximab.

5.2.2.1.1 Daclizumab (Zenapax®)

Daclizumab (Zenapax®) is a humanized anti-CD25 monoclonal antibody in clinical use since 1997 for prophylaxis against acute organ rejection in subjects receiving a kidney allograft. Please refer to applicable product labeling and the Package Insert for its known and potential risks to human subjects.

Five I.V. doses of daclizumab will be given with all subsequent islet transplants. The first dose will be 2 mg/kg and will be given within two hours prior to islet transplant. Doses 2 to 5 will be 1 mg/kg will be given every 2 weeks starting on day 14 after the subsequent transplant.

If a third transplant is deemed necessary and performed between 30 and 70 days after the second transplant, no additional doses of daclizumab will be given.

If a third islet transplant is deemed necessary and performed more than 70 days after the second transplant (see Section 7.5 for indications for subsequent transplants), all five doses of daclizumab will be repeated.

5.2.2.1.2 Basiliximab (Simulect®)

Two I.V. doses of basiliximab will be given with the first and second (if necessary) transplants. The first dose will be 20 mg and will be given within two hours prior to islet transplant on the day of islet transplantation. The second dose will be given on Day 4 after the transplant.

If a third transplant is deemed necessary and performed between 30 and 70 days after the second transplant, no additional doses of basiliximab will be given.

If a third islet transplant is deemed necessary and performed more than 70 days after the second transplant (see Section 7.5.1 for indications for subsequent transplants), both doses of basiliximab will be repeated.

5.3 Concomitant Medications

5.3.1 Antibacterial, Antifungal, and Antiviral Prophylaxis

Broad spectrum antimicrobial prophylaxis should be administered preoperatively according to site-specific standards, or as the Transplant Infectious Disease. consultant recommends.

5.3.1.1 Trimethoprim/Sulfamethoxazole (Septra SS®/Bactrim®)

Trimethoprim / sulfamethoxazole will be administered at a dose of 80 mg/400 mg PO QD starting on Day +1 for the duration of study follow-up. In the event that a subject is unable to take trimethoprim/sulfamethoxazole, he/she will be treated on a case-by-case basis as is medically indicated.

5.3.1.2 Clotrimazole (Mycelex Troche®)

Clotrimazole will be administered as 1 troche PO QID starting on Day –2 relative to transplant, to be continued for 3 months after transplantation. Alternatively, antifungal prophylaxis per standard practice at each site may be administered instead of clotrimazole.

5.3.1.3 Valganciclovir (Valcyte®)

Valganciclovir will be administered starting on Day -2 at a dose of 450 mg PO QD, increasing to 900 mg QD by day 12 and continuing for 14 weeks post-transplant (irrespective of CMV status of donor or recipient).

5.3.2 Anticoagulation Prophylaxis/Hematological Agents

5.3.2.1 Heparin

Heparin will be administered at a dose of 70 U/kg body weight of recipient, divided equally among the islet bags, given with islet infusion, followed by 3 U/kg/hr IV for the next 4 hrs. From the 5th through the 48th hr post-transplant, heparin will be titrated to achieve and maintain partial thromboplastin time (PTT) between 50-60 seconds. If the site does not use PTT to titrate heparin, a comparable site-specific method and value should be used.

5.3.2.2 Enoxaparin (Lovenox®)

Enoxaparin will be administered at a dose of 30 mg SC BID through day 7 post-islet transplant, with the first dose given 48 hours after the transplant procedure (when heparin is discontinued).

5.3.2.3 Pentoxifylline

Pentoxifylline will be administered at a dose of 400 mg slow release TID beginning 2 days prior to transplant (Day -2) and continuing for 7 days post-transplant (Day 7).

5.3.2.4 Aspirin

Enteric coated aspirin will be administered at a dose of 81 mg PO qPM starting 24 hrs post-transplant, and continued as medically indicated.

5.3.3 Insulin Therapy

Glucose levels will be targeted to 80-120 mg/dL. Insulin (*e.g*. Regular, Lispro, NPH, Glargine) will be administered as needed to maintain glucose levels in the target range. The subject will test BG five times per day (AM fasting, before lunch, 2 hours after lunch, before supper, and at bedtime). The subject's daily BG levels will be reviewed by a study nurse and/or one of the investigators three times per week during the first two weeks after discharge, and then weekly during the next month. Exogenous insulin will be withdrawn or adjusted as needed. Patients able to maintain fasting BG levels below 140 mg/dL and 2-hour post-prandial levels below 180 mg/dL after insulin discontinuation will be considered insulin independent.

5.3.4 Other Standard Therapies

Anti-hypertensive, anti-hyperlipidemia and other approved therapies for pre-existing and new medical conditions will be provided per standard of care. Pre- and post-islet transplant procedure drug regimens (*e.g.,* pre-transplant sedation and anesthetic) will be given per standard of care.

5.4 Rescue Medications

Rescue therapy will not be initiated in this protocol to treat suspected rejection. Immunologic surveillance methods that would allow diagnosis of islet allograft rejection early enough for timely intervention have yet to be identified and validated.

5.5 Prohibited Medications

Prohibited medications for this protocol, except as specifically indicated in this protocol include:

- steroid medication (save topicals and prednisone at a dose of ≤5 mg daily, or an equivalent dose of hydrocortisone, for physiological replacement only)
- any medications in the macrolide antibiotic class

- other investigational products
- other immunosuppressive therapies
- immunomodulatory agents
- other anti-diabetic agents
- Dapsone

5.6 Assessment of Compliance with Study Treatment

Assessment of subject compliance will be determined by the completion of scheduled study visits and required documentation that the specific subject is responsible for (*e.g.,* Blood Glucose Logs, AE and Insulin Use recording) as well as their willingness to comply with the recommendations of the study investigators. Glucometers will be downloaded for verification of the blood glucose values recorded in the subjects' logs. Any aberration of trough levels of immunosuppressive agents that could indicate nonadherence, lack of compliance that poses a significant clinical risk and or derangement of protocol data collection will be documented. Please refer to Section 5.7.2 for a description of possible indications for premature discontinuation of study treatment.

Compliance with the maintenance immunosuppression medications used in this protocol (tacrolimus and rapamycin) will be assessed by regular monitoring of their plasma trough concentrations (please refer to section 6).

5.7 Modification or Discontinuation of Study Treatment

5.7.1 Modification of Consensus Immunosuppression Regimen

Should an islet product become unsuitable for transplantation subsequent to recipient randomization and treatment with induction immunosuppression, the subject will remain on maintenance therapy for 2 weeks. This will provide sufficient time for organ procurement, islet isolation, and infusion of an islet product without any additional induction treatment of the recipient. An emergency request will be placed through UNOS to ensure that the next available pancreas for islet transplantation is directed to the selected manufacturing site. Should an islet product not be available during this 2 week period, the subject will continue maintenance immunosuppression, but induction with a monoclonal antibody IL-2 receptor blocker will be administered at the time of the islet transplant. If an adequate islet product does not become available within a 30 day period, immunosuppression will be discontinued, as per site specific standards, and the case will be considered a treatment failure.

In the event that protocol-regulated concomitant medications are not tolerated, the subject will continue taking the immunosuppressive therapy in order to protect the islet graft. In the event that the immunosuppression regimen is not tolerated, the Site Principal Investigator (PI) may elect to prescribe an alternative immunosuppression regimen. The intent would be for the alternative regimen to be temporary in nature where possible. Any non-protocol directed study

treatment modification that the site PI determines is necessary should be reported as a protocol deviation.

5.7.1.1 Rabbit Anti-Thymocyte Globulin-Induced Anaphylaxis

In rare instances, anaphylaxis has been reported with Thymoglobulin® use. In such cases, the infusion should be terminated immediately. Medical personnel should be available to treat subjects who experience anaphylaxis. Emergency treatment such as 0.3 mL to 0.5 mL aqueous epinephrine (1:1000 dilution) subcutaneously and other resuscitative measures including oxygen, IV fluids, antihistamines, corticosteroids, pressor amines, and airway management, as clinically indicated, should be provided. Thymoglobulin® or other rabbit immunoglobulins should not be administered again for such subjects.

5.7.1.2 Rabbit Anti-Thymocyte Globulin and Rituximab-Induced Cytokine Release

Thymoglobulin® and rituximab infusions may cause cytokine release-related fever and chills. To minimize these, the first dose should be infused over a minimum of 6 hours into a high-flow vein. Also, premedication with corticosteroids, pentoxifylline, acetaminophen, and/or an antihistamine will be provided in order to minimize the reaction incidence and/or intensity. At any sign of the above reaction, slowing the infusion rate by 50% will also occur.

5.7.1.3 Neutropenia

Neutropenia is an expected consequence of the administration of several medications in this protocol. Subject safety is of utmost importance. Clinical treatment decisions take precedence over recommended guidelines.

If a subject's absolute neutrophil count is less than 1000 cells/μL and the patient is afebrile, then the following will be done:

- Reduce rabbit ATG by 50%.
- Reduce the prophylactic use of valganciclovir from 900 grams per day to 450 mg per day or hold valganciclovir.
- Reduce trimethoprim/sulfamethoxazole to 80/400 mg on Monday, Wednesday, and Friday or hold trimethoprim/sulfamethoxazole.
- Review and obtain current sirolimus trough levels and consider dosage adjustment if trough level are >12ng/mL
- If subject is using mycophenolate mofetil or mycophenolate sodium in lieu of sirolimus or tacrolimus consider dose reduction.
- Consider administration of G-CSF.
- Monitor temperature BID.

- Follow-up within 48-72 hours to obtain: repeat complete blood count (CBC) with differential, patient symptoms, and measured temperatures.

If a subject's absolute neutrophil count is less than 1000 cells/μL and the subject is febrile, then the following will be done:

- Obtain Infectious Disease Consult.
- Hold rabbit ATG.
- Hold valganciclovir and trimethoprim/sulfamethoxazole.
- Review and obtain current sirolimus trough levels and consider dosage adjustment if trough level are >12ng/mL.
- If subject is using mycophenolate mofetil or mycophenolate sodium in lieu of sirolimus or tacrolimus consider dose reduction.
- Administer G-CSF.
- Monitor temperature BID.
- Follow-up within 48-72 hours to obtain: repeat CBC with differential, subject symptoms, and measured temperatures.

If a subject's absolute neutrophil count is measured as less than 500 cells/μL and the subject is afebrile, then the following will be done:

- Hold rabbit ATG.
- Hold administration of trimethoprim/sulfamethoxazole and/or valganciclovir.
- Review and obtain current sirolimus trough levels and hold dose if trough level are >12ng/mL.
- If subject is using mycophenolate mofetil or mycophenolate sodium in lieu of sirolimus or tacrolimus consider holding dose.
- Obtain CMV antigenemia or PCR for CMV.
- Consider fluoroquinolones in afebrile patients.
- Consider clotrimazole.
- Administer G-CSF.
- Monitor temperature BID.
- Follow up within 24 hours to obtain repeat CBC, subject symptoms, and measured temperatures.

If a subject's absolute neutrophil count is measured as less than 500 cells/μL and the subject is febrile, then the following will be done:

- The subject will be hospitalized under neutropenic precautions and Infectious Disease/Hematology consult will be obtained.

- Hold rabbit ATG.
- Hold administration of trimethoprim/sulfamethoxazole and/or valganciclovir.
- Review and obtain current sirolimus trough levels and hold dose if trough level are >12ng/mL .
- If subject is using mycophenolate mofetil or mycophenolate sodium in lieu of sirolimus, or tacrolimus consider holding dose.
- Obtain CMV antigenemia or PCR for CMV.
- Administer G-CSF.

5.7.1.4 Thrombocytopenia

If the subject is found to have a platelet count (PLT) of <50 x 10^9/L, ATG will be withheld until PLT > 50 x 10^9/L, then resume at a 50% reduced dose. If the PLT is <50 x 10^9/L, sirolimus will be withheld for 24 hours, then resumed at a 50% reduced dose. If PLT fails to return to >50 x 10^9/L within one week, sirolimus is to be withheld until PLT > 50 x 10^9/L, after which sirolimus is resumed at 50% of the dose that preceded the drop in PLT to <50 x 10^9/L. If the PLT is between 50 and 75 x 10^9/L, reduce ATG dose by 50% until PLT is > 75 x 10^9/L.

5.7.1.5 Nephrotoxicity:

A sustained 33% increase in serum creatinine or a 33% decrease in GFR warrants a prompt referral to a nephrologist for evaluation. If it is determined that the decrease in renal function is attributable to CNI immunosuppressive therapy, the treating physician should chose ONE of the therapeutic alternatives shown in the following table:

Table 2: Response to nephrotoxicity

Allowable therapeutic responses to CNI-induced nephrotoxocity	Rationale
Discontinue sirolimus, and replace it with mycophenelate mofetil or mycophenolate sodium.	The nephrotoxic effect of CNIs is increased by concomitant administration of sirolimus[123, 157].
If the trough sirolimus level is maintained at >10 ng/mL without adverse effects, discontinue the CNI and replace it with mycophenolate mofetil or mycophenolate sodium.	CNI should be discontinued only if the subject can tolerate a trough level of sirolimus that will result in adequate immunosuppression.
Decrease the target CNI trough level by 25%.	CNI toxicity is dose-related.

A repeat assessment of GFR should be performed 3 months after the change in immunosuppression.

Anti-hypertensives, anti-hyperlipidemics and other approved therapies for pre-existing and new medical conditions will be provided per standard of care.

5.7.2 Premature Discontinuation of Study Treatment (Transition to "Off-Protocol" Treatment)

Study treatment may be prematurely discontinued for any subject for any of the following reasons:

1. The subject is unwilling or unable to comply with the protocol.
2. The investigator believes that the study treatment is no longer in the best interest of the subject.
3. Graft Failure: Islet allograft failure will be defined as absence of insulin production by transplanted islets, as evidenced by absence of c-peptide. This will be determined by (1) undetectable c-peptide on random testing, followed by (2) undetectable c-peptide at baseline, and at 60 and 90 minutes after MMTT. C-peptide levels obtained in the course of the MMTT will be run at the core lab in Seattle, WA. All study treatment will be tapered and discontinued, at the discretion of the site Principal Investigator, when it is determined that graft failure has occurred.
4. An unexpected related SAE. The agent(s) to which the event is attributed will be discontinued.

Subjects who prematurely discontinue study treatment will remain in the study until normal termination, for the purpose of monitoring safety and efficacy parameters and will enter the reduced follow-up schedule outlined in Appendix 2. Data from these subjects will be used in the intent-to-treat analysis. These subjects are permitted to simultaneously enroll in a CIT or site-specific graft failure follow-up protocol, if available.

6. CRITERIA FOR PREMATURE TERMINATION OF THE STUDY

6.1 Subject Withdrawal Criteria

Subjects may be prematurely terminated from study for the following reasons:

1. The subject elects to withdraw consent from all future study activities, including follow-up.
2. The subject is "lost to follow-up" (*i.e.,* no further follow-up is possible because attempts to reestablish contact with the subject have failed).
3. The subject dies.

Subjects who prematurely terminate from this study will not be replaced. Data from such subjects obtained before withdrawal of consent or before being lost to follow up will be used in the intent-to-treat analysis. If a subject with functioning transplanted islets chooses to withdraw from the protocol, s/he must be informed of their risk for losing his/her islet graft and becoming sensitized if s/he chooses to discontinue immunosuppressive therapy and return to his/her original method of insulin management.

6.2 Study Stopping Rules

6.2.1 Protocol Suspension and Review

Study enrollment at all participating clinical sites will be suspended pending expedited review of all pertinent data by the institutional review board (IRB), the National Institute of Allergy and Infection Disease (NIAID), the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), and the NIDDK Data Safety Monitoring Board (DSMB), if any one of the following occurs:

1. The Medical Monitor finds any unexpected fatal or life-threatening AE possibly related to the use of the test therapy;
2. Primary non-function occurs in 3 or more consecutive subjects at 2 or more participating clinical sites. Primary non-function (PNF) is defined by undetectable c-peptide levels (1-3 hours post-prandial) between 3-7 days post transplant;
3. There are 6 consecutive study subjects with a c-peptide less than 0.3 ng/mL (on random testing, at baseline and 1-3 hrs post MMTT) at 75 days post-transplant;
4. Any event(s) which in the opinion of the Medical Monitor or Protocol Chair indicates the need for DSMB review; or
5. The DSMB recommends termination of protocol enrollment and further transplants on a study-wide basis based on a review of the data and finding evidence that such action is necessary. Statistical guidelines for terminating the study based on monitoring guidelines are provided in section 10.

After the protocol is placed on hold, no additional transplants within the trial will be performed at any participating clinical site until the CIT Steering Committee and DSMB meet either in person or by conference call to review in depth the results and circumstances surrounding the islet functional failure or SAE to determine whether the trial enrollment of new subjects and conduct of additional transplants could be safely resumed.

6.2.2 Site Suspension and Review

Study enrollment and initial islet transplants will be suspended (placed on hold) at a participating clinical site, pending expedited review of all pertinent data by the IRB, the NIAID, the NIDDK, and the NIDDK DSMB, if any one of the following occurs:

1. Any possibly study-related grade 5 AE; or

2. Two SAEs related to the islet transplant procedure (*e.g*. bleeding, thrombosis, gall bladder injury); or

3. Two consecutive primary non-functioning transplants defined by undetectable c-peptide levels (1-3 hours postprandial) between 3-7 days post transplant.

After any site is placed on hold, no additional transplants will be performed at that site until the CIT Steering Committee and DSMB meet either in person or by conference call to review in depth the results and circumstances surrounding the islet functional failure or SAE to determine whether the trial enrollment of new subjects and conduct of additional transplants could be safely resumed at that site, or whether there could be implications for the continuation of the entire proposed pilot protocol also at other affiliated sites testing the same protocol.

In all cases of PNF, subjects will be asked to temporarily continue immunosuppression to decrease the risk of sensitization that could increase the risk of poor outcome should future transplants occur. A tapering schedule will be applied until immunosuppressants are completely discontinued.

7. STUDY PROCEDURES

7.1 Enrollment

This research study will be explained in lay terms to each potential research subject. The potential subject will sign an informed consent form before undergoing any screening study procedures. Subjects who are deemed eligible for the study (see sections 4.0) will be enrolled and assigned a unique subject number.

Once an Islet Transplant has been provided, the patient becomes a part of the intention-to-treat population. All transplanted subjects will be included in the primary analysis.

This will be an open-label, prospective, single-arm, single-center study. The addition of rituximab to the CIT-07 strategy with elimination of the CNI tacrolimus will be evaluated. All islets will be processed at the University of Pennsylvania where all subjects will be recruited.

Unless the study is terminated early, recruitment will continue until the maximum sample size is reached, at which point recruitment will be terminated. It is anticipated that accrual of subjects will take 24 months.

7.2 Screening Visit

Prior to scheduling the screening visit, potentially eligible candidates for participation will have their medical records reviewed by one of the investigators. The following must be made available by the potential subject:

1. his/her age, gender, height, weight, age of diabetes onset, insulin type and requirements;
2. completed questionnaire inquiring for the presence of contraindicated medical conditions;
3. statement from his/her diabetologist regarding the type and stability of diabetes, the duration of the doctor-patient relationship, the current treatment regimen, blood pressure control, and presence of micro- or macrovascular complications (retinopathy, nephropathy, neuropathy, artherosclerosis), including their severity and treatment; and
4. recent (last 6 months) laboratory reports for HbA1c, urinary albumin, kidney and liver function, and fasting lipids.

If review of these materials indicates that the potential subject is not eligible, s/he will be notified by a study coordinator and explained why this is so (*e.g.* age > 65 years); if review indicates potentially unsuitable diabetes care, the potential subject will be referred to Penn's Rodebaugh Diabetes Center for further assessment and management; if review indicates that the potential subject may be eligible, s/he will be contacted by a study coordinator to schedule the screening visit.

At the screening visit, the study details and procedures will be discussed with a coordinator. If the potential subject remains interested, s/he will review the informed consent document with the coordinator, and an investigator will join the discussion and answer any questions. Once

satisfied that all questions have been answered, the potential subject will either decline to participate or sign the informed consent document. This may occur at a subsequent visit if the potential subject desires, in order to think further about what participation means and/or to consult with family and/or friends. If at any time the suitability of diabetes care is questioned, the potential subject will be referred to Penn's Rodebaugh Diabetes Center for further assessment and management.

Once informed consent has been obtained, eligibility will be confirmed through the performance of a history and physical examination (that includes a neurologic assessment), hypoglycemia and glycemic lability questionnaires, EKG, fasting serum biochemistries (glucose, c-peptide, electrolytes, creatinine, liver function tests [LFTs], and lipid panel), HbA1c, TSH, complete blood count (CBC, including WBC differential), coagulation studies (PT/INR, PTT, Fibrinogen), blood type, HLA type, panel reactive anti-HLA antibodies, serum HCG (females), serologies (HBV, HCV, HIV 1&2, EBV, HTLV 1&2), PPD, PSA (men over 40-years or with a family history of prostate cancer), Boost® stimulated serum glucose and c-peptide, urine collection for creatinine and albumin, chest X-ray, abdominal ultrasound, and cardiac persantine thallium of electrocardiogram. More than one visit will certainly be necessary to collect this information. Additional reports will be required from each subject's diabetologist, retinologist, dentist (to exclude infection), and gynecologist (indicating results of Pap smear and mammography for women older than 35 years). A psychosocial evaluation may be requested if either a coordinator or investigator is unsure whether a potential subject may be mentally unfit to undergo the procedure or to determine whether a psychosocial problem may be responsible for the instability of diabetes; such an evaluation would be performed by an experienced transplant social worker and/or psychiatrist. Subjects with a history or examination evidence of a central neurologic disease (*e.g.* multiple sclerosis) will be considered ineligible at Penn so that monitoring for neurologic warning signs of possible PML, such as changes in vision, loss of balance or confusion will not erroneously be attributed to an underlying neurologic condition. Once all eligibility criteria (inclusion and exclusion) are met, the subject will be placed on the transplant waiting list.

7.3 Baseline Visits

Once enrolled, subjects will return every 3months to assess the adequacy of their diabetes care, determine the HbA1c, and complete hypoglycemia and glycemic lability questionnaires in order to ensure continued eligibility for transplantation. Additional tests will be repeated as indicated in the timeline provided in the appendix.

Hypoglycemia will be assessed by completing 1) the Clarke survey of reduced hypoglycemia awareness[158], 2) the Ryan hypoglycemia score[81], and 3) the Hypoglycemia Fear Survey[58, 145].

Glycemic lability will be assessed by calculating 1) the mean amplitude of glycemic excursions from 2 consecutive days of 7 blood glucose measurements each day[8, 77], and 2) the Ryan lability index[81].

On one occasion (repeated annually as necessary), a hypoglycemic clamp will be performed to evaluate glucose counter-regulation after an overnight admission to the GCRC[159].

On one occasion (repeated annually as necessary), a continuous glucose monitoring system will be employed for 72-hours to assess the duration of hypo- and hyperglycemic episodes.

Insulin sensitivity will be assessed by a frequently-sampled intravenous glucose tolerance test (FSIGT)[160, 161] and euglycemic clamp. The euglycemic clamp will serve to validate the FSIGT-derived index of insulin sensitivity[153] and provide normoglycemic control data for the hypoglycemic clamp[162]. Both the FSIGT and a euglycemic clamp will be done annually, as will a hypoglycemic clamp. One clamp will be performed on the initial overnight GCRC admission. The remaining clamp will be done on the second overnight GCRC admission. The performance of the euglycemic clamp will occur in randomized order with the hypoglycemic clamp (above). Retinopathy will be evaluated by photography on an annual basis.

During this time of eligibility prior to transplant, additional baseline immunologic mechanistic assays will be performed as indicated in the timeline provided in the appendix. Their descriptions can be found in a subsequent section titled Mechanistic Assays.

7.4 Randomization

Once a compatible islet prep becomes available, subject eligibility will be re-confirmed. Eligible subjects will be randomized on Day -2 relative to transplant, between CIT-05 and CIT-07. Subjects randomized will receive immunosuppressive therapy beginning on Day -2 (see Section 5 for full description of Study Treatment Regimen). Subjects will receive the initial islet transplant on Day 0 and will continue the immunosuppression regimen detailed in Section 5.

7.5 Study Treatment Visits

The first study treatment visit will occur at the time a suitable islet preparation is available for transplantation. Once a suitable donor pancreas becomes available, the potential recipient will be notified and requested to arrive promptly (< 2-hours) for admission to the Kidney/Pancreas Transplant service of the Hospital of the University of Pennsylvania. On admission, blood work (glucose, HbA1c, electrolytes, creatinine, LFTs, CBC and differential, PT/INR, PTT, blood type and cross-match [including to HLA antigens]), urinalysis, EKG (unless performed in last 3 months), and chest X-ray (unless performed in last 3 months) will be performed. Once subject eligibility is confirmed by the above tests and the islet preparation meets release criteria, the induction of immunosuppression will begin on Day -2 with the administration of Thymoglobulin® ± rituximab, and the islet preparation will remain in culture for ~ 48-hours until infusion on Day 0. On Day 0, once the islet preparation has been determined to still meet release criteria, the subject will proceed to the operating room for the transperitoneal infusion of islets to the portal vein under general anesthesia.

The immunosuppression and islet transplants will be delivered during the hospitalization as detailed in the above section titled Study Design. Blood work will be monitored every 6-12 hours for 48-hours post-infusion for glucose, electrolytes, creatinine, LFTs, CBC and differential, and levels of tacrolimus and rapamycin. BG will be performed every 2-hours during 12-hours post-infusion and dextrose and/or insulin will be administered as needed to maintain the BG 100 – 140 mg/dL. Thereafter, BG will be performed at least 7 times daily and basal (for insulin pumps) or glargine insulin used at ~ 30 – 50 % of the pre-transplant dose for fasting BG > 100 mg/dL, and bolus (lispro or aspart) insulin used before meals for post-prandial BG > 140 mg/dL.

Hospital discharge is anticipated by Day +2, after which four weekly followed by four biweekly clinic visits will occur for the first 3 months to monitor glucose, electrolytes, creatinine, LFTs, CBC and differential, and trough levels of tacrolimus and rapamycin, and to titrate insulin therapy based on prior week's 4-7 daily BG measurements (pre- and 2-hours post-meals and bedtime). BG targets will be < 110 mg/dL fasting and < 140 mg/dL after meals for the first month, followed by < 126 mg/dL fasting and < 180 mg/dL after meals thereafter. Insulin-independence (defined in Section 3.1.1) will be determined at 75 ± 5 days post-transplant based in part on the ability to achieve those latter glycemic targets.

Subjects who do not meet the criteria for insulin-independence at any time after 75 days following the initial transplantation will be eligible for a second (or third) transplantation of islets from one or more donor pancreata, unless sensitization to donor antigen, as indicated by the development of >20% panel-reactive anti-HLA antibodies, has occurred. A similar procedure for hospital admission will be followed for a subsequent infusion of islets, except that daclizumab will replace Thymoglobulin® and start on Day 0; thus, no period of islet culture will be required. Post-infusion care will be identical for the first 2 months.

7.5.1 Criteria and Timing for Subsequent Islet Transplants

Islet transplant recipients who do not meet criteria for insulin independence (see section 3.1), but have either a basal or stimulated c-peptide level ≥0.3 ng/mL (0.1 nmol/L), will be considered **insulin-dependent with partial islet graft function**, and will be considered for a **second islet islet transplant**. Their insulin requirements will be determined by the total daily dose necessary to maintain the HbA1c ≤7.0%, the fasting capillary glucose level ≤ 140 mg/dL (7.8 mmol/L), and the 2-hour post-prandial capillary glucose levels ≤180 mg/dL (10.0 mmol/L), based on measuring capillary glucose levels a minimum of 21 times in a seven day period, and having at least one MAGE in that week.

In order to be eligible for a second islet transplant the following requirements must be met:

1. Subject received ≥5,000 IE/kg with the first transplant, but failed to achieve or maintain insulin independence
2. Subject has been compliant with study monitoring and prescribed immunosuppressive therapy
3. Subject has no unresolved SAEs
4. No evidence of progressive renal dysfunction, with blood creatinine rising above 2.0 mg/dL (177 µmol/L)
5. No evidence of hypersensitization, allergic responses, or other potentially serious drug reactions to medications required by the protocol
6. PRA ≤50% by flow cytometry (assessment performed locally) and the alloantibody specificity not cross-reactive with antigen(s) present in the subsequent islet preparation in order to avoid unacceptable antigen(s).

A second islet transplant will be considered after the 75 ± 5 days visit and metabolic assessment is completed, but before 8 months from the first transplant.

Islet transplant recipients who do not meet the criteria for insulin independence after the first infusion, and have both basal and stimulated c-peptide levels <0.3 ng/mL (0.1 nmol/L), will be considered for a second islet transplant only after review of the potency testing from the first transplant product and post-transplant clinical data by the Steering Committee that will have to give the final approval for the additional islet transplant.

If after the second islet transplant both basal and stimulated c-peptide levels remain <0.3 ng/mL (0.1 nmol/L), these recipients will be considered **failures** with no islet graft function, and immunosuppression will be withdrawn.

The option of a **third islet transplant** under this protocol will be considered only if all of the following conditions are met:

1. The subject received greater than 4,000 IE/kg with the second transplant, but remains dependent on insulin for longer than one month after the second transplant.
2. There is evidence of partial graft function.
3. The CIT PIs, Site PIs, and the Steering Committee have determined that there were no relevant protocol deviations at the site.
4. The subject has been compliant with study monitoring and prescribed immunosuppressive therapy.
5. No evidence of a serious and life-threatening infection, AE, or other condition that precludes attempting an intraportal injection or continuation of the post-transplant treatment regimen.
6. No evidence of PTLD.
7. No evidence of progressive renal dysfunction, with blood creatinine rising above 2.0 mg/dL (177 μmol/L).
8. No evidence of hypersensitization, allergic responses, or other potentially serious drug reactions to medications required by the protocol.
9. No evidence of abnormal liver ultrasound and LFTs within 1.5 times the ULN range.
10. PRA <50 by flow cytometry (assessment performed locally) and the alloantibody specificity not cross-reactive with antigen(s) present in the subsequent islet preparation in order to avoid unacceptable antigen(s).

Subjects who have completed 8 months of follow-up post-first transplant will no longer be eligible for additional islet transplants funded under this protocol. Subjects that do not meet the criteria for a subsequent transplant and do not have a functioning graft will enter a reduced follow-up schedule (Appendix 2).

7.6 Follow-up Visits

At 75 ± 5 days following each islet transplant, insulin-independence will be assessed as the primary endpoint as defined above (please see Section 3.1.1) based on one week of frequent capillary blood glucose monitoring ending with a fasting clinic visit for determination of HbA1c, fasting serum glucose and c-peptide, and serum glucose and c-peptide 90-minutes after ingestion of Boost®. This information also allows for the calculation of the β-score, a composite index of

islet graft function. Other self-glucose monitoring information captured at this visit will enable calculation of the Clarke and HYPO scores of hypoglycemia awareness and severity, respectively, the hypoglycemia fear survey, and the MAGE and LI scores of glycemic lability. This assessment will be repeated at 6, 9, 12, 15, 18, 21, and 24 months post-transplant.

The acute c-peptide and insulin responses to IV glucose and arginine will be performed during a two night admission to the GCRC scheduled at 3 months following each transplant, and at 12 and 24 months after the last transplant. The IV glucose test will be administered as a frequently-sampled intravenous glucose tolerance test in order to calculate insulin sensitivity (S_I) and the disposition index (DI). The IV arginine test will be performed with glucose-potentiation in insulin-independent recipients and those taking ≤0.1 U/kg of daily insulin to optimize glycemic control in order to determine the β-cell secretory capacity at day 75 and at 12 months. Also at 3, 12, and 24 months, 72-hours of continuous glucose monitoring will be performed.

At 6 and 14 months post-transplant, paired hypoglycemic and euglycemic clamps will be performed one week apart, in randomized order, each after a one night admission to the GCRC.

At 12 months following the first transplant retinal photography will be performed.

Clinic visits for immunosuppression drug monitoring will occur monthly for the first two years and quarterly thereafter. Additional tests will be repeated as indicated in the timeline provided in the appendix.

7.7 Visit Windows

The visit for determining the primary endpoint of insulin-independence will occur at 75 ± 5 days following each islet transplant. All other visits are scheduled on a monthly basis from the last transplant (for the first 9 months; quarterly thereafter ± 14 d until 24 months, then biannually ± 30 d), and should occur within 7 days (plus or minus) of the calendar date for the transplant. For example, a subject receiving a transplant on the 14th of one month should have follow-up visits between the 7th and 21th of subsequent months. A second transplant resets this schedule, except for the retinal photography.

Study visits should take place within the time limits specified on the Schedule of Events (Appendix 1).

7.8 Post-Study Follow-up

Upon completion of the protocol-specified follow-up (Appendix 1), subjects will be asked to enroll in a separate safety/efficacy follow-up protocol. Subjects who agree to participate in the follow-up protocol and sign informed consent, will be followed for an additional (5) five years. Follow-up assessments will be conducted at pre-defined intervals as specified in the follow-up protocol. A sample schedule of events for the follow-up protocol is provided in Appendix 4. If a subject chooses to withdraw from the follow-up protocol prior to completion of the 5years, every attempt will be made to complete at Year 5 assessments immediately prior to withdrawal.

8. SAFETY MONITORING

AEs that are classified as serious according to the definition set forth by the health authorities must be reported promptly to NIAID/NIDDK, Clinical Research Organization (CRO) / Data Coordinating Center (DCC), health authorities, PIs, and IRBs. This section defines the types of AEs and outlines the procedures for appropriately collecting, grading, recording, and reporting them. Information in this section complies with *International Conference on Harmonization (ICH) Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting* and *ICH E6: Guideline for Good Clinical Practice,* and applies the standards set forth in the *CIT-TCAE* . This document, created by the CIT Consortium, modifies the National Cancer Institute (NCI), *Common Terminology Criteria for Adverse Events (CTCAE)* version 3.*0* (June 10, 2003), to ensure applicability in the setting of Islet Transplantation.

8.1 Definitions

8.1.1 Adverse Event

An AE is any occurrence or worsening of an undesirable or unintended sign, symptom (including an abnormal laboratory finding), or disease that is temporally associated with the use of a medicinal product whether considered related to the medicinal product or not.

8.1.2 Serious Adverse Event

An SAE is defined per 21CFR§312.32 as "any AE occurring at any dose that suggests a significant hazard, contraindication, side effect, or precaution." This includes but is not limited to any of the following events:

1. Death.
2. A life-threatening event. A life-threatening event is any adverse therapy experience that, in the view of the investigator, places the patient or subject at immediate risk of death from the reaction as it occurred.
3. Inpatient hospitalization or prolongation of existing hospitalization. Please note that hospital admissions for the purpose of conducting protocol-mandated procedures do not need to be reported as SAEs, unless the hospitalization is prolonged due to complications.
4. Persistent or significant disability.
5. Congenital anomaly or birth defect.
6. An event that required intervention to prevent permanent impairment or damage. An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based on appropriate medical judgment, it may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

7. Other conditions specified in the protocol.

In addition, events that occur at a higher than expected frequency, as determined by appropriate medical judgment, may be considered SAEs.

Regardless of the relatedness of the AE to study drug, the event must be identified as an SAE if it meets any of the above definitions.

8.1.3 Unexpected Adverse Event

An AE is considered "unexpected" when its nature (specificity) or severity is not consistent with available product information, such as safety information provided in the package insert, the protocol, or the investigator's brochure.

8.2 Adverse Events

8.2.1 Collecting Procedure

AEs that are associated with a protocol mandated procedure, which is not part of the normal standard of care of the participant, and hypoglycemic events, will be collected beginning immediately after enrollment consent has been obtained. All other AEs will be collected beginning immediately after randomization. All AEs will continue to be collected until study completion, or for 30 days after the subject prematurely withdraws from the study. AEs will be followed until the time the event is resolved, stabilized, or the subject completes or withdraws from the study, whichever comes first.

AEs may be discovered through any of these methods:

- Observing the subject.
- Questioning the subject, which should be done in an objective manner.
- Receiving an unsolicited complaint from the subject.
- 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 this is the case, then the evaluation that produced the value or result should be repeated until the value or result returns to normal or can be explained and the subject's safety is not at risk. If an abnormal value or result is determined by the investigator to be clinically significant, it must be reported as an AE.

8.2.2 Recording Procedure

Throughout the study the investigator will record all AEs on the appropriate AE case report form (CRF) regardless of their severity or relation to study medication or study procedure. The investigator will treat subjects experiencing AEs appropriately and observe them at suitable intervals until their symptoms resolve or their status stabilizes.

8.2.3 Reporting of AE Information Following Study Completion

Collection of safety information following the end of investigational product administration is important in assisting in the identification of possible delayed toxicities or withdrawal effects. In this trial, all SAEs must be collected that occur within 30 days following discontinuation of dosing. In addition, the investigators should report any SAE that may occur after this time period which they believe to be certainly, probably, or possibly related to the investigational product. Finally, all events of death, graft loss, malignancy, PTLD, and serious infections (i.e., otherwise meeting SAE reporting requirements) must be reported for all randomized subjects until the end of the study, irrespective of study drug discontinuation or investigator-deemed causality.

8.2.4 Grading and Attribution

8.2.4.1 Grading Criteria

The study site will grade the severity of AEs experienced by CIT study subjects according to the criteria set forth in the *CIT-TCAE*... This document provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all AEs.

AE severity will be graded on a scale from 1 to 5 according to the following standards in the *CIT-TCAE* manual:

Grade 1 = Mild AE.

Grade 2 = Moderate AE.

Grade 3 = Severe and undesirable AE.

Grade 4 = Life-threatening or disabling AE.

Grade 5 = Death.

AEs, not included in the *CIT-TCAE* listing, should be recorded and their severity graded from 1 to 5 according to the General Grade Definition provided below:

Table 3: General severity definition of adverse event

Grade 1	**Mild**	Transient or mild discomforts (< 48 hours), no or minimal medical intervention/therapy required, hospitalization not necessary (non-prescription or single-use prescription therapy may be employed to relieve symptoms, *e.g.*, aspirin for simple headache, acetaminophen for post-surgical pain).
Grade 2	**Moderate**	Mild to moderate limitation in activity some assistance may be needed; no or minimal intervention/therapy required, hospitalization possible.
Grade 3	**Severe**	Marked limitation in activity, some assistance usually required; medical intervention/therapy required hospitalization possible.
Grade 4	**Life-threatening**	Extreme limitation in activity, significant assistance required; significant medical/therapy intervention required hospitalization or hospice care probable.
Grade 5	**Death**	Death.

All AEs will be reported and graded, by the PI or designee whether they are or are not related to disease progression or treatment.

8.2.4.2 Definition of Attribution

The relatedness, or attribution, of an AE to islet transplantation, which includes the transplant procedure and/or the islet product, the secondary investigational agent (Rituximab), or to the immunosuppression and/or infection prophylaxis will be determined by the site investigator. The site investigator will also record the determination of attribution on the appropriate eCRF and/or SAE report form. The relationship of an AE (attribution of AE) to islet transplantation (islets or transplant procedure), Rituximab, or immunosuppression/infection prophylaxis will be defined by using the descriptors provided below.

Table 4: Attribution of Adverse Events

Code	**Descriptor**	**Definition**
UNRELATED CATEGORY		
1	Unrelated	The AE is clearly not related to allogeneic islets; the islet transplant procedure; immunosuppression or infection prophylaxis.
RELATED CATEGORIES		
2	Unlikely	The AE is doubtfully related to allogeneic islets; the islet transplant procedure; immunosuppression or infection prophylaxis.
3	Possible	The AE may be related to allogeneic islets; the islet transplant procedure; immunosuppression or infection prophylaxis.
4	Probable	The AE is likely related to allogeneic islets; the islet transplant procedure; immunosuppression or infection prophylaxis.
5	Definite	The AE is clearly related to allogeneic islets; the islet transplant procedure; immunosuppression or infection prophylaxis.

For additional information and a printable version of the CIT-TCAE manual, consult the CIT website: http://isletstudy.org.

8.3 Serious Adverse Events

8.3.1 Collecting Procedure

SAEs will be collected following the subject's signing of the enrollment consent until 30 days after the subject completes or withdraws from the study. SAEs will be followed until the time the event is resolved, stabilized, or until 30 days after the subject completes or withdraws from the study, whichever comes first.

In addition, SAEs that occur after this time period that are believed to be certainly, probably, or possibly related to the investigational agent will be collected.

8.3.2 Recording Procedure

SAEs will be recorded on the AE eCRF.

If the investigator believes that an SAE is not related to the investigational product, but is potentially related to the conditions of the study (such as withdrawal of previous therapy, or complication of a trial procedure), the relationship should be specified in the narrative section of the SAE page of the eCRF.

8.3.3 Reporting Procedure

The following process for reporting an SAE ensures compliance with the ICH guidelines and 21CFR §312.32.

8.3.3.1 Reporting Criteria From Sponsor to Health Authority

After the SAE has been assessed, the event will be reported to the appropriate health authorities in the required manner based on the following criteria:

- **No reporting.** This requirement applies if the AE is deemed not serious by the DCC medical reviewer and, NIDDK/NIAID medical monitor.
- **Standard reporting** (*i.e.,* should be included in the investigational new drug [IND] annual report to the health authorities). This requirement applies if the AE is classified as any of the following:

  Serious, expected, and drug related.

  Serious, expected, and *not* drug related.

  Serious, *unexpected,* and not drug related.
- **Expedited reporting.** This requirement applies if the AE is considered serious, unexpected, and drug related as defined in 21 CFR 312.32. This type of SAE must be reported by the sponsor to the appropriate health authorities within 15 days; fatal or life-threatening events must be reported within 7 days.

8.3.3.2 Reporting Timeline– From the Site to the DCC

When an investigator identifies an SAE (as defined in section 8.1.2), he or she must notify the DCC Safety Reporting Center within 24 hours of discovering the event by submitting an initial electronic SAE CRF. In the event that the eCRF cannot be submitted (*i.e.* computer failure), the site must fax a paper SAE report to the DCC within 24 hours of discovering the event.

AEs as defined in Section 8.1.1 other than serious AEs will be reported to the DCC by the sites on at least a monthly basis.

8.3.3.3 Reporting Timeline – From the DCC to the Sponsor and Health Authorities

The DCC is responsible for notifying the sponsor within 2 business days of receiving the report by the clinical site. The sponsor is responsible for disseminating reports to the health authorities, and all investigators in the study and the manufacturer of the secondary study drug. SAEs per 21 CFR 312.32 definitions, except elective hospitalizations, will be reported to the Health Authority by the study sponsor (NIAID) in accordance with applicable regulations.

8.3.3.4 Notifying the Data and Safety Monitoring Board

The NIDDK/NIAID will provide the DSMB with listings of all AEs/SAEs on an ongoing basis at least yearly.

8.3.3.5 Notifying the Institutional Review Board and Ethics Committee

The investigator will ensure the timely dissemination of SAE information, including expedited reports, to the IRB and Ethics Committee (EC) in accordance with applicable regulations and guidelines.

8.3.3.6 Pregnancy

Sexually-active women of child bearing potential (WOCBP) must use an effective method of birth control during the course of the study, in a manner such that risk of failure is minimized. Prior to study enrollment, WOCBP must be advised of the importance of avoiding pregnancy during trial participation and the potential risk factors for an unintentional pregnancy. The subject must sign an informed consent form documenting this discussion.

In addition, all WOCBP should be instructed to contact the investigator immediately if they suspect that they might be pregnant (*e.g.*, missed or late menstrual period) at any time during study participation.

8.3.3.7 Reporting Pregnancy as a Serious Adverse Event

Any pregnancy that occurs during a clinical study that is using an investigational drug must be reported to the DCC utilizing the SAE report form. This report is *for tracking purposes only*. All pregnancies that are identified during the study must be followed to conclusion and the outcome of each must be reported. The investigator should be informed immediately of any pregnancy and should instruct a pregnant subject to stop taking study medication. The investigator should report all pregnancies within 24 hours (as described in section 8.3.3.2) using the SAE report form. The investigator should counsel the subject and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the subject should continue until the conclusion of the pregnancy, and a follow-up SAE report form detailing the outcome of the pregnancy should be submitted.

8.4 Updating Source Documentation

Documents describing the safety profile of a drug, such as the investigator's brochure, will be amended as needed by the study drug manufacturer to ensure that the description of safety information adequately reflects any new clinical findings. Until these documents are updated, expedited reporting will be required for additional occurrences of a reaction.

9. MECHANISTIC ASSAYS

The following specimens may be used in future assays to reevaluate biological responses as research tests are developed over time. Study subjects will be informed that they may be approached about additional clinical evaluations or studies that have received the full approval of the NIAID/NIDDK as new evaluations are identified. If additional evaluations are determined to be desirable, this protocol (and other appropriate study documents, *e.g.*, the informed consent and the statistical analysis plan [SAP]) will be amended and submitted to the appropriate regulatory authorities, ECs, and IRBs for approval. Each subject's signature will be obtained on the revised informed consent form before additional evaluations are performed. The specimens from these evaluations may be stored up to the end of the contract— approximately 5 years, or longer if the contract is extended.

9.1 Metabolic Testing

9.1.1 Study Endpoints

Designated primary and secondary endpoints will be employed to compare subjects in this study to the group in CIT-07. Because the assessment of islet graft function is dependent on complex physiologic relationships between the graft and its recipient, no single test adequately addresses the viability of the transplant. Therefore, insulin-independence will be used as a clinically relevant measure of islet graft function for the primary endpoint, and additional stimulatory tests of islet graft function utilizing meal (MMTT) and glucose (FSIGT) challenges will be performed to assess secondary endpoints. Also, the effect of islet graft function on glycemic control (HbA1c), glycemic lability (MAGE and LI), hypoglycemia (Clarke and HYPO scores), glucose variability (CGMS), and QOL will be assessed as additional secondary endpoints.

9.1.2 Metabolic Assessments

All subjects will use a study provided One Touch® Ultra glucometer for measuring capillary glucose levels. The timing of all metabolic assessments is provided in the Schedule of Events (Appendix 1).

9.1.2.1 Insulin Requirements

Subjects will record their total daily insulin dose on self-monitoring diaries. Subject should be given exogenous insulin as needed to maintain fasting capillary glucose levels ≤140 mg/dL (7.8 mmol/L) at a minimum of 4 out of 7 days a week; 2-hour post-prandial capillary glucose levels should not exceed 180 mg/dL (10.0 mmol/L) more than 3 times per week.

9.1.2.2 Glycemic control

Glycemic control will be assessed by HbA1c (%), which will be analyzed centrally at the University of Washington.

9.1.2.3 Glycemic lability

Glycemic lability will be assessed by both the MAGE[77] and the LI[81] .

The MAGE requires 14 – 16 BG measurements over two consecutive days taken before and 2-hours after breakfast, lunch, and dinner, and at bedtime with an optional measurement at 3 AM. A glycemic excursion is calculated as the absolute difference in peak and subsequent nadir (or vice versa) glucose values, with the direction (peak to nadir versus nadir to peak) determined by the first quantifiable excursion in the two day period. All excursions > 1 S.D. of the 7 – 8 glucose readings for the day in which they occurred qualify for the analysis, where they are summed and divided by the number of qualified excursions to give the MAGE in mmol/L (or mg/dL) glucose. A MAGE >11.1 mmol/L (200 mg/dL) is indicative of marked glycemic lability.

The LI requires 4 or more daily BG measurements over a 4 week period and is calculated as the sum of all the squared differences in consecutive glucose readings divided by the hours apart the readings were determined (range 1 to 12 hours) in $mmol/L^2/hr \cdot wk^{-1}$. A LI greater than or equal to the 90th percentile (433 $mmol/L^2/hr \cdot wk^{-1}$) of values derived from an unselected group of T1D patients is evidence for severe glycemic lability.

9.1.2.4 Hypoglycemia

An episode of severe hypoglycemia is defined as an event with one of the following symptoms: memory loss; confusion; uncontrollable behavior; irrational behavior; unusual difficulty in awakening; suspected seizure; seizure; loss of consciousness; or visual symptoms, in which the subject was unable to treat him/herself and which was associated with either a blood glucose level <54 mg/dL (3.0 mmol/L) or prompt recovery after oral carbohydrate, IV glucose, or glucagon administration[48].

In addition, composite indices of hypoglycemia frequency, severity, and symptom recognition will be assessed by both the Clarke survey[158] and the HYPO score[81].

The Clarke survey involves subject completion of eight questions scored by the investigator according to an answer key that gives a total score between 0 and 7 (most severe), where scores of 4 or more indicate reduced awareness of hypoglycemia and increased risk for severe hypoglycemic events.

The HYPO score involves subject recording of BG readings and hypoglycemic events (BG <3.0 mmol/L [54 mg/dL]) over a 4-week period and recall of all severe hypoglycemic episodes in the previous 12 months. A HYPO score greater than or equal to the 90th percentile (1047) of values derived from an unselected group of T1D patients indicates severe problems with hypoglycemia.

9.1.2.5 Mixed-Meal Tolerance Test (MMTT)

Basal (fasting) and stimulated glucose and c-peptide levels will be determined using the MMTT. Subjects will be instructed not to eat or inject short-acting (or bolus) insulin after 8 PM the night before the test. Evening or bedtime administration of long-acting insulin will be permitted, as will consumption of water. Subjects receiving CSII (insulin "pump" therapy) may remain on the basal rate of insulin. Subjects will arrive fasting to the transplant or diabetes clinic where the capillary BG will be checked. If the BG is <70 mg/dL (3.89 mmol/L) or >180 mg/dL (10 mmol/L), the test will be rescheduled for the next possible day. If the BG is 70 – 180 mg/dL (3.89 – 10 mmol/L), basal glucose and c-peptide levels will be drawn. Immediately after, the subject will receive 6 mL per kg body weight (to a maximum of 360 mL) of Boost® High Protein

Drink (or a nutritionally equivalent substitute) to consume in 5 minutes starting at time = 0. Then, at time = 90 minutes, stimulated glucose and c-peptide levels will again be drawn.

Each blood sample collected for c-peptide and glucose determination will be drawn according to University of Washington (Seattle, WA) SOP and will be shipped frozen to U of W for measurement in the core laboratory.

9.1.2.6 β-Score: A Composite Index of Post-Transplant Graft Function

The β-score will be determined from the HbA1c, insulin requirements, fasting (basal), glucose, and basal or stimulated c-peptide as developed by Ryan *et al*[151] . The score may range from 0 (no graft function) to 8, with all subjects reported with a score of 8 also having 90-minute glucose levels during a MMTT that are ≤10.0 mmol/L (180 mg/dL), indicative of excellent graft function.

9.1.2.7 The C-Peptide: (Glucose X Creatinine) Ratio

The c-peptide: (glucose X creatinine) ratio (CPGCR) will be determined from the fasting (basal) glucose and c-peptide, and a simultaneous serum creatinine. This measure accounts for both the dependence of c-peptide secretion on the ambient glucose concentration and the dependence of c-peptide clearance on kidney function[163, 164]. The CPGCR is calculated as [c-peptide (ng/mL) * 100]/[glucose (mg/dL) * creatinine (mg/dL)]. An index of islet graft function, this measure correlates well with both the 90-minute glucose levels during a MMTT and with the β-score[165].

9.1.2.8 Insulin-Modified Frequently-Sampled Intravenous Glucose Tolerance (FSIGT) Test

The AIR_{glu}, insulin sensitivity (SI), and disposition index (DI) will be determined using the FSIGT test. This assessment provides a composite measure of β-cell function, the DI, which relates the effect of SI on first-phase insulin secretion (AIR_{glu}). Understanding the effect of insulin sensitivity on insulin secretory dynamics post-transplant is important because insulin resistance imposes an increased demand on β-cell function to maintain the same level of glycemia. Whether insulin resistance, possibly attributable to immunosuppressive drugs, is an important problem post-transplant is not known. Preliminary data indicate that insulin sensitivity may actually be improved post-transplant, despite immunosuppression, possibly due to improved glucose and free fatty acid metabolism[152]. These results require confirmation by longitudinal analysis.

The insulin-modified FSIGT test[153] involves blood sampling at baseline (t = -10, -5, and -1 min) and at t = 1, 2, 3, 4, 5, 7, 10, 12, 14, 16, 18, 20, 22, 25, 30, 40, 50, 70, 100, 140, & 180 minutes post-injection of glucose at t = -30 seconds with an injection of insulin at t = 20 min. Each blood sample collected for insulin, c-peptide, and glucose determination will be drawn according to University of Washington (Seattle, WA) SOP and will be shipped frozen to U of W for measurement in the core laboratory. The AIR_{glu} is calculated as the incremental area-under-the-curve for insulin between 0 and 10 minutes post-injection (the same calculation can be performed for c-peptide). Glucose effectiveness (SG), a measure of insulin-independent glucose disposal, and SI, a measure of insulin-dependent glucose disposal, are derived from Bergman's minimal model using MinMod Millenium® software, and further allow for determination of the disposition index ($DI = AIR_{glu} \cdot SI$).

9.1.2.9 Continuous Glucose Monitoring System® (CGMS)

Glucose variability and hypoglycemia duration will be determined using CGMS® (Medtronic Minimed, Northridge, CA). CGMS involves the SC placement of a glucose sensor connected by tubing to a pager-sized monitoring device that stores glucose data over a 72-hour period. Subjects will have the sensor placed in the diabetes clinic and wear it continuously for 72 – 84 hours. Then they will drop the monitoring device off or ship it to the clinic 4 days later for analysis. Subjects will need to calibrate the sensor to their capillary BG readings 4 times daily with no interval between readings exceeding 12-hours. Data from each 72-hour period will be analyzed for mean glucose concentration, mean glucose variability (absolute value of measured glucose minus 5.5 mmol/L [100 mg/dL]), number and duration of hyper- (> 10.0 mmol/L [180 mg/dL]) and hypo- (<3.0 mmol/L [54 mg/dL]) glycemic episodes, and total duration of hypoglycemia[79, 119].

9.1.2.10 Quality of Life (QOL)

Generic and disease-specific measures will be used to assess quality of life.

Generic Measures

Version 2 of the SF-36® Health Survey, standard (4-week) recall form

This widely used, generic instrument derives eight scales (physical functioning, role-physical, bodily pain, general health, vitality, social functioning, role-emotional, mental health) and two summary components (physical and mental). Changes to version 2 in relation to version 1 include simplified wording, simplified layout, and changes to the number of response options to selected items. Additionally, current normative data for version 2 are based on more recent, 1998 general US population data and norm-based scoring has been developed for the eight individual scales in addition to the summary components (for which it was available in version 1). The current manual contains US population norms by gender and age group within gender. The publisher states that the next printing, which is scheduled for the fall of 2005, will contain disease-specific norms including diabetes and kidney disease. If the 36-item version of the instrument were felt to be too lengthy, version 2 of the SF-12 (standard recall form) would be an option. This shorter version would derive eight scales and two summary components and would be also be normed to the 1998 data (general population and disease-specific groups).

EQ-5D (EuroQoL)

This instrument is a utility measure that generates a descriptive profile and single index value for health status. The descriptive portion addresses five health dimensions (mobility, self care, usual activities, pain/discomfort, and anxiety/depression) with respondents indicating one of three possible responses for each dimension. Summary data can be reported as the proportion of respondents with problems in each dimension. Additionally, the multidimensional "health state" can be converted to a single weighted health status index that reflects the valuation of various possible health states from general population samples, including one that has been developed in a nationally representative US sample. The second portion of the EQ-5D is a (0-100) visual analogue scale that is used to report overall health status. Advantages of this instrument include its brevity and particular application in cost-effectiveness research. The EQ-5D is a public domain instrument. Projects may be registered and instruments obtained through the EQ-5D website, www.euroqol.org.

Disease-targeted Measures

Diabetes Distress Scale

The Diabetes Distress Scale (DDS) represents the latest iteration of the Problem Areas in Diabetes (PAID) scale. This is a 17-item self-administered questionnaire culled from a longer battery of 28 items. Psychometric properties for the DDS were recently published in Diabetes Care (March 2005). The DDS measures four diabetes-related distress domains: emotional-burden (EB), physician-related interpersonal distress (PD), regimen-related distress (RD), and diabetes-related interpersonal distress (ID). Internal consistency as measured by Cronbach's coefficient alpha ranged between 0.88 and 0.93 for the multi-item scales. The developers tested for and demonstrated construct validity using exploratory factor analysis.

Hypoglycemic Fear Survey

The Hypoglycemic Fear Survey (HFS) is a 23-item self-administered survey for measuring the fear experienced with respect to hypoglycemia. The HFS measures hypoglycemia avoidance behavior and worry about hypoglycemia. Different versions of the instrument can be found in the literature, varying in length from 15 to 33 items. We have used the 33-item recommended by Daniel Cox. Coefficient alpha for the behavioral and the worry scales were found to exceed 0.90.

9.1.2.11 Glucose-potentiated arginine (GPA) test

Rationale:

Both the glucose-potentiation slope and β-cell secretory capacity are ~ 25% of normal in insulin-independent transplant recipients [11] suggesting the presence of a very limited engrafted mass even in successful cases. This is consistent with the increasing rate of return to insulin therapy that occurs with each year following successful transplantation. Transplantation strategies that improve the engrafted β-cell mass should be identified by an increased β-cell secretory capacity in insulin-independent recipients, and should allow for more durable graft survival. Alternatively, immunosuppressive strategies that are free of calcineurin-inhibitors may lead to further improvements of both the glucose-potentiation slope and β-cell secretory capacity since calcineurin-inhibitors impair glucose-dependent insulin secretion and reduce β-cell insulin content.

Approach:

The standard IV arginine stimulation test (AST) involves blood sampling at baseline (t = -5 & -1 min) and at t = 2, 3, 4, & 5 min post-injection of a maximally stimulating dose of arginine hydrochloride (5 g or 50 mL of 10% solution, Pharmacia & Upjohn, Kalamazoo, MI) over 1 min starting at t = 0. After collection, the protease inhibitors trasylol and leupeptin are immediately added to the blood samples, which are placed on ice, centrifuged at 4 °C, and frozen at -80 °C until biochemical analysis. The acute C-peptide and insulin responses to arginine (ACR_{arg} & AIR_{arg}), defined as the mean of the post-injection C-peptide or insulin values minus the mean of their baseline values, provide measures of β-cell function. The total amount of blood sampled is 24 mL.

The AST may be extended to a glucose-potentiated arginine (GPA) test.[11] This will only be done in insulin-independent subjects or those taking ≤ 0.1 U/kg of insulin daily to optimize their glycemic control. After the baseline AST described above, a hyperglycemic clamp technique utilizing a variable rate of 20% and 10% glucose solutions is performed to achieve a glucose

level of 230 mg/dL. Blood samples are taken every 5 min, centrifuged, and measured at bedside with a portable glucose analyzer (YSI 1500 Sidekick; Yellow Springs Instruments, Yellow Springs, OH) in order to adjust the infusion rates and achieve the desired glucose concentration. After 45-minutes of the glucose infusion, the 5 g arginine pulse is injected again with identical blood sampling. It has been demonstrated that the first administration of arginine has no effect on the subsequent response to arginine using this protocol. Then, a 2-hour period without glucose infusion takes place to avoid the priming effects of hyperglycemia on insulin release. At the end of the 2-hour period, a hyperglycemic clamp is performed to achieve a glucose level of 340 mg/dL. Forty-five minutes after initiation of the glucose infusion, another arginine pulse is injected with identical blood sampling. The total amount of blood sampled is 92 mL.

The AIR_{arg} performed during the 230 mg/dL glucose clamp allow for determination of the glucose-potentiation slope, a measure of glucose-dependent insulin secretion, defined as the difference in the AIR_{arg} at fasted and 230 mg/dL glucose levels, divided by the difference in glucose ($\Delta AIR_{arg}/\Delta$ PG). The AIR_{arg} performed during the 340 mg/dL glucose clamp allows for estimation of the β-cell secretory capacity (AIR_{max}), a measure of functional β-cell mass, since the AIR_{arg} is maximal at glucose concentrations > 315 mg/dL[17]. The same calculations can be performed using ACR_{arg}[11].

9.1.2.12 Glucose Counter-Regulation

Rationale:

Islet transplantation is being evaluated as a potential therapy for patients with T1D experiencing severe problems with hypoglycemia. These problems result from the defective glucose counter-regulation present in these patients. Recent work has demonstrated normal suppression of endogenous insulin secretion during hypoglycemia and significantly greater glucagon secretion during hypo- when compared to euglycemia in islet transplant recipients. The presence of less endogenous insulin and more glucagon should increase endogenous glucose production by the liver and serve to prevent or correct low blood glucose. Additionally, some islet transplant recipients have normal epinephrine responses to hypoglycemia[11, 68, 69], which may contribute to increasing glucose production. Because increasing endogenous glucose production is the final common pathway for these hormonal responses and is what is clinically important in the defense against hypoglycemia (not the levels of the hormones themselves), we propose to evaluate endogenous glucose production in T1D patients before and > 6 months following islet transplantation using a hyperinsulinemic hypoglycemic clamp and a paired hyperinsulinemic euglycemic clamp.

Approach:

Hyperinsulinemic hypoglycemic clamp: Subjects will avoid strenuous exercise for 3 days prior to testing. On the day before testing, the last injection of long-acting insulin will occur either that morning or the night before. Subjects will be admitted to the General Clinical Research Center (GCRC) of the University of Pennsylvania in the afternoon and fed a standard diabetic dinner with their last injection of rapid acting insulin by 1800 and fasting started at 2000. At 2100 an antecubital venous catheter will be placed for the infusion of regular insulin (0.5 U/mL solution) as needed overnight to maintain the blood glucose between 100 – 140 mg/dL. Any CSII will be discontinued at that time. At 600 a contralateral retrograde venous hand catheter will be placed and the hand warmed in a thermoregulated box (~ 50 ºC) for arterialized venous blood sampling. Patency of the intravenous catheters will be maintained with slow infusions of 0.9% saline.

At 0700 (t = -120 min) a primed (5 mg/kg · fasting glucose/90 for 5 min) continuous (0.05 mg/kg·min for 355 min) infusion of 6,6 $^2H_2$ glucose (99% enriched; Cambridge Isotopes

Laboratories, Andover, MA) will be administered to assess endogenous glucose production before and during the induction of hypoglycemia[166]. After baseline blood samples at t = -20, -10, and -1 min, at 0900 (t = 0 min) a continuous (1.0 mU/kg·min for 240 min) infusion of insulin will be administered to produce hyperinsulinemia. Subsequently, a variable rate infusion of 20% glucose will be initiated to achieve hourly glucose plateaus of 80, 65, 55, and 45 mg/dL. To reduce changes in plasma enrichment of 6,6 $^2H_2$ glucose during the clamp, the variable glucose infusion will be enriched to ~ 2.0 % with 6,6 $^2H_2$ glucose[166].

Blood samples will be taken every 5 min, centrifuged, and measured at bedside with a portable glucose analyzer (YSI 1500 Sidekick; Yellow Springs Instruments, Yellow Springs, OH) to adjust the glucose infusion rate and achieve the desired glucose concentration. Additional blood samples will be taken at t = 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220, and 240 min for biochemical analysis and verification of the glucose levels. The total amount of blood sampled will not exceed 168 mL. A questionnaire will be administered every 20 min during the study in order to quantitate autonomic symptoms as the sum of scores ranging from 0 (none) to 5 (severe) for each of the following symptoms: anxiety, palpitations, sweating, tremor, hunger, and tingling[167].

Hyperinsulinemic euglycemic clamp: Because hyperinsulinemia inhibits endogenous insulin secretion, glucagon secretion, and endogenous glucose production, a euglycemic clamp will be performed to assess the impact of insulin per se on endogenous glucose production independent from hypoglycemia. Subjects will undergo the euglycemic clamp in randomized order with the hypoglycemic clamp at least 1 week but not longer than 1 month apart. The hyperinsulinemic euglycemic clamp will be conducted as described for the hypoglycemic clamp above, but with the target glucose 90 mg/dL for the entire 240 min study.

Biochemical analysis: Blood samples will be collected on ice into chilled tubes containing EDTA with protease inhibitors added to protect islet hormones, centrifuged at 4 ºC, separated, and frozen at -80 ºC within 60 min of collection for subsequent analysis. Glucose will be measured in duplicate by the glucose oxidase method using an automated glucose analyzer (YSI 2300; Yellow Springs Instruments). Plasma immunoreactive insulin, C-peptide, glucagon, growth hormone and cortisol will be measured in duplicate by double-antibody radioimmunoassay (Linco Research, St. Charles, MO) performed at the Diabetes Endocrinology Research Center of the University of Pennsylvania. Plasma epinephrine and norepinephrine will be measured by high-performance liquid chromatography with electrochemical detection at the GCRC laboratory of the University of Pennsylvania. Enrichment of 6,6 $^2H_2$ glucose will be measured by gas chromatography-mass spectrometry analysis at Metabolic Solutions, Inc. (Nashua, NH). Samples from paired euglycemic-hypoglycemic experiments for each subject will be assayed simultaneously.

Calculations: Incremental C-peptide, glucagon, growth hormone, cortisol, epinephrine, norepinephrine, autonomic symptom, and endogenous glucose production responses will be calculated by subtracting basal values from those obtained during the last 20 min of hypo- or euglycemia.

Basal rates of endogenous glucose production (EGP) will be calculated using the formula: basal EGP = IR ([enrichment_{inf}/$\text{enrichment}_{plasma}$] – 1), where IR is the basal 6,6 $^2H_2$ glucose infusion rate in mg/kg·min, enrichment_{inf} is the percent enrichment of the 6,6 $^2H_2$ glucose infusate, and $\text{enrichment}_{plasma}$ is the percent basal plasma 6,6 $^2H_2$ glucose enrichment.

The rate of appearance (Ra) of glucose during the clamps will be calculated using Steele's non-steady state equation modified for the use of stable isotopes: Ra = F-(V[((C2+C1)/2)][(E2-E1)/(t2-t1)]/(E2-E1)/2) where C2 and C1 are the glucose concentrations at the times (t) 2 and 1

respectively; V is the effective volume of distribution of glucose (40 mL/kg), F is the infusion rate (0.05 mg/kg·min), and E represents the isotopic enrichment at the respective time points. EGP during the clamps will be calculated from the difference between the rate of appearance of glucose in the plasma and the glucose infusion rate.

The difference in the incremental responses obtained during the hypo- and euglycemic experiments will be compared within subject from before to after islet transplantation with one-way or repeated measures ANOVA as appropriate with significance considered at $P < 0.05$ (two tailed).

9.2 Immunologic Testing

Although insulin independence can be achieved via transplantation of an adequate number of viable, functional islets, a gradual reduction in the percent insulin independent patients occurs over time, with approximately 25% of patients still insulin free at 4 years post-transplant. Immune mediated islet destruction in the form of allorejection and/or recurrent autoimmunity, as well as attrition of a marginal islet mass due to exhaustion and/or toxicity of immunosuppressive agents, have all been postulated to play a role in islet loss. In order to begin to dissect the role of immune mediated reactions in allograft loss, tests will be done to determine if sensitization to donor allo- or islet autoantigens has occurred. In addition, maintenance of protective immunity in the setting of immunosuppression will be addressed, as will the role of innate immune reactions in the early post-transplant period.

While methods for determination of allo- and autoantibody have been extensively studied and are fairly well-established, reliable, reproducible and validated methods for assessment of T cell immunoreactivity to allo and/or autoantigens do not exist. For the most part, these techniques are time-consuming, technically demanding and require large blood volumes and significant staff time for set up and analysis of the resultant data. Several methods are undergoing testing in multiple T1D consortia (*e.g.,* ELISPOT, tetramer staining, T cell proliferation assays) to determine which tests provide the most reliable data with regards to distinguishing between patients with T1D vs normal controls (for autoantigen) and to improve techniques for assessing recipient anti-donor reactivity.

9.2.1 Immune Assays

9.2.1.1 HLA typing of donors and recipients, crossmatching

HLA typing of donors and recipients, as well as crossmatching, will be done at individual centers. A negative crossmatch is required in order for transplantation to occur.

9.2.1.2 Alloantibody

Development of alloantibody is generally associated with longer term graft loss. Each sample will be tested using flow PRA screening beads to determine the presence of class I or class II antibodies. If results are negative for class 1 and class 2 antibodies, no further HLA antibody testing will be performed on that sample. If results are positive for class 1 and/or class 2, flow specificity beads will be used to determine the PRA and specificities of anti-HLA antibodies present. If antibody specificity cannot be defined using this technique, single antigen beads may be tested. Serum samples will be stored in the laboratory in the event that additional testing is

required. Dr. Kamoun at the University of Pennsylvania will provide core laboratory services for alloantibody analysis. Two mL blood samples for collection of serum will be obtained prior to initiation of immune suppression and transplant and at 3, 6, 9, and 12 months post-infusion, including 365 days post-initial transplant. Quarterly sample collection will continue for the duration of follow-up.

9.2.1.3 Autoantibody

The role of autoantibody in graft loss remains unclear. George Eisenbarth's lab in Denver will provide core lab service for autoantibody assessments.

9.2.1.4 Measures of innate immunity

In order to correlate expression of pro-inflammatory or pro-coagulant markers on islets with recipient response in the early post-transplant period, ethylenediaminetetraacetic acid (EDTA) anti-coagulated blood will be collected for assessment of thrombin-antithrombin (TAT), C3a, and c-peptide levels.

9.2.1.5 Archived serum, cells and RNA

In order to ensure that we will ultimately gain as much information as possible from these trials, and due to the ongoing development of assays such as T cell assays. Serum, cells and RNA will be archived for future analyses. Details for subjects regarding the archiving of samples and use for future assays are contained in the study's informed consent form. Subjects will have the option of whether or not they want to have samples archived and will indicate their choice on the informed consent form. A subject's choice regarding archiving samples will not affect his/her participation in the study.

Serum: Blood will be collected obtain serum, and archived in the NIDDK repository.

Peripheral Blood Mononuclar Cell (PBMC) and Plasma: Blood will be collected to obtain recipient PBMC, processed and archived in the NIDDK repository.

RNA: Blood will be collected for RNA isolation and archival in the NIDDK repository.

9.2.1.6 Alloimmunization Studies

HLA antibody analysis will be performed in the laboratory of Dr. Malek Kamoun at the University of Pennsylvania using Luminex (One Lambda, Inc. 21001 Kittridge Street, Canoga Park, CA 91303-2801).

Subjects will be screened for anti-HLA class I/II antibodies using Luminex screening assays (LS1 and LS2). If either assay is positive, a Luminex Single Antigen (LSA) assay will be performed to define PRA antigen specificity. A Luminex Specificity Bead (LSB) assay will be performed only if LSA results are not definitive. LSA and LSB assays include the following:

LSA1- high definition class I screening for specificity
LSB1- class I screening for specificity
LSA2- high definition class II screening for specificity
LSB2- class II screening for specificity

9.2.2 University of Pennsylvania Specific Assays

Additional assays that are being developed at individual centers will be performed at those sites. Protocols and results will be shared to determine if an assay is gaining validity as a measure that should be incorporated into future trials.

9.2.2.1 Flow Cytometry

Basic immunophenotyping is especially important for monitoring of immunotherapies that involve agents that delete and/or alter the function of specific leukocyte subsets (*e.g.,* T or B cells). The four-color panel that will be used for the routine evaluation of lymphocyte subsets at Penn is shown below.

In brief, samples for routine flow cytometry are processed as follows: All samples are processed within 24 hours of the blood draw and usually within 4 hours. Briefly, 150 microliters of anticoagulated whole blood (sodium EDTA, equilibrated for a minimum of 30 minutes at room temperature with gentle mixing) are aliquoted in Beckton Dickinson FACS tubes. The cells are washed three times, incubated with blocking antibody (if required) and subsequently with extracellularly staining antibodies. Red blood cells are lysed and the white cells are resuspended in a buffer containing 1% paraformaldehyde. Samples are analyzed by flow cytometry within 24 hours of processing and staining.

Table 5: Four Color Lymphocyte Immunophenotyping Panel

Tube	Description	Acquisition Gate	FITC	PE	PerCP	APC
1-5	**Setup and Compensation**		**CD8 (2)**	**CD8 (3) (10u)**	**CD8 (4)**	**CD8 (5)**
6	**Control**	**Lymphocytes (10K)**	**IgG1**	**IgG1**	**CD4**	**CD8**
7	**Immature**	**CD19+ Ly (10K or ET)**	**CD24**	**CD138**	**CD19**	**Class II**
8*	**Transitional**	**CD19+ Ly (10K or ET)**	**IgM**	**CD23**	**CD19**	**CD21**
9	**Transitional**	**CD19+ Ly (10K or ET)**	**CD10**	**CD38**	**CD19**	**CD27 (5ul)**
10	**Transitional**	**CD19+ Ly (10K or ET)**	**CD24**	**CD38**	**CD19**	**CD27 (5ul)**
11*	**Marginal/Isotype**	**CD19+ Ly (10K or ET)**	**IgD**	**CD24 (5ul)**	**CD19**	**IgM**
12	**B1**	**CD19+ Ly (10K or ET)**	**CD24**	**CD38**	**CD19**	**CD5**
13*	**MBL/Lymphoma**	**CD19+ Ly (10K or ET)**	**CD10**	**CD23**	**CD19**	**CD5**

14*	**Light Chains**	**CD19+ Ly (10K or ET)**	**Kappa**	**Lambda**	**CD19**	**CD5**
15*	**Activated/Plasmablast**	**CD19+ Ly (10K or ET)**	**CD27**	**CD38**	**CD19**	**CD23(20ul)**
16*	**Memory**	**CD19+ Ly (10K or ET)**	**IgM**	**CD38**	**CD19**	**CD27 (5ul)**
17*	**Plasma**	**CD19+ Ly (10K or ET)**	**Kappa/ Lambda**	**CD138**	**CD20**	**CD19 (5ul)**
18	**T cell**	**CD3+ Ly (10K or ET)**	**CD3**	**CD8 (10ul)**	**CD4**	**CD45**
19	**CD4**	**CD3+ Ly (10K or ET)**	**CD3**	**CD62L**	**CD4**	**CD45RA**
20	**CD8**	**CD3+ Ly (10K or ET)**	**CD3**	**CD62L**	**CD8**	**CD45RA**
21*	**Treg**	**CD3+ Ly (20K or ET)**	**CD3**	**CD127**	**CD4**	**CD25**
22	**T activation**	**CD3+ Ly (10K or ET)**	**CD28**	**CD62L**	**CD4**	**CD3**
23	**T activation**	**CD3+ Ly (10K or ET)**	**CD28**	**CD95**	**CD4**	**CD8**
24	**NK cells**	**Lymphocytes (50K)**	**CD3**	**CD56**	**CD8**	**CD16**
25*	**NK activation**	**Lymphocytes (50K)**	**NKG2d**	**CD56**	**CD3**	**NKp44(15ul)**
26*	**NK clones**	**Lymphocytes (50K)**	**CD158b**	**CD158a**	**CD3**	**NKB1 (10ul)**

Notes (Table 5):
All antibodies are commercially available from BD Pharmingen, Biolegend, and e-Bioscience. The minimum numbers of gated events to be collected are indicated in parentheses. In the acquisition gate column, "Lymph" or Ly means lymphocyte gate, ET means entire tube. Blocking antibodies are added to the starred tubes. 20 μL of of each fluorophore-conjugated antibody are added per tube except to tubes in which microliter amounts of antibody added are indicated in values in parentheses. These amounts are based either on titration experiments performed in pilot experiments or manufacturer recommendations (data not shown).

9.2.2.2 High Definition Flow Cytometry

In addition to basic phenotyping, more detailed analysis of B cell phenotype and repertoire has been developed. The ultimate goal of these studies will be to improve monitoring of autoreactive B cells in T1D patients. These studies will assist in achieving the following aims:

1. Better definition of transitional B cell subsets in humans.

2. Analysis of peripheral lymphocyte dynamics in humans. The serial analysis of B cell depletion and repletion following rituximab therapy will provide insights into the turnover and dynamics of different peripheral B cell subsets. Plasma cells and other cells that may be resistant to B cell depletion are of special interest in this study as well.

3. Provide a technical platform for attempting to isolate and characterize (auto) antigen-specific B cells. The frequency of antigen-specific B cells to a conventional T dependent protein antigen ranges from one in ten thousand to less than one in a million lymphocytes. Therefore any method that is used to characterize these cells must be very stringent about separating these cells from the background.

To begin to address these questions, we have developed high definition (HiD) flow cytometry panels (Table 6). Key elements of the panel include the ability to remove unwanted populations of irrelevant cells types through the use of a dump channel and rigorous gating for doublet exclusion as well as live-dead cell discrimination.

Table 6: Ten Color HiD Flow Panel

Tube	FITC	PE	PE-TR/ PE-AF610	PerCP	PE-Cy7	AF-750	APC	APC-Cy5.5/ AF-700	PE-Cy5	PE-Cy5.5	Pacific Blue	Live
1	No stain											
2	CD8 (20ul)											
3		CD8 (3ul)										
4			CD4 (1ul)									
5				CD20 (20ul)								
6					CD19 (1ul)							
7						CD3 (5ul)						
8							CD8 (20ul)					
9								CD38 (5ul)				
10									CD21 (20ul)			
11										CD5 (5ul)		
12											CD8 (10ul)	
13 *	IgM (20ul)	CD10 (20ul)	CD24 (5ul)	CD20 (20ul)	CD19 (1ul)	CD27 – (20ul)	CD23 (20ul)	CD38 (5ul)		CD5 (5ul)	Dump 1	Dapi
14 *	IgM (20ul)	IgD (1ul)	CD24 (5ul)	CD20 (20ul)	CD19 (1ul)	CD27 - (20ul)	CD23 (20ul)	CD38 (5ul)	CD21 (20ul)		Dump 1	Dapi
15 *	λ (20ul)	κ (20ul)	CD24 (5ul)	CD20 (20ul)	CD19 (1ul)	CD27 – (20ul)	CD23 (20ul)	CD38 (5ul)	CD138 (10ul)		Dump 1	Dapi
16 *	IgD (20ul)	Kappa (20ul)		9G4 (1ul)	CD19 (1ul)	CD27 - (20ul)	IgM (20ul)	CD38 (5ul)		CD5 (5ul)	Dump 1	Dapi
17 *	IgM (20ul)	CD267 (20ul)			CD19 (1ul)	CD27 – (20ul)	CD23 (20l)	CD38 (5ul)	CD95 (20ul)		Dump 1	Dapi
18 *	NKG2D (20ul)	CD8 (3ul)	CD4 (1ul)	CD45 (20ul)	CD16 (1ul)	CD3 (5ul)	NKp44 (10 ul)	CD56 (15ul)	CD62L (20ul)		Dump 2	Dapi

Dump 1 – CD3 (10ul), CD8 (10ul), CD14 (10ul)
Dump 2 – CD19 (5ul)

Abbreviations for table 6 are the same as in table 5. Single color controls are used for digital compensation. Flow cytometry data are acquired on an LSRII flow cytometer and analyzed using

FlowJo (Treestar Inc.) software. Four-color and ten-color immunophenotyping studies will be performed in Dr. Luning Prak's laboratory.

9.2.2.3 CDR3 Spectratyping, Clone tracking, and Light Chain Receptor Editing

Heavy chain spectratyping will be performed using "global" primers (FR3 + J_H cocktail) to screen for clonal expansions. Candidate expanded clones will be sequenced and V_H/J_H-specific primer sets which will be used to track specific clones in individual subjects. V_H/J_H primer sets will also be used for reading frame analysis and size distribution analysis to gain further insights into the stringency of B cell repertoire selection. Light chain receptor editing frequency will be monitored by iRS-RS quantitative PCR and diversity of editing products will be monitored in parallel by spectratyping. Results will be normalized for the amount of input DNA by actin PCR and absolute quantification will be performed using titrations of a cloned iRS-RS rearrangement into genomic DNA lacking antibody gene rearrangements (standard curve). Rearrangement diversity and frequency estimates will be normalized for the B cell fraction and kappa/lambda ratio (these measurements will be obtained from the immunophenotyping studies). CDR3 spectratyping, clone tracking and quantitative PCR assays will be performed in the Luning Prak laboratory.

9.2.2.4 B and T Lymphocyte Functional Assays

B lymphocytes will be monitored periodically for functional competence to respond to light chain Fab, CpG and Fab + CpG stimulation. Mononuclear cell preparations from peripheral blood of study subjects will be frozen and tested in batches in the same assay on the same day. LPS will be included as a positive control and media will be included as a negative control. B cell activation will be monitored by calcium flux, lymphocyte activation marker expression and cell proliferation (CFSE peaks will be evaluated using ModFit software). These assays will be performed in the laboratory of Jean Boyer.

T cells will be stimulated in culture with islet-derived peptides from GAD65, IAA, and IA-2. Mononuclear cell preparations from peripheral blood of study subjects will be frozen and tested in batches in the same assay on the same day. Controls include media alone, concanavalin stimulation, PMA/ionomycin and tetanus toxoid. Assay read-outs will include cell proliferation (monitored by CFSE dilution) and expression of cytokines (IL-10, TNFα and IFNγ) by ELISpot assay. These assays will be performed in the laboratory of Jean Boyer.

9.2.2.5 Monitoring of Rituximab

Also relevant to treatment with antibodies, chimeric molecules or other proteins is analysis for development of antibodies to the therapeutic protein; such a response can limit the efficacy of the treatment. Rituximab levels will be monitored by PK assays and the development of anti-rituximab antibodies (human anti-chimeric antibodies [HACAs]) will be monitored using the same assays that are currently in place for other rituximab trials on campus. The tests will be performed by Genentech.

9.2.2.6 Antibody repertoire

We hypothesize that the B cell repertoire in T1D patients will contain expanded clones and have evidence of increased receptor editing. We have developed a series of antibody gene rearrangement assays to test these assertions including CDR3 spectratyping with global and VH-gene specific primers and a new kind of L chain assay that monitors the diversity of RS recombination events. We predict that CDR3 spectratyping will reveal the reproducible presence of rearrangements of particular sizes, suggestive of clonal expansion. Clonal expansion can be confirmed by DNA sequence analysis. If no evidence of clonal expansion is obtained with "global" VH primers, we will survey specific VH gene rearrangements. Of particular interest in this connection is the autoimmune-associated V gene 4-34. This H chain is found at increased frequency in a variety of autoimmune conditions and is associated with multireactivity. Furthermore, a monoclonal antibody, 9G4, can be used to isolate B cells that express VH 4-34 H chains. If expanded clones are recovered, we can monitor their longevity/turnover in the setting of B depletion therapy using clone-specific PCR assays. For receptor editing, we anticipate seeing VH gene rearrangements with increased CDR3 length, suggestive of reiterative H chain rearrangement (this can be verified by DNA sequence analysis). We also expect, if receptor editing is increased, to see a greater level of RS junctional diversity in B cells from T1D patients compared to non-autoimmune controls. It will be interesting to analyze these features of the B cell repertoire before and after B cell depletion. Drawing from our experience in B cell repertoire analysis in normal and auto-immune prone strains of mice, we look forward to applying these assays alone or in conjunction with B cell separation methods to analyze B lymphocyte repertoire in T1D patients.

9.2.2.7 T cell ELISpot Assays

T cell responses will be monitored by ELISpot assay. A screening assay will use dominant T cell epitopes against GAD65, IAA and IA-2 using IFN-g and TGFb secretion as read-outs. If the screening ELISpot is positive, the specificity of reacting T cells (checked against a panel of peptides) will be profiled and the secretion of multiple cytokines will be monitored. A multiplex cytokine assay is currently under development.

9.2.2.8 Protective Immunity

Determining whether or not protective immunity is maintained in immunosuppressed patients is important for understanding the risks associated with various agents. We will monitor the memory response to influenza virus by serology, B and T cell ELISpot and, if available, B cell flow cytometry in patients at baseline and 6 months after islet transplantation. Cell proliferation and cytokine profiling will help evaluate the nature of the immune response. Functional assays of antigen-specific B cells and T cells to influenza are in development.

10. STATISTICAL CONSIDERATIONS AND ANALYTICAL PLAN

10.1 Statistical Analyses

The goal of this study is to provide strong scientific evidence that the rate of favorable outcome in transplanted subjects is high enough to justify the risks of the procedure and the immunosuppression. All efficacy analyses will be based on the intention-to-treat principle. Once a subject has been transplanted s/he will be included in the final analysis. All transplanted subjects will also be included in the safety analysis. There will be planned interim analyses for safety and efficacy at the direction of the DSMB. Details of all statistical analyses will be given in the formal statistical analysis plan (SAP).

10.2 Study Endpoint Assessment

10.2.1 Primary Endpoint

The primary objective of the analysis is to estimate the rate of insulin independence at 75 days after the first Islet Transplant among those in the study. The primary endpoint is insulin independence (yes/no) at day 75 after Islet Transplant as defined in section 3.1. The primary analysis will compute an exact binomial estimate and a 95% confidence interval for the true rate.

The primary endpoint should be available for all randomized subjects. An exception will be if a death occurs or if the subject withdraws consent to be followed, in these cases the endpoint will be classified as failure to achieve insulin independence. Should the endpoint not be evaluated for a particular individual for other reasons, a failure will be imputed unless an evaluation is done at a time longer than 75 days after transplant and before an additional islet transplant, in which case that later value will be imputed. All imputations will be reported with the primary analysis.

10.2.2 Secondary Endpoint

As a secondary analysis the proportion of subjects with insulin independence at 75 days after transplant will be compared to the proportion of subjects with insulin independence at the University of Pennsylvania in the CIT-07 study using an exact test for two proportions. The same proportion will also be compared to pooled analysis of all subjects in the CIT-07 study from the four CIT centers (see the Statistical Analysis Plan, SAP).

Dichotomous variables such as HbA1c classified as normal (≤7.0%) and non-normal (>7.0%) will be analyzed in a manner similar to the primary analysis. Continuous variables such as HbA1c, AIRg and c-peptide response will be examined for normality of the distribution and if there is no compelling evidence that the distribution is non-normal, will be analyzed by computing the mean and calculating a 95% confidence interval using the student's t-distribution

If there is compelling evidence that the normal distribution does not apply a logarithmic, square root or other transformation will be done before analyzing the data. An estimate and a 95% confidence interval for each response will be calculated. In addition, the responses will be

compared to the responses in subjects at the University of Pennsylvania in CIT-07 (using an exact test for two proportions, or a t-test, possibly after transformation, for continuous variables).

The additional hypothesis generating endpoints of 3.1.2 and the additional endpoints in 3.1.3 will be analyzed similarly and all the data will be subject to exploratory analysis. For variables that are collected at regular follow up visits, regression models for longitudinal data will be used to examine the responses over time for both dichotomous and continuous variables.

Values will not be imputed for missing secondary endpoints. The frequency of missing data will be reported.

There are a very large number of secondary endpoints. By examining multiple secondary endpoints it is likely that some variables will be found significantly different between the subjects in this study and those from the University of Pennsylvania in CIT-07, but these findings may be Type I errors. Appropriate qualifiers will be reported with any findings which are nominally significant at the 0.05 level or less and such results should be interpreted with extreme caution.

The sample size is small and so confidence intervals will be large and the power to detect differences between the subjects in this study and the subjects in CIT-07 is small.

10.3 Patient and Demographic Data

10.3.1 Baseline Characteristics and Demographics

Summary descriptive statistics for baseline and demographic characteristics will be provided for all enrolled subjects in the ITT sample. Demographic data will include age, sex, race, ethnicity, sex, height and body weight; these data will be presented in the following manner:

- Continuous data (*i.e.,* age, body weight, and height) will be summarized descriptively by mean, standard deviation, median, and range;
- Categorical data (*i.e.,* sex and race) will be presented as enumerations and percentages.

Statistical presentation for baseline and demographic characteristics may be further summarized by values of important baseline predictors of outcome and will be further defined in the SAP.

10.3.2 Medical History

Medical history will be collected, including the existence of current signs and symptoms and clinical significance for each body system.

10.3.3 Use of Medications

All medications used will be coded using the World Health Organization (WHO) drug dictionary. The number and percentage of subjects receiving concomitant medications or therapies will be presented. Statistical presentation of concomitant medications or therapies may

be further summarized by withdrawal status, favorable outcome status at one year, and other characteristics to be determined by the study investigators.

The percent of subjects who complete the study, losses to follow-up, times to lost to follow-up, and reasons for loss to follow up (*e.g.,* AEs) will be presented. Statistical presentation of study completion may be further summarized by demographic variables and baseline predictors of outcome and will be further defined in the statistical analysis plan SAP.

10.4 Sample Size and Power Calculations

The purpose of this pilot study is to estimate the true rate of insulin independence at 75 days under an experimental immunosuppression regimen. The selected sample size is 12 subjects. The point estimate of the true insulin independence rate will be the proportion of the 12 patients that achieve insulin independence. The precision of the estimate depends on the observed number of subjects achieving insulin independence. The following table displays the confidence intervals that would be computed for each possible outcome. If 6 of the 12 subjects achieve insulin independence then the estimated rate will be 50% and a 95% confidence interval will be 0.21 to 0.79. That is, we are 95% confident that the true rate is at least 21% and no more than 79%. The confidence interval rules out any rate less than 21% or greater than 79%.

Number of Subjects insulin Independent at 75 Days	Estimated Rate	Exact 95% Confidence Interval	
		Lower Bound	Upper Bound
0	0	0.0	0.26
1	0.08	0.002	0.38
2	0.17	0.20	0.48
3	0.25	0.05	0.57
4	0.33	0.10	0.65
5	0.42	0.15	0.72
6	0.50	0.21	0.79
7	0.58	0.28	0.85
8	0.67	0.35	0.90
9	0.75	0.43	0.94
10	0.83	0.52	0.98
11	0.92	0.62	0.998
12	1.0	0.74	1.00

10.5 Interim Analyses to Ensure Patient Safety

The DSMB will be convened to review safety and efficacy data following NIH policy. Formal interim analyses will include distributions of endpoints, biomarkers and AEs.

Because this is a small study and because it is important to collect as much safety data as possible, it is not likely that the DSMB or the investigators will recommend stopping early for evidence of efficacy. The monitoring plan will therefore recommend early stopping only if there is sufficient evidence to conclude that the investigational treatment in either arm is harmful or not effective. Because there is no appropriate historical control rate with which to compare

outcomes in this study, effectiveness will be measured by comparing the subjects in this study to those concurrently enrolled in CIT-07 at The University of Pennsylvania. Should the primary outcome in this study be clearly significantly worse than the primary outcome in subjects in CIT-07 at the same institution, then early termination should be considered.

Because the primary outcome of insulin independence at 75 days after transplant is not necessarily predictive of longer term outcomes, the monitoring boundary will be conservative. It will also be designed to maintain the type I error rate at the final analysis. Interim analyses will be performed after 9 subjects have been enrolled in each group, and also after 10 and 11 subjects have been enrolled in each group. If the unadjusted p-value from the exact one-sided test is less than 0.05 in any of the 3 interim analyses the boundary will be crossed. Because of the discrete distribution of the p-value from the exact test, the probability of early stopping if there is no difference between CIT-05 and CIT-07 is less than 0.05. If the boundary is crossed, the probability of the primary endpoint in CIT-05 is significantly less than the corresponding probability in CIT-07 at the University of Pennsylvania.

In the unlikely event that the boundary is crossed before enrollment is completed the DSMB will be asked for a recommendation on whether to terminate enrollment in the study. If this boundary is crossed the DSMB will also be presented with an analysis comparing the study outcomes to a pooled analysis of all the subjects in CIT-07, not just those at the University of Pennsylvania.

10.6 Reporting Deviations from Original Statistical Plan

The principal features of the study design and of the plan for statistical analysis of the data are outlined in this protocol and in the subsequent SAP. Any changes in these principal features will require a protocol or an SAP amendment, which would be subject to review by the Steering Committee, the independent DSMB, the study sponsor, and the health authorities. These changes will be described in the final report as appropriate.

11. IDENTIFICATION AND ACCESS TO SOURCE DATA

11.1 Identifying Source Data

The investigator is required to keep accurate records to ensure that the conduct of the study is fully documented (see section 12). The results of all clinical and clinical laboratory evaluations will be maintained in the subject's medical records and the data will be transferred to electronic web based CRF's.

Safety data will be recorded electronically in a system specifically designed for this purpose. All data will be reviewed periodically by the DSMB and IRB. The DSMB and/or the IRB have the authority to withdraw any subjects and/or terminate the study because of safety findings.

11.2 Permitting Access to Source Data

The investigational site participating in this study will maintain the highest degree of confidentiality permitted for the clinical and research information obtained from the subjects and donors 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 site must permit authorized representatives of the sponsor(s) including, pharmaceutical collaborators and their commercial partners, and health authorities to examine (and when required by applicable law, to copy) clinical records for the purpose of quality assurance reviews, audits, and evaluations of the study safety and progress. Unless required by the laws that permit copying of records, only the coded identity associated with documents or with other subject data may be copied (and all personally identifying information must be obscured). Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that is linked to identified individuals. The investigational site will normally be notified before auditing visits occur.

12. 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 sponsor is responsible for regularly reviewing the conduct of the trial, for verifying adherence to the protocol, and for confirming the completeness, consistency, and accuracy of all documented data.

12.1 Compliance, Access, Entry and Handling of Study Data

The site PI is required to keep accurate records to ensure that the conduct of the study is fully documented, and to ensure that ECRFs are completed for all subjects according to study guidelines outlined in the study protocol and the Data System Users Instruction Manual.

Access to the data entry screens will be user ID and password protected. Each user will be provided with a unique personal ID and password. The investigational site participating in this study will maintain the highest degree of confidentiality permitted for the clinical and research information obtained from the subjects in this clinical trial. Medical and research records should be maintained at each site in the strictest confidence. However, as part of the quality assurance and legal responsibilities of an investigation, the investigational site must permit authorized representatives of the sponsor(s) and health authorities to examine (and when required by applicable law, to copy) clinical records for the purpose of quality assurance reviews, audits, and evaluations of the study safety and progress. Unless required by the laws that permit copying of records, only the coded identity associated with documents or with other subject data may be copied (and all personally identifying information must be obscured). Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that is linked to identified individuals. The investigational site will normally be notified before auditing visits occur.

All data will be entered, stored, and managed in a relational database supported by database servers at the DCC. The results of all clinical and laboratory evaluations will be maintained in the subjects medical records and the data will be transferred from these source documents directly to the electronic study CRFs. In order to maintain security, all data will be encrypted using the Secure Sockets Layer protocol. This protocol allows an encrypted link to be established between the DCC web server and the computer at each center. In addition, the data will be verified by a series of computerized edit checks, and all relevant data queries will be resolved regularly. All discrepancies will be reviewed, and any resulting queries will be resolved with the site personnel and amended in the database.

All changes made to CRFs will be recorded in an electronic audit trail to allow all data changes in the data system to be monitored and maintained in accordance with federal regulations. Once a CRF is entered into the database and the person entering the data indicates that CRF is complete, any change to that data will be entered into the system's audit trail. The audit trail will record the CRF and variable that is changed, the old value, the new value, the date and time the change was made, reason change was made, and the user ID of the person making the change. Once a change is completed, the data system will re-validate all variables on that CRF. The changed CRF will be required to pass all validity and logic consistency checks. If any edit

criteria fail, the system will generate appropriate queries. The clinical center coordinator will be asked to resolve the questions before the changes are completed.

The change system will allow certified DCC personnel and certified clinical center coordinators to make changes. Changes can be initiated by DCC monitors, DCC coordinators, and certified site personnel. Site personnel can access only the data for their own center. The system will generate weekly summary listings of all changes made to the database, the person making each change, and the reason for each change. These reports will be carefully reviewed by the DCC coordinator to monitor for unnecessary changes and/or problems with the data system.

13. ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE

13.1 Statement of Compliance

This clinical study will be conducted using cGCP as delineated in *Guidance for Industry: E6 Good Clinical Practice Consolidated Guidance*[168], and according to the criteria specified in this study protocol. Before study initiation, the protocol and the informed consent documents will be reviewed and approved by an appropriate EC or IRB, and NIAID/NIDDK. Any amendments to the protocol or to the consent materials must also be approved by the IRB/EC and submitted to the applicable Health Authorities before they are implemented.

13.2 Informed Consent and Assent

The informed consent form is a means of providing information about the trial to a prospective subject and allows for an informed decision about participation in the study. All subjects (or their legally acceptable representative) must read, sign, and date a consent form before entering the study, taking study drug, or undergoing any study-specific procedures. Consent materials for subjects who do not speak or read English must be translated into the subjects appropriate language.

The informed consent form must be 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 subject for review. The attending physician, in the presence of a witness, will review the consent and answer questions. The prospective subject will be told that being in the trial is voluntary and that he or she may withdraw from the study at any time, for any reason.

13.3 Privacy and Confidentiality

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

14. PUBLICATION POLICY

The CIT policy on the publication of study results will apply to this trial.

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Appendix 1: Schedule of Events for CIT-05

Time points (specified in Days relative to transplant)	**SCR**	**WL / BL[1]**	**0[2]**	**3**	**7**	**14**	**21**	**28**	**56**	**75**	**120**	**150**	**180**	**270**	**365**	365 post initial tx
Visit Number	**01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**	**10**	**11**	**12**	**13**	**14**	**15**	**Y1**
Visit Windows (specified in days)	N/A	N/A	N/A	N/A	+/-3	+/-3	+/-3	+/-3	+/-7	+/-5	+/-7	+/-7	+/-7	+/-14	+/-14	+/-14
Equivalent Week/Month	N/A	N/A	N/A	N/A	**W1**	**W2**	**W3**	**W4**	**M2**	**M2.5**	**M4**	**M5**	**M6**	**M9**	**M12**	Varies
GENERAL ASSESSMENTS																
Informed Consent	X[3]	X[4]														
Med/Diabetes Hx & Demographics	X															
Evaluation of Inclusion / Exclusion	X	X														
PAP Smear (females)	X	X-yrly														
Mammogram (females >35)	X	X-yrly														
Colorectal cancer screen (subj ≥50)	X[5]															
Retinopathy Evaluation[6]	X	X-yrly														X
Physical Exam	X	X-yrly	X		X	X	X	X	X	X	X	X	X	X	X	
QOL		X-q3mo								X			X		X	X
Chest X-Ray	X	X-yrly													X	
Abdominal US (including Pelvis/Liver)	X	X-yrly			X										X	
ECG	X	X-yrly													X	
Cardiac Stress Test or Angiogram	X															
PPD	X	X-yrly													X	
AE/Hypoglycemic Events/Toxicity Assess		X	X	X	X	X	X	X	X	X	X	X	X	X	X	
LOCAL LABORATORY ASSESSMENTS																
CBC (WBC + Diff & Plat)	X	X-q6mo	X		X	X	X	X	X	X	X	X	X	X	X	
Coagulation (PT, PTT, INR)	X	X-yrly	X													
Chemistry[7]	X	X-q6mo	X		X	X	X	X	X	X	X	X	X	X	X	
Lipids	X	X-q6mo								X			X	X	X	
Thyroid Function (TSH)	X	X-yrly														
Pregnancy test (females)	X	X[8]														
PSA	X	X-yrly													X	
Serology[9] (Hep B, Hep C, HIV, HTLV)	X	X-yrly														X
EBV IgG	X															

Time points (specified in Days relative to transplant)	**SCR**	**WL / BL[1]**	**0[2]**	**3**	**7**	**14**	**21**	**28**	**56**	**75**	**120**	**150**	**180**	**270**	**365**	365 post initial tx
Visit Number	**01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**	**10**	**11**	**12**	**13**	**14**	**15**	**Y1**
Visit Windows (specified in days)	N/A	N/A	N/A	N/A	+/-3	+/-3	+/-3	+/-3	+/-7	+/-5	+/-7	+/-7	+/-7	+/-14	+/-14	+/-14
Equivalent Week/Month	N/A	N/A	N/A	N/A	**W1**	**W2**	**W3**	**W4**	**M2**	**M2.5**	**M4**	**M5**	**M6**	**M9**	**M12**	Varies
CMV IgG, CMV IgM		X-yrly[10]														X[10]
Blood Type		X[11]														
HLA		X														
Crossmatch		X[12]														
Fasting & post-prandial c-peptide[13]				X	X											
Glucose (immediately post-transplant)			X[14]													
PRA by flow cytometry		X[15]														
CENTRAL LABORATORY METABOLIC ASSESSMENTS																
First morning spot urine[16]	X	X						X		X					X	X
GFR	X	X-yrly						X		X					X	X
CMV and EBV by PCR[17]		X				X		X	X	X	X	X	X[18]	X[19]	X	X
HbA1c	X	X-q3mo								X			X	X	X	X
Fasting serum gluc/c-pep & serum creat	X	X						X	X	X	X	X	X[18]	X[19]	X	X
90 min[20] c-pep/glucose (MMTT)	X									X			X	X	X	X
Insulin modified FSIGT		X-yrly								X					X	X
LOCAL METABOLIC ASSESSMENTS																
Glycemic Stability (CGMS)		X-yrly								X					X	X
BSR eCRFs[21]	X	X-q3mo								X			X	X	X	X
CIT05-SPECIFIC LOCAL METABOLIC ASSESSMENTS																
Glucose Potentiated Arginine[33]										X					X	
Glucose Counter-Regulation		X-yrly											X		X	
CALCULATED METABOLIC ASSESSMENTS																
MAGE		X-q6mo								X			X	X	X	X
LI	X	X-q6mo								X			X	X	X	X
Clarke Score	X	X-q6mo											X		X	X
CALCULATED METABOLIC ASSESSMENTS (Con't)																
HYPO	X	X-q6mo								X			X	X	X	X

Time points (specified in Days relative to transplant)	**SCR**	**WL / BL[1]**	**0[2]**	**3**	**7**	**14**	**21**	**28**	**56**	**75**	**120**	**150**	**180**	**270**	**365**	365 post initial tx
Visit Number	**01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**	**10**	**11**	**12**	**13**	**14**	**15**	**Y1**
Visit Windows (specified in days)	N/A	N/A	N/A	N/A	+/-3	+/-3	+/-3	+/-3	+/-7	+/-5	+/-7	+/-7	+/-7	+/-14	+/-14	+/-14
Equivalent Week/Month	N/A	N/A	N/A	N/A	**W1**	**W2**	**W3**	**W4**	**M2**	**M2.5**	**M4**	**M5**	**M6**	**M9**	**M12**	Varies
Beta Score		X								X			X	X	X	X
C-peptide (glucose X creatinine) ratio	X	X						X	X	X	X	X	X[22]	X[22]	X	X
IMMUNOSUPRESSION LEVELS																
Sirolimus 24-hour trough levels			X	X	X	X	X	X	X	X	X	X	X	X	X	X
Tacrolimus 12-hour trough levels[23]				X	X	X	X	X	X	X	X	X	X	X	X	X
CIT05-SPECIFIC IMMUNOSUPPRESSION LEVELS																
Rituximab PK			X					X					X	X		
HACA evaluation			X					X					X	X		
MECHANISTIC ASSAYS																
Alloantibody	X	X-q6mo[24]								X			X	X	X	X
Autoantibody (GAD, IA-2, IAA)		X								X			X	X	X	X
TAT, C3a, & c-peptide		X	X[25]													
CIT05-SPECIFIC MECHANISTIC ASSAYS																
General B and T cell flow cytometry[26]		X-q 6 mo			X			X		X		X	X	X	X	X
HiD B cell tube[27]		X-q 6 mo			X			X				X	X			X
CD19+ separation for B cell spectratyping[28]		X											X	X	X	
Clone tracking[29]		X-q3mo			X	X	X	X	X	X	X	X	X	X	X	X
T cell ELISpot screen[30]		X-q6mo								X			X	X	X	
T cytokine profile[31]		X-q6mo								X			X	X	X	
B cell ELISpot[32]		X						X					X			
ARCHIVED SAMPLES																
Serum		X								X			X	X	X	X
PBMC & Plasma		X								X			X	X	X	X
RNA		X								X			X	X	X	X

[1] WL = Waiting List. BL = Baseline. Repeat assessments as indicated (i.e. yrly, q3mo), while subject is on the waiting list. All one-time WL/BL assessments should be completed on Day -2 whenever possible, but always prior to start of immunosuppression. For repeat WL/BL assessments, results from test done closest to the start of immunosuppression will be used as the baseline value.
[2] Day 0 = the day of transplant. This SOE applies to the 1st, 2nd, and 3rd transplant as applicable. The SOE is restarted at Day 0 for each subsequent transplant.
[3] *Informed Consent #1* includes information on CIT-07 and CIT-05 protocols.
[4] *Informed Consent #2* includes information specific to CIT-05. IC #2 must be signed immediately after randomization.
[5] FOBT (take-home multiple sample method) or FIT w/in last yr, flexible sigmoidoscopy w/in last 5 yrs, double-contrast barium enema w/in last 5 yrs, colonoscopy w/in last 10 yrs. All positive tests should be followed up with colonoscopy.
[6] Retinopathy eval includes fundoscopic pictures for WL/BL assessments and Y1. Screening retinopathy evaluation should be done per site-specific standards.
[7] Chemistry includes: Sodium, albumin, magnesium, chloride, potassium, alk phosphatase, total bilirubin, CO_2, creatinine, ALT (SGPT), BUN, gamma GT, glucose, AST (SGOT), calcium, phosphorus.
[8] Complete pregnancy test within 72 hours prior to randomization.
[9] Serology includes: HBc Ab, HBs Ab, HBs Ag, HCV Ab, HIV, and HTLV-I/II. Do not repeat Hepatitis B tests if HBs Ab was previously positive.
[10] Repeat only if previous test was negative.
[11] Repeat for subsequent transplant(s).
[12] Sample used for crossmatch may be obtained up to 60 days prior to the start of immunosuppression, as long as there is no evidence of infections or transfusions since the time the sample was drawn. Repeat crossmatch for subsequent transplants.
[13] C-peptide should be done locally and drawn fasting, and twice between 1-3 hours post-prandial on Day 3 and Day 7 post-transplant.
[14] Finger stick glucose should be done locally and drawn every hour for the first 6 hours immediately post-transplant.
[15] Subsequent transplants only. Local result used to determine eligibility for subsequent transplant only.
[16] First morning spot urine includes: albumin, protein, and creatinine.
[17] CMV & EBV by PCR done monthly post-transplant. At the time of a subsequent transplant, a more intensified protocol will be utilized.
[18] If blood drawn locally at Months 7 & 8 (Visits 13a, 13b respectively), sample should be sent from local lab to study site and then shipped to the central laboratory (Univ of Washington for fasting glucose/c-peptide and serum creatinine; University of Alberta for EBV & CMV by PCR).
[19] If blood drawn locally at Months 10 & 11 (Visits 14a, 14b respectively), sample should be sent from local lab to study site and then shipped to the central laboratory (University of Washington for fasting glucose/c-peptide and serum creatinine; University of Alberta for EBV & CMV by PCR).
[20] MMTT should include 60 and 90 minute c-peptide and glucose measurements for the screening visit and as necessary when determining graft failure.
[21] Blood Sugar Record (BSR) eCRF is completed using information gathered from subject diary logs, glucometer download data, and insulin requirements.
[22] C-peptide, glucose creatinine ratio calculated monthly.
[23] Tacrolimus levels will be drawn for CIT-07, and will replace sirolimus levels only in subjects from CIT-05 who cannot tolerate sirolimus and are changed to tacrolimus.
[24]For each transplant, complete alloantibody assessment every 6 months and again on Day -2, regardless of the most recent draw. Central PRA result, current within 6 months, is used to determine subject eligibility for first transplant.

[25] TAT, C3a & C-peptide: pre-tx, 15, 60, 180 min post-tx.
[26] General flow cytometry on T and B lymphocyte subsets will be performed using a four-color panel. In order to obtain absolute cell counts and for quality control purposes, a CBC with differential will be performed in parallel with every flow cytometry experiment. General flow will be performed every 6 months while subjects are on the waitlist. In addition to the time points listed in the table above, late time points (months 15, 18, and 21 after the first islets transplant) will be added if the subject is still in study follow-up.
[27] The HiD B cell tube refers to using a large number of different fluorochrome conjugated markers in the same tube (11 colors). High definition flow will be performed every 6 months while subjects are on the waitlist. In addition to the time points listed in the table above, high definition flow will be performed at late time points (months 15, 18, and 21 after the first islets transplant) if the subject is still in study for follow-up.
[28] CD19 separation will be performed twice while subject is on the waiting list prior to transplantation and at least once during a late time point (after 12 months).
[29] DNA will be extracted from peripheral blood leukocytes every three months while subjects are on the waitlist to be transplanted and will be banked at the indicated time points following transplant.
[30] The T cell ELISpot screen will be performed at baseline and repeated every six months while the subject is on the wait list (scheduling can be done at the discretion of other studies and the subject; also, testing will not be performed if the subject has a viral infection.) The screen will be performed using dominant T cell epitopes and pools of peptides for islet antigens. The read-outs will be IFN-γ and TGF-β secretion.
[31]If the T cell ELISpot is positive, the specificity of reacting T cells (checked against a panel of peptides) will be profiled and the secretion of different cytokines will be monitored. The sample volume for the T cell ELISpot screen and specificity is 18 ml per time point.
[32] A B cell ELISpot assay will be performed at baseline and repeated every six months while the subject is on the wait list (scheduling can be done at the discretion of other studies and the subject; also testing will not be performed if the subject has a viral infection.) Mononuclear cells will be purified from samples for functional assays and frozen until all samples from a given patient can be performed in the same assay on the same day. 10 ml of anticoagulated whole blood (EDTA) will be required per time point for this assay. Note that the time points have been changed from early to late (BL, M9, M12 and beyond M12, if available.) Later time points are essential because B lymphocyte recovery is expected to be delayed in patients receiving combined B and T cell depleting agents. The 9 month time point will only be assayed if the circulating B lymphocyte count is above 50 B cells per microliter of whole blood. The CIT-07 time points will parallel the time points performed in CIT-05.
[33]Subjects requiring >0.1 U/kg insulin will undergo an Arginine Stimulation Test alone (AST).

Appendix 2: Reduced Follow-up Schedule of Events

Subjects withdrawn from study therapy should be followed according to the reduced follow-up schedule provided below. All reduced follow-up assessments should be scheduled relative to the day on which the study treatment is discontinued. The last follow-up visit will vary depending on when the subject discontinues study therapy and should be done at 1 year post the subject's **last** transplant.

REDUCED FOLLOW-UP SCHEDULE

Complete the following assessments at the intervals (+/-7 days) indicated below relative to the day the subject discontinued study treatment. Continue conducting these assessments at the defined intervals until the subject reaches one year post **last** transplant.

- Assess SAEs and hypoglycemic events: q1 month. If subject does not come to the study site for the visit, attempt to obtain information via a phone contact.
- Alloantibody (central lab): q 1 month for the first 3 months and q 3 months thereafter.

Complete the following assessments at 1 year (+/- 14 days) post **initial** transplant:

- Assess SAEs and hypoglycemic events
- Alloantibody (central lab)
- HbA1c (central lab)
- 90 minute c-peptide post MMTT (central lab)
- Serum creatinine (central lab)
- QOL questionnaire (via mail or in-person)

Complete the following assessments at 1 year (+/- 7 days) post **last** transplant:

- Assess SAEs and hypoglycemic events
- QOL questionnaire (via mail or in-person)

Appendix 3: Sample of Events for Follow-up Protocol

Time Point (months [M] relative to final islet transplant; years [Y] relative to initial transplant)	M15	M18	M21	M24	Y2	M30	M36	Y3	M42	M48	Y4	M54	M60	Y5
Visit Number (relative to final islet transplant)	**16**	**17**	**18**	**19**		**20**	**21**		**22**	**23**		**24**	**25**	
Visit Window (specified in days)	**± 14**	**± 14**	**± 14**	**± 14**	**± 90**	**± 30**	**± 30**	**± 90**	**± 30**	**± 30**	**± 90**	**± 30**	**± 30**	**± 90**
Medical and Diabetes History	X	X	X	X		X	X		X	X		X	X	
Physical Exam	X	X	X	X		X	X		X	X		X	X	
Retinopathy Evaluation														X
QOL		X		X	X		X	X		X	X		X	X
AE /Hypoglycemic Events/Toxicity Assessment	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Nutritional assessment		X		X		X	X		X	X		X	X	
CBC (WBC + Diff & Plat)	X	X	X	X		X	X		X	X		X	X	
Chemistry (P18 +Mg or P20)	X	X	X	X		X	X		X	X		X	X	
Lipids		X		X			X			X			X	
Autoantibody[1]	X	X	X	X		X	X		X	X		X	X	
Alloantibody[2]	X	X	X	X		X	X		X	X		X	X	
First morning spot urine[2]		X		X	X		X	X		X	X		X	X
GFR					X			X			X			X
HbA1c	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Fasting & Post-Prandial Glucose & C-peptide	X	X	X	X	X	X	X	X	X	X	X	X	X	X
MTT	X	X	X	X		X	X		X	X		X	X	
Insulin-modified FSIGT				X										
Glycemic Stability (CGMS)	X	X	X	X		X	X		X	X		X	X	
BSR eCRF[3]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
MAGE	X	X	X	X	X	X	X	X	X	X	X	X	X	X
LI	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Clarke Score	X	X	X	X	X	X	X	X	X	X	X	X	X	X
HYPO	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Beta Score	X	X	X	X	X	X	X	X	X	X	X	X	X	X
C-peptide (glucose X creatinine) ratio	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Sirolimus Levels	X	X	X	X		X	X		X	X		X	X	
Tacrolimus Levels	X	X	X	X		X	X		X	X		X	X	
Serum[4]	X	X	X	X		X	X		X	X		X	X	
PBMC & Plasma[4]	X	X	X	X		X	X		X	X		X	X	
RNA[4]	X	X	X	X		X	X		X	X		X	X	

[1] Central laboratory assessment
[2] First morning spot urine includes: albumin, protein, and creatinine
[3] BSR eCRF is completed using information gathered from subject diary logs, glucometer download data, and insulin requirements.
[4] Archived samples

CLINICAL ISLET TRANSPLANTATION (CIT)
PROTOCOL CIT-08
Extended Follow Up after Islet Transplantation in Type 1 Diabetes
Version 6.0 (25 April 2017)

Study Sponsors:
The National Institute of Allergy and Infectious Diseases (NIAID)
The National Institute of Diabetes & Digestive & Kidney Diseases (NIDDK)

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(as defined in RFA-DK-04-005)

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Confidentiality Statement

The information contained within this document is not to be disclosed in any way without prior permission of the CIT PIs, the Division of Allergy, Immunology, and Transplantation, or the National Institute of Diabetes & Digestive & Kidney Diseases.

INVESTIGATOR SIGNATURE PAGE	
Protocol Number: CIT-08	**Version/Date:** Version 6.0 / April 25, 2017
IND: Exempt	**CIT Principal Investigators:** Bernhard Hering, MD; Xunrong Luo, MD, PhD, FACS; Olle Korsgren, MD, PhD; Nicole Turgeon, MD; Ali Naji, MD, PhD ; Andrew Posselt, MD, PhD; Camillo Ricordi, MD; James Shapiro, MD, PhD, Dixon Kaufman, MD, PhD, FACS; James Markmann, MD, PhD
Title: *Extended Follow-Up after Islet Transplantation in Type 1 Diabetes*	
Study Sponsors: The National Institute of Allergy and Infectious Diseases (NIAID) The National Institute of Diabetes & Digestive & Kidney Diseases (NIDDK)	

INSTRUCTIONS: Please have the Principal Investigator print, sign, and date at the indicated location below. A copy should be kept for your records and the original signature page sent to the Data Coordinating Center.
After signature, please return the original of this form by surface mail to:

ATTN: Clinical Trials Statistical & Data Management Center Department of Biostatistics
201 S Clinton St
Iowa City, IA 52240-4034

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 United States Code of Federal Regulations (CFR) – 21 CFR Parts 45, 50, 54, 56, and 312, and 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 Site Principal Investigator, I agree to conduct protocol CIT-08, "Islet Transplantation in Type 1 Diabetes" according to good clinical practices. 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 NIAID and NIDDK.

_____

Site Principal Investigator (Print)

_____ _____

Site Principal Investigator (Signature) **Date**

Protocol Synopsis

Title	Islet Transplantation in Type 1 Diabetes
Clinical Phase	Phase 3
IND Sponsor	DAIT/NIAID/NIH
IND Number	Exempt
Activation Date	June 2011
Accrual Objective	Approximately 75 subjects
Accrual Period	N/A
Follow-up Period	Varies; the CIT08 follow-up period begins after termination from the CIT parent study and ends on the final date of the CIT08 study, 01Jul2017.
Study Design	A single-arm, multi-center cohort study in islet transplantation
Treatment Description	Subjects who have received an islet transplant during participation in CIT02, CIT03, CIT04, CIT05, CIT06, or CIT07 will undergo additional follow-up, including annual assessments of graft function (if applicable) and safety.
Primary Endpoint	The primary endpoint is duration of sustained **islet allograft function** as determined by evidence from MMTT of C-peptide production at each anniversary of the final transplant. A C-peptide level greater than or equal to 0.3 ng/mL at 0, 60, or 90 minutes will be considered evidence of islet allograft function.
Secondary Endpoints	Secondary endpoints include the following: • Serum creatinine and calculated eGFR at each annual study visit • Incidence of serious adverse events during the 12-month period preceding each annual study visit • Insulin requirements during a one-week period preceding each annual study visit • Incidence of severe hypoglycemic events during the 12-month period preceding each annual study visit • HbA1c levels at each annual study visit • All causes of mortality
Inclusion Criteria	1. Participation in any of the following CIT parent studies: CIT02, CIT03, CIT04, CIT05, CIT06, and CIT07. 2. Willingness of participants to continue to use an approved method of contraception during and 4 months after study participation. 3. Ability to provide written informed consent.
Exclusion Criteria	1. For female subjects: Positive pregnancy test, presently breast-feeding, or unwillingness to use effective contraceptive measures for the duration of the study and 4 months after discontinuation.

For male subjects: intent to procreate during the duration of the study or within 4 months after discontinuation or unwillingness to use effective measures of contraception. Oral contraceptives, Norplant®, Depo-Provera®, and barrier devices with spermicide are acceptable contraceptive methods; condoms used alone are not acceptable.

2. Received an islet transplant in a non-CIT research study.
3. Any medical condition that, in the opinion of the investigator, will interfere with safe participation in the trial.

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Glossary of Abbreviations

AE	Adverse Event
ATG	Anti-thymocyte Globulin
BG	Blood Glucose
CBC	Complete Blood Count
CFR	Code of Federal Regulations
cGCP	Current Good Clinical Practice
cGMP	Current Good Manufacturing Practices
CIT	Clinical Islet Transplantation Consortium
CRF	Case Report Form
CRO	Clinical Research Organization
CTCAE	Common Terminology Criteria for Adverse Events
DAIT	Division of Allergy, Immunology, and Transplantation
DCC	Data Coordinating Center
DCCT	Diabetes Control and Complications Trial
DSMB	Data Safety Monitoring Board
EC	Ethics Committee
FDA	Food and Drug Administration
GFR	Glomerular Filtration Rate
HbA1c	Glycosylated hemoglobin
HLA	Histocompatability Antigen
HSV	Herpes Simplex Virus
ICH	International Conference on Harmonization
IND	Investigational New Drug
IRB	Institutional Review Board
ITN	Immune Tolerance Network
IV	Intravenous
MMTT	Mixed-Meal Tolerance Test
NIAID	National Institute of Allergy and Infectious Disease
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NIH	National Institutes of Health
PI	Principal Investigator
PTLD	Post-transplant Lymphoproliferative Disorder
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan

SOP	Standard Operating Procedure
T1D	Type 1 Diabetes
TCAE	Terminology Criteria for Adverse Events

Study Definitions

Graft failure: Islet allograft failure will be defined as absence of insulin production by transplanted islets, as evidenced by C-peptide < 0.3 ng/mL. This will be determined by (1) C-peptide <0.3 ng/mL on random testing, followed by (2) C-peptide <0.3 ng/mL at baseline, and at 60 and 90 minutes after MMTT. C-peptide levels obtained in the course of the MMTT will be run at the core lab in Seattle, WA; allow 72 hours for results. Participants with confirmed graft failure do not need to complete subsequent metabolic assessments.

Islet allograft function: A C-peptide ≥ 0.3 ng/mL at 0, 60, or 90 minutes after MMTT will be considered evidence of insulin production by transplanted islets. C-peptide levels obtained in the course of the MMTT will be run at the core lab in Seattle, WA; allow 72 hours for results.

Parent studies: CIT02, CIT03, CIT04, CIT05, CIT06, and CIT07.

Severe hypoglycemia: An event with one of the following symptoms: memory loss; confusion; uncontrollable behavior; irrational behavior; unusual difficulty in awakening; suspected seizure; seizure; loss of consciousness; or visual symptoms, in which the subject was unable to treat him/herself and which was associated with either a blood glucose level < 54 mg/dL [3.0 mmol/L] or prompt recovery after oral carbohydrate, IV glucose, or glucagon administration).

1. BACKGROUND AND RATIONALE

1.1 Background

The Clinical Islet Transplant Consortium opened in October 2004 under a research initiative (RFA-DK-04-005) sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and the National Institute of Allergy and Infectious Diseases (NIAID). This consortium conducts seven trials in islet transplantation, six of which (CIT02, CIT03, CIT04, CIT05, CIT06, and CIT07) will be the source of study subjects in CIT08. Approximately 75 subjects are expected to be enrolled and followed in this trial.

1.2 CIT Parent Studies

The CIT studies aim to determine the safety and efficacy of islet transplantation with the goal of obtaining licensure in the U.S.

CIT07 (phase III licensure study): The primary objective is to demonstrate, in a multicenter, single-arm study, the safety and efficacy of islet transplantation for the treatment of T1D in subjects with hypoglycemia unawareness and a history of severe hypoglycemic episodes.

CIT06 (phase III licensure study): The primary objective is to demonstrate that islet transplantation in patients with established kidney transplants leads to improved metabolic control as measured by serial HbA1c levels and a reduced occurrence of hypoglycemic events.

CIT02 (phase II pilot study): The primary objective is to determine the proportion of subjects who are insulin independent at 75 ± 5 days posttransplant after one islet transplant among subjects treated with lisofylline in addition to the standard islet transplant regimen used in the CIT-07 protocol.

CIT03 (phase II pilot study): The primary objective of this protocol is to assess the safety and efficacy of an immunosuppressive regimen consisting of ATG (1st transplant only), basiliximab (subsequent transplants only), etanercept, DSG, sirolimus, and low-dose tacrolimus on posttransplant islet function in subjects with T1D.

CIT04 (phase II pilot study): The primary objective of this protocol is to assess the safety and efficacy of an immunosuppressive medication consisting of a monoclonal antibody IL-2 receptor blocker (daclizumab or basiliximab), belatacept and mycophenolate mofetil in islet transplantation. The primary efficacy measure will be the proportion of insulin-independent subjects at day 75 (± 5 days) following the first islet transplant.

CIT05 (phase II pilot study): The primary objective is to determine the proportion of subjects who are insulin independent at 75 ± 5 days following the first islet transplant among subjects treated with an experimental islet transplant immunosuppression regimen which includes rituximab and excludes tacrolimus.

1.3 Rationale for Current Protocol

The purpose of this protocol is to collect long-term follow-up information on the safety and efficacy of islet transplantation in CIT subjects after their completion in their CIT parent study.

1.4 Known and Potential Risks and Benefits to Human Subjects

Administration of all immunosuppressive and immunomodulatory therapies used presently to prevent rejection of transplanted tissues carry general risks of opportunistic infection and malignancy, including lymphoma (~1%), and skin cancers. These agents are not recommended for nursing mothers, and it is recommended (and mandated in the current protocol) that women of childbearing potential use effective contraception before, during and for at least 4 months following administration of these agents.

The agents listed below are those currently used in the parent trials. We anticipate that these will be used in CIT08. However, it is possible that this can change due to changes in drug availability.

1.4.1.1 SIROLIMUS (RAPAMUNE®)

The FDA approved sirolimus (rapamycin, Rapamune®) as an immunosuppressive agent in 1999 (see product monograph for details). In 208 kidney transplant recipients receiving 5 mg of sirolimus daily compared to 124 receiving placebo, there was an increased incidence of hypercholesterolemia (46 vs. 23%), hyperlipemia (57 vs. 23%), rash (20 vs. 6%), arthralgia (31 vs. 18%), diarrhea (35 vs. 27%), anemia (33 vs. 21%), leucopenia (13 vs. 8%), thrombocytopenia (30 vs. 9%), and hypokalemia (17 vs. 9%). Side effects are related to drug concentration and are improved with maintenance of the sirolimus 24-hour trough level between 10–20 ng/mL.

Of infections, only mucosal herpes simplex virus (HSV) occurred at a greater rate with sirolimus. There was no increase in rate of malignancy (3.4 vs. 3.1%). While sirolimus was originally proposed as a non-nephrotoxic agent, it is becoming apparent that sirolimus-associated nephrotoxicity does occur in clinical practice. Crew *et al.* described two patients with thrombotic microangiopathy secondary to sirolimus exposure[2]. Sirolimus alters the pharmacokinetic profiles of other CNIs (*e.g.*, tacrolimus) and may thereby potentiate nephrotoxicity[3]. Fervenza *et al.* described nephrotoxicity from sirolimus in patients with chronic glomerulopathies that was non-reversible on cessation of therapy[4]. Nephrotoxicity from combined sirolimus and tacrolimus has been described in patients with T1D undergoing islet transplantation, particularly where there is underlying pre-existing renal damage from diabetes[5,6].

The majority of islet transplant recipients receiving sirolimus in conjunction with tacrolimus have experienced transient mouth ulceration and lower extremity edema[6,7]; perinephric edema and a high incidence of benign ovarian cysts have also been described in islet recipients in association with sirolimus[8]. Pneumonitis and colitis have also occurred[9,10]. The most common (> 30%) adverse reactions are: oral apthous ulcers, peripheral edema, hypertriglyceridemia, hypertension, hypercholesterolemia, increased creatinine, abdominal pain, diarrhea, headache, fever, urinary tract infection, anemia, nausea, arthralgia, pain, and thrombocytopenia.

Concerns have been raised by the FDA regarding trials of combined sirolimus/tacrolimus in liver transplant recipients, where there has been a statistically increased risk of hepatic artery thrombosis and late death in sirolimus-treated recipients. A careful analysis of these events does not establish causative association between sirolimus/tacrolimus and thrombosis or death events. There was no increased association with portal venous thrombosis in the liver transplant trials. While sirolimus continues to be used off-label in islet recipients, there is not

presently felt to be an association between portal thrombus formation in islet recipients and the use of sirolimus or tacrolimus.

1.4.1.2 TACROLIMUS (PROGRAF®)

Tacrolimus (Prograf®, FK506) has been in wide clinical use for the prevention of allograft rejection since 1994 when the FDA approved it after several years of testing. Tacrolimus is a macrolide antibiotic which inhibits calcineurin after binding intracellularly to FKBP12 within T cells, inhibiting IL-2 transcription. Tacrolimus is invariably administered with other immunosuppressive agents but is known to be associated with several side effects including hypertension, diabetes, nephrotoxicity, hyperkalemia, dyslipidemia, pruritis, neurotoxicity, neurologic sequelae (including tremor, ataxia, and extremely rarely central pontine myelinolysis), posterior reversible encephalopathy syndrome (PRES), progressive multifocal leukoencephalopathy (PML), interstitial lung disease, BK nephropathy, nausea, vomiting and diarrhea (see product monograph for details). In 205 kidney transplant recipients receiving tacrolimus, the principal AEs were neurologic (tremor [54%], headache [44%], insomnia [32%], paresthesia [23%]) and gastrointestinal (diarrhea [44%], nausea [38%], constipation [35%]) complaints, hypertension (50%), and kidney dysfunction (52%); hyperkalemia (31%) and hyperglycemia (22% in previous non-diabetics) also occurred. The severity of these events appears to be dose dependent, with very high plasma levels also producing delirium, seizures, and coma. Complications can be minimized with the relatively low dose long-term therapy typically used in islet transplant trials.

1.4.1.3 CYCLOSPORINE (NEORAL®)

Cyclosporine is associated with renal dysfunction, tremors, hirsutism, hypertension, and gum hyperplasia.

1.4.1.4 MYCOPHENOLATE MOFETIL (CELLCEPT®) AND MYCOPHENOLATE SODIUM (MYFORTIC®)

CellCept® and Myfortic® are associated with: diarrhea, leucopenia, vomiting, and evidence of higher frequency of certain types of infections, some of which can be fatal. CellCept® and Myfortic® may increase the risk of developing lymphoproliferative disease, lymphomas, and other malignancies, particularly of the skin, and have been known to cause fetal harm (congenital malformations and pregnancy loss) when administered to a pregnant woman. Cases of progressive multifocal leukoencephalopathy, sometimes fatal, and pure red cell aplasia have been reported in patients treated with CellCept® or Myfortic® in combination with other immunosuppressive agents.

Contraception requirements are outlined in the eligibility criteria.

1.4.2 Risk of Study Procedures

The procedures involved with the care of research subjects undergoing clinical islet transplantation include risks pertaining to: a) blood draw testing, b) metabolic stimulation testing, and c) specific follow-up testing.

1.4.2.1 METABOLIC STIMULATION TESTING

The risks associated with metabolic testing are generally regarded as minor. Placement of IV cannulae may be associated with pain and discomfort at the puncture site, bruising, bleeding, displacement, interstitial infusion of fluids, local vein thrombosis, infection or thrombophlebitis.

The administration of bolus glucose by mouth or intravenously may lead to acute hypoglycemia or hyperglycemia, or rarely may induce ketoacidosis.

1.4.2.2 BLOOD DRAW TESTING

Peripheral blood draws performed during these research studies will not exceed 450 mL per six-week period. The subject may experience some discomfort at the site of the needle entry, and there is risk of bruising at the site. There is a remote risk of fainting or local infection.

1.4.3 Benefits

The major benefit of this study will be to provide further information on the duration and quality of function of islet grafts beyond the CIT parent study's follow up period.

2. OBJECTIVES

2.1 Primary Objective

The primary objective is to provide extended follow-up for safety and efficacy and to support continued islet graft function to participants previously enrolled in CIT02, CIT03, CIT04, CIT05, CIT06, or CIT07.

3. SELECTION OF SUBJECTS

3.1 Inclusion Criteria

Patients who meet all of the following criteria are eligible for participation in the study:

1. Participation in any of the following CIT parent studies: CIT02, CIT03, CIT04, CIT05, CIT06, and CIT07.
2. Willingness of participants to continue to use an approved method of contraception during and 4 months after study participation.
3. Ability to provide written informed consent.

3.2 Exclusion Criteria

Patients who meet any of these criteria are not eligible for participation in the study:

1. For female subjects: Positive pregnancy test, presently breast-feeding, or unwillingness to use effective contraceptive measures for the duration of the study and 4 months after discontinuation. For male subjects: intent to procreate during the duration of the study or within 4 months after discontinuation or unwillingness to use effective measures of contraception. Oral contraceptives, Norplant®, Depo-Provera®, and barrier devices with spermicide are acceptable contraceptive methods; condoms used alone are not acceptable.
2. Received an islet transplant in a non-CIT research study.
3. Any medical condition that, in the opinion of the investigator, will interfere with safe participation in the trial.

4. STUDY DESIGN

This is an open-label, multi-center cohort study for participants from the CIT02, CIT03, CIT04, CIT05, CIT06, and CIT07 studies who continue to have islet graft function. These participants will continue immunosuppressive medications under CIT08 and will be assessed for islet function on an annual basis.

4.1 Study Endpoints

4.1.1 Primary Endpoint

The primary endpoint is duration of sustained **islet allograft function** as determined by evidence from MMTT of c-peptide production at each anniversary of the final transplant. A c-peptide level greater than or equal to 0.3 ng/mL at 0, 60, or 90 minutes will be considered evidence of islet allograft function.

4.1.2 Secondary Endpoints

Secondary endpoints include the following:

- Serum creatinine and calculated eGFR at each annual study visit
- Incidence of serious adverse events during the 12-month period preceding each annual study visit
- Insulin requirements during a one-week period preceding each annual study visit
- Incidence of severe hypoglycemic events during the 12-month period preceding each annual study visit
- HbA1c levels at each annual study visit
- All causes of mortality
- Presence of alloantibody after graft failure, in the absence of immunosuppression

5. STUDY TREATMENT REGIMEN

5.1 Immunosuppression Medications

The marketed immunosuppressive medications in this protocol will be obtained by prescription unless provided by the study through the drug distributor. Generic brands are allowed, when available.

5.1.1 Drug Accountability

Under Title 21 of the Code of Federal Regulations (21CFR §312.62), the investigator is required to maintain adequate records of the disposition of immunosuppressants provided for the study through the drug distributor, including the date and quantity of the drug received, to whom the drug was dispensed (subject-by-subject accounting), and a detailed accounting of any drug accidentally or deliberately destroyed.

Records for receipt, storage, use, and disposition will be maintained by the study site. A drug-dispensing log will be kept current for each subject. This log will contain the identification of each subject and the date and quantity of drug dispensed.

All records regarding the disposition of the study-provided immunosuppressants will be available for inspection by the clinical trial monitor.

5.2 Concomitant Medications

Antibacterial, antifungal, and antiviral prophylaxis, insulin therapy, and other standard therapies will be provided per site-specific practices. The cost of these drugs will not be covered under this protocol. Substitution of non-brand name generic equivalents for those protocol required medicines is permitted to reduce cost to the patients and/or their insurance companies.

5.3 Rescue Medications

Rescue therapy will not be initiated in this protocol to treat suspected rejection. Immunologic surveillance methods that would allow diagnosis of islet allograft rejection early enough for timely intervention have yet to be identified and validated.

5.4 Prohibited Medications

None.

6. CRITERIA FOR PREMATURE TERMINATION OF THE STUDY

6.1 Subject Withdrawal Criteria

Subjects may be prematurely terminated from study for the following reasons:

1. The subject elects to withdraw consent from all future study activities, including follow-up.
2. The subject is "lost to follow-up" (*i.e.*, no further follow-up is possible because attempts to reestablish contact with the subject have failed).
3. The subject dies.
4. The investigator determines that it is not in the subject's best interest to continue.
5. The subject enrolls and begins study treatment in another investigational protocol for islet transplantation while enrolled in this study.

Subjects who prematurely terminate from this study will not be replaced. If possible, assessment of adverse events will be collected prior to termination (see section 8). Data from such subjects obtained before withdrawal of consent or before being lost to follow-up will be used in the intent-to-treat analysis. If a subject with functioning transplanted islets chooses to withdraw from the protocol, s/he will be informed of their risk for losing his/her islet graft and becoming sensitized if s/he chooses to discontinue immunosuppressive therapy and return to his/her original method of insulin management.

6.2 Subject Stopping Rules

6.2.1 Subject Stopping Rules

None.

6.2.2 Study Stopping Rules

None.

7. STUDY PROCEDURES

7.1 Enrollment and Screening

Patients who meet the general inclusion criteria for this study will be approached regarding their participation in this study. The study procedures, risks, and potential benefits will be discussed with the potential study subject in lay language. The potential study subject will have an opportunity to review the informed consent and ask questions.

Once informed consent has been obtained, the subject will be enrolled. Subject eligibility will be confirmed through information collected from their most recent CIT study visit. If more than 90 days have elapsed since the subject's last CIT study visit, then the screening visit assessments should be performed in order to confirm eligibility.

7.2 Follow-up Visits

Subjects will be followed in this study after termination from the CIT parent study until the end date of the CIT08 study. Follow-up is comprised of quarterly visits done locally for safety monitoring and annual visits at the study center to assess graft function (if applicable) and safety.

Retrospective medical chart review will be conducted as needed to collect available follow-up information due to:

- delayed enrollment in CIT08 after termination from the CIT parent study
- the time period between termination from CIT08 prior to extension of the duration of follow-up and subsequent re-enrollment

This retrospective chart review will collect evidence of graft failure in addition to the follow-up assessments outlined in Appendix 1.

Subjects are allowed to concurrently enroll in and be screened for a non-CIT islet transplant study at any point during participation. Once study treatment in the non-CIT study is initiated, they will be withdrawn from CIT08 (see section 6.1).

Subjects with confirmed **graft failure** will not complete metabolic assessments. Subjects who experience graft failure and subsequently stop immunosuppression will have alloantibody assessed 3 months after their last dose of immunosuppression.

7.3 Visit Windows

If the screening visit occurs within 90 days of the subject's final parent study visit, the results from the final parent study visit should be used. If the screening visit occurs more than 90 days after the final parent study visit, then the screening assessments must be repeated. Annual study visits will occur within plus or minus 30 days of the anniversary of the subjects' last parent study visit. Quarterly local visits will occur within plus or minus 14 days.

8. SAFETY MONITORING

8.1 Overview

This section defines the types of AEs and outlines the procedures for appropriately collecting, grading, recording, and reporting them. Information in this section complies with 21CFR 312; ICH Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting; and ICH Guideline E-6: Guidelines for Good Clinical Practice; and applies the standards set forth in the CIT Common Terminology Criteria for Adverse Events.

The investigator is responsible for the detection and documentation of events meeting the criteria and definition of an AE or SAE as described in section 8.2 of this protocol. AEs and SAEs will be recorded in the source documents and on the appropriate electronic CRF(s). All data will be reviewed periodically by the independent study monitor, who may provide recommendations to NIAID about withdrawing any participant and/or terminating the study because of safety concerns.

8.2 Definitions

8.2.1 Adverse Event

An adverse event (AE) is defined as any untoward or unfavorable medical occurrence associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research (ICH E-6 Guidelines for GCP).

For this study, an adverse event will include any untoward or unfavorable medical occurrence associated with the following.

- **Study therapy:**
  - None
- **Study mandated procedures:**
  - Blood draws – Any AE occurring within 24 hours after a protocol mandated blood draw.
  - Metabolic testing – Any AE occurring within 24 hours after study-required metabolic testing.

Adverse events occurring outside the designated time parameters should also be reported if the investigator deems a possible association with a study mandated procedure.

Recording of adverse events in this trial will be limited to:

- cirrhosis
- renal insufficiency
- malignancy
- hypoglycemia
- all adverse events meeting the serious criteria outlined in section 8.2.4.

8.2.2 Suspected Adverse Reaction (SAR)

A suspected adverse reaction (SAR) is any adverse event for which there is a reasonable possibility that the investigational study therapy or procedure caused the adverse event. For the purposes of safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the study therapy or procedure and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a study therapy or procedure (21 CFR 312.32(a)).

8.2.3 Unexpected Adverse Event

An adverse event or suspected adverse reaction is considered "unexpected" if it is not consistent with the risk information described in the protocol or other experience pertaining to study procedures in this population.

8.2.4 Serious Adverse Event

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or DAIT/NIAID, it results in any of the following outcomes (21CFR312.32(a)):

1) Death.
2) A life-threatening event. An AE or SAR is considered "life-threatening" if, in the view of either the investigator or DAIT/NIAID, its occurrence places the subject at immediate risk of death. It does not include an AE or SAR that, had it occurred in a more severe form, might have caused death.
3) Inpatient hospitalization greater than 24 hours or prolongation of existing hospitalization.
4) Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
5) Congenital anomaly or birth defect.
6) An event that required intervention to prevent permanent impairment or damage.
7) An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based on appropriate medical judgment, it may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

8.3 Grading and Attribution of Adverse Events

8.3.1 Grading Criteria

The study site will grade the severity of AEs experienced by CIT study subjects according to the criteria set forth in the *CIT-TCAE*. This document provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all AEs.

AE severity will be graded on a scale from 1 to 5 according to the following standards in the *CIT-TCAE* manual:

Grade 1 = Mild AE.

Grade 2 = Moderate AE.

Grade 3 = Severe and undesirable AE.

Grade 4 = Life-threatening or disabling AE.

Grade 5 = Death.

Table 4: General severity definition of adverse event

Grade 1	**Mild**	Transient or mild discomforts (< 48 hours), no or minimal medical intervention/therapy required, hospitalization not necessary (non-prescription or single-use prescription therapy may be employed to relieve symptoms, *e.g.*, aspirin for simple headache, acetaminophen for post-surgical pain).
Grade 2	**Moderate**	Mild to moderate limitation in activity some assistance may be needed; no or minimal intervention/therapy required, hospitalization possible.
Grade 3	**Severe**	Marked limitation in activity, some assistance usually required; medical intervention/therapy required hospitalization possible.
Grade 4	**Life-threatening**	Extreme limitation in activity, significant assistance required; significant medical/therapy intervention required hospitalization or hospice care probable.
Grade 5	**Death**	Death.

AEs, not included in the *CIT-TCAE* listing, should be recorded and their severity graded from 1 to 5 according to the General Grade Definition provided above.

All AEs will be reported and graded, by the PI or designee, whether they are or are not related to disease progression or study protocol.

8.3.2 Definition of Attribution

Attribution will only be determined and collected for serious adverse events.

The relatedness, or attribution, of an SAE to a study procedure will be determined by the site investigator. The site investigator will also record the determination of attribution on the appropriate eCRF and/or SAE report form. The relationship of an SAE (attribution of SAE) to a study procedure will be defined by using the descriptors provided below.

Table 5: Attribution of adverse events

Code	Descriptor	Definition
UNRELATED CATEGORY		
1	Unrelated	The adverse event is definitely not related to the study treatment.
RELATED CATEGORIES		
2	Possible	The adverse event might or might not be related to the study treatment. (This grade is assigned when uncertainty exists)
3	Definite	The adverse event is definitely related to the study treatment.

For additional information and a printable version of the CIT-TCAE manual, consult the CIT website: http://isletstudy.org.

8.4 Collecting and Recording of Adverse Events

8.4.1 Collection Period

AEs will be followed until resolution, stabilization, or until 30 days after a participant terminates from the study, whichever comes first.

8.4.2 Collecting Adverse Events

Adverse Events (including SAEs) may be discovered through any of these methods:

- Observing the subject.
- Receiving an unsolicited complaint from the subject.
- During their annual study visits and/or at the time of premature withdrawal from the study, subjects will be asked whether, in the past year:
    - They were hospitalized;
    - They had a medical issue requiring a visit to the emergency room or an urgent care clinic;
    - They experienced any severe hypoglycemic events;
    - They have been diagnosed with a malignancy; and
    - They have become pregnant or have plans for pregnancy.
- In addition, an abnormal value or result from a clinical or laboratory evaluation can also indicate an adverse event, as defined in Section 8.3, Grading and Attribution of Adverse Events.

8.4.3 Recording Adverse Events

The investigator will record adverse events and serious adverse events as described previously (Section 8.2, Definitions) on the appropriate case report form regardless of the relationship to study procedure.

Adverse events must be recorded by the site on the appropriate AE/SAE CRF within 5 business days of awareness.

Adverse events collected on a case report form.

Once recorded, an AE/SAE will be followed until it resolves with or without sequelae, or until the end of study participation, or until 30 days after the subject prematurely withdraws (without withdrawing consent)/or is withdrawn from the study, whichever occurs first.

8.4.4 Reporting Serious Adverse Events

8.4.4.1 REPORTING OF SERIOUS ADVERSE EVENTS TO SPONSOR

This section describes the responsibilities of the site investigator to report serious adverse events to the sponsor via the DCC eCRF. Timely reporting of adverse events is required by 21 CFR and ICH E6 guidelines.

Site investigators must report all serious adverse events (see Section 8.2.4, Serious Adverse Event), regardless of relationship or expectedness within 24 hours of discovering the event.

For serious adverse events, all requested information on the AE/SAE eCRF should be provided to the DCC. However, unavailable details of the event should not delay submission of the known information. As additional details become available, the AE/SAE eCRF should be updated and submitted.

8.4.4.2 REPORTING OF ADVERSE EVENTS TO IRBS

All investigators must report adverse events in a timely fashion to their respective IRBs in accordance with applicable regulations and guidelines.

8.4.4.3 REPORTING PREGNANCY

Any pregnancy that occurs during a clinical study that is using an investigational drug must be reported to the DCC utilizing the SAE report form. This report is for tracking purposes only. The investigator will counsel the subject and discuss the risks of continuing with the pregnancy and the possible effects on the fetus, and will encourage the subject to discuss those choices with her obstetrician. All pregnancies that are identified during the study must be followed to conclusion and the outcome of each must be reported using the follow-up SAE report form. A woman who wishes to become pregnant while on the study will be counseled as to her choices and, if she decides to stop using contraception, will be dropped from the study.

8.5 Review of Safety Information

8.5.1 Medical Monitor Review

The DAIT/NIAID and NIDDK Medical Monitors will receive monthly reports compiling new and accumulating information on AEs, SAEs, and pregnancies recorded by the sites on appropriate eCRFs.

In addition, the Medical Monitor will review and triage SAE and pregnancy reports received from the DCC.

8.5.2 DSMB Review

The Data and Safety Monitoring Board (DSMB) will review safety data yearly during planned DSMB Data Review Meetings. Data for the planned safety reviews will include, at a minimum, a listing of all reported AEs and SAEs.

9. MECHANISTIC ASSAYS

9.1 Metabolic Testing

9.1.1 Study Endpoints

Because the assessment of islet graft function is dependent on complex physiologic relationships between the graft and its recipient, no single test adequately addresses the viability of the transplant. The primary endpoint of duration of graft function addresses the clinically important outcome.

9.1.1.1 GLYCEMIC CONTROL

Glycemic control will be assessed by HbA1c (%), which will be analyzed at the central laboratory.

9.1.1.2 HYPOGLYCEMIA

An episode of severe hypoglycemia is defined as an event with one of the following symptoms: memory loss; confusion; uncontrollable behavior; irrational behavior; unusual difficulty in awakening; suspected seizure; seizure; loss of consciousness; or visual symptoms, in which the subject was unable to treat him/herself and which was associated with either a blood glucose level <54 mg/dL (3.0 mmol/L) or prompt recovery after oral carbohydrate, IV glucose, or glucagon administration.[15]

9.1.1.3 MIXED-MEAL TOLERANCE TEST (MMTT)

Basal (fasting) and stimulated glucose and C-peptide levels will be determined using the MMTT. Subjects will be instructed not to eat or inject short-acting (or bolus) insulin after 8 PM the night before the test. Evening or bedtime administration of long-acting insulin will be permitted, as will consumption of water. Subjects receiving CSII (insulin "pump" therapy) may remain on the basal rate of insulin. Subjects will arrive fasting to the transplant or diabetes clinic where the capillary BG will be checked. If the BG is <70 mg/dl (3.89 mmol/L) or >180 mg/dl (10 mmol/L), the test will be rescheduled for the next possible day. If the BG is 70 – 180 mg/dl (3.89 – 10 mmol/L), basal glucose and c-peptide levels will be drawn. Immediately after, the subject will receive 6 mL per kg body weight (to a maximum of 360 mL) of Boost® High Protein Drink (or a nutritionally equivalent substitute) to consume in 5 minutes starting at time = 0. Then, at time = 60 minutes and time = 90 minutes, stimulated glucose and C-peptide levels will again be drawn.

Each blood sample collected for c-peptide and glucose determination will be drawn according to University of Washington (Seattle, WA) SOP and will be shipped frozen to U of W for measurement in the core laboratory.

9.2 Immunologic Testing

Although insulin independence can be achieved via transplantation of an adequate number of viable, functional islets, a gradual reduction in the percent insulin independent patients occurs over time, with approximately 25% of patients still insulin free at 4 years post-transplant. Immune mediated islet destruction in the form of allorejection and/or recurrent autoimmunity, as well as attrition of a marginal islet mass due to exhaustion and/or toxicity of immunosuppressive agents, have all been postulated to play a role in islet loss. In order to begin to dissect the role of immune mediated reactions in allograft loss, tests will be done to determine if sensitization to donor allo- or islet autoantigens has occurred. In addition, maintenance of protective immunity in the setting of immunosuppression will be addressed.

While methods for determination of allo- and autoantibody have been extensively studied and are fairly well-established, reliable, reproducible and validated methods for assessment of T cell immunoreactivity to allo and/or autoantigens do not exist. For the most part, these techniques are time-consuming, technically demanding and require large blood volumes and significant staff time for set up and analysis of the resultant data. Several methods are undergoing testing in multiple T1D consortia (*e.g.*, ELISPOT, tetramer staining, T cell proliferation assays) to determine which tests provide the most reliable data with regards to distinguishing between patients with T1D vs. normal controls (for autoantigen) and to improve techniques for assessing recipient anti-donor reactivity.

9.2.1 Immune Assays

9.2.1.1 ALLOANTIBODY

Development of alloantibody is generally associated with longer term graft loss. Development of alloantibody specific for 1 or 2 HLA antigens can now be defined using assays that incorporate HLA specific monoclonal antibodies. Alloantibody assessments will be performed at each site's laboratory for subjects who experience graft failure during participation in CIT08 and subsequently discontinue immunosuppression.

9.2.1.2 ARCHIVED SERUM

In order to ensure that we will ultimately gain as much information as possible from these trials, and due to the ongoing development of assays such as T cell assays, serum will be archived for future analyses. Details for subjects regarding the archiving of samples and use for future assays are contained in the study's informed consent form. Subjects will have the option of whether or not they want to have samples archived and will indicate their choice on the informed consent form. A subject's choice regarding archiving samples will not affect his/her participation in the study.

Serum: Blood will be collected to obtain serum and archived in the NIDDK repository.

10. STATISTICAL CONSIDERATIONS AND ANALYTICAL PLAN

10.1 Study Endpoint Assessment

10.1.1 Primary Endpoint

The primary endpoint is time to islet graft failure. The purpose of the analyses will be to estimate the probability of islet graft failure as a function of time from entry into the study . Life table methods will be used to estimate the survival curve and provide confidence intervals for the probability of islet graft survival for selected time points.

10.1.2 Secondary Endpoints

Insulin usage will be estimated from the one-week self report values. Estimates of population means and confidence intervals for those means will be reported for each follow-up visit. Linear mixed models methods will be used to describe the profile of change with time.

Numbers of severe hypoglycemic events will be estimated from the self report values obtained at each follow-up visit. Estimates of population means and confidence intervals for those means will be reported for each follow-up visit. Linear mixed models methods with appropriate likelihood functions will be used to describe trends with time.

HbA1c and serum creatinine levels will be measured at central laboratories at study entry and at the annual follow-up visit. GFR will be estimated using the updated CKD-EPI method. Estimates of population means and confidence intervals for those means will be reported for each follow-up visit. Linear mixed models methods will be used to describe trends with time.

Incidence of serious adverse experiences will be tabulated by body system and MeDRA code.

Life table methods will be used to estimate mortality rates.

The overall incidence of alloantibody conversion will be reported as a rate per 100 days of follow-up. A 95% confidence interval for the rate will be computed using boot-strap methods.

10.2 Patient and Demographic Data

10.2.1 Baseline Characteristics and Demographics

Summary descriptive statistics for baseline and demographic characteristics will be provided for all subjects in the ITT sample. Demographic data will include age, race, sex, body weight, and height; these data will be presented in the following manner:

- Continuous data (*i.e.*, age, body weight, and height) will be summarized descriptively by mean, standard deviation, median, and range.
- Categorical data (*i.e.,* sex and race) will be presented as enumerations and percentages.

Statistical presentation for baseline and demographic characteristics may be further summarized by values of important baseline predictors of outcome and will be further defined in the SAP.

10.3 Reporting Deviations from Original Statistical Plan

The principal features of the study design and of the plan for statistical analysis of the data are outlined in this protocol and in the subsequent SAP. Any changes in these principal features will require a protocol or an SAP amendment, which would be subject to review by the independent DSMB, the study sponsor, and the health authorities. These changes will be described in the final report as appropriate.

11. IDENTIFICATION AND ACCESS TO SOURCE DATA

11.1 Identifying Source Data

The investigator is required to keep accurate records to ensure that the conduct of the study is fully documented (see section 12). The results of all clinical and clinical laboratory evaluations will be maintained in the subject's medical records and the data will be transferred to clinical CRFs.

Safety data will be recorded on CRFs specifically designed for this purpose. All data will be reviewed periodically by the DSMB and IRB. The DSMB and/or the IRB have the authority to withdraw any subjects and/or terminate the study because of safety findings.

11.2 Permitting Access to Source Data

The investigational site participating in this study will maintain the highest degree of confidentiality permitted for the clinical and research information obtained from the subjects 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 site must permit authorized representatives of the sponsor(s), including pharmaceutical collaborators and their commercial partners, and health authorities to examine (and when required by applicable law, to copy) clinical records for the purpose of quality assurance reviews, audits, and evaluations of the study safety and progress. Unless required by the laws that permit copying of records, only the coded identity associated with documents or with other subject data may be copied (and all personally identifying information must be obscured). Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that is linked to identified individuals. The investigational site will normally be notified before auditing visits occur.

12. QUALITY CONTROL AND QUALITY ASSURANCE

Site monitoring will be conducted to ensure that human subject protection, study procedures, lab procedures, study intervention administration are performed to comply with pertinent regulations, sponsor requirements, and GCP/ICH guidelines, and in accordance with the site and sponsor SOPs. DAIT, NIAID, or a designee will conduct site monitoring visits related to the protocol procedures and GCP standards.

12.1 Compliance, Access, Entry and Handling of Study Data

The site PI is required to keep accurate records to ensure that the conduct of the study is fully documented, and to ensure that CRFs are completed for all subjects according to study guidelines outlined in the study protocol and the Data System Users Instruction Manual.

Access to the data entry screens will be user ID and password protected. Each user will be provided with a unique personal ID and password. The investigational site participating in this study will maintain the highest degree of confidentiality permitted for the clinical and research information obtained from the subjects in this clinical trial. Medical and research records should be maintained at each site in the strictest confidence. However, as part of the quality assurance and legal responsibilities of an investigation, the investigational site must permit authorized representatives of the sponsor(s) and health authorities to examine (and when required by applicable law, to copy) clinical records for the purpose of quality assurance reviews, audits, and evaluations of the study safety and progress. Unless required by the laws that permit copying of records, only the coded identity associated with documents or with other subject data may be copied (and all personally identifying information must be obscured). Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that is linked to identified individuals. The investigational site will normally be notified before auditing visits occur.

All data will be entered, stored, and managed in a relational database supported by database servers at the DCC. The results of all clinical and laboratory evaluations will be maintained in the subjects' medical records and the data will be transferred from these source documents directly to the electronic study CRFs. In order to maintain security, all data will be encrypted using the Secure Sockets Layer protocol. This protocol allows an encrypted link to be established between the DCC web server and the computer at each center. In addition, the data will be verified by a series of computerized edit checks, and all relevant data queries will be resolved regularly. All discrepancies will be reviewed, and any resulting queries will be resolved with the site personnel and amended in the database.

All changes made to CRFs will be recorded in an electronic audit trail to allow all data changes in the data system to be monitored and maintained in accordance with federal regulations. Once a CRF is entered into the database and the person entering the data indicates that CRF is complete, any change to that data will be entered into the system's audit trail. The audit trail will record the CRF and variable that is changed, the old value, the new value, the date and time the change was made, reason change was made, and the user ID of the person making the change. Once a change is completed, the data system will re-validate all variables on that CRF. The changed CRF will be required to pass all validity and logic consistency checks. If any edit

criteria fail, the system will generate appropriate queries. The clinical center coordinator will be asked to resolve the questions before the changes are completed.

The change system will allow certified DCC personnel and certified clinical center coordinators to make changes. Changes can be initiated by DCC monitors, DCC coordinators, and certified site personnel. Site personnel can access only the data for their own center. The system will generate weekly summary listings of all changes made to the database, the person making each change, and the reason for each change. These reports will be carefully reviewed by the DCC coordinator to monitor for unnecessary changes and/or problems with the data system.

13. ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE

13.1 Statement of Compliance

This clinical study will be conducted using cGCP, as delineated in *Guidance for Industry: E6 Good Clinical Practice Consolidated Guidance* [16], and according to the criteria specified in this study protocol. Before study initiation, the protocol and the informed consent documents will be reviewed and approved by an appropriate EC or IRB, and NIAID/NIDDK. Any amendments to the protocol or to the consent materials must also be approved by the IRB/EC and submitted to the applicable Health Authorities before they are implemented.

13.2 Informed Consent and Assent

The informed consent form is a means of providing information about the trial to a prospective subject and allows for an informed decision about participation in the study. All subjects (or their legally acceptable representative) must read, sign, and date a consent form before entering the study, taking study drug, or undergoing any study-specific procedures. Consent materials for subjects who do not speak or read English must be translated into the subjects' appropriate language.

The informed consent form must be 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 subject for review. The attending physician, in the presence of a witness if required by the IRB, will review the consent and answer questions. The prospective subject will be told that being in the trial is voluntary and that he or she may withdraw from the study at any time, for any reason.

13.3 Privacy and Confidentiality

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

14. PUBLICATION POLICY

The CIT policy on the publication of study results will apply to this trial.

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Appendix 1. Schedule of Events for Extended Follow-up

Visit schedule based on anniversary of last islet transplant in CIT parent study; visit schedule repeats each year.	**Timing**	**SC**	Local Visits[1]			On-Site Visit
			3 month	6 month	9 month	**Anniversary of Final Islet Transplant**
	Visit #	**0**	See MOP for Table of Visit Numbers			
Visit Window (specified in days)		**- 90**	±14	±14	±14	**± 30**
GENERAL ASSESSMENTS						
Informed Consent		X				
Evaluate Inclusion/Exclusion Criteria		X				
Physical Exam		X[2]				X
AE /Hypoglycemic Events/Toxicity Assessment[3]		X[2]	X	X	X	X
Calculated GFR		X[2]				X
Insulin use		X[2]	X	X	X	X[4]
Urine pregnancy test (females)		X				
LOCAL LABORATORY ASSESSMENTS						
CBC (WBC + Diff & Plat)[5]		X[2]	X	X	X	X
Chemistry[5,6]		X[2]	X	X	X	X
Lipids[5]		X[2]				X
Alloantibody		Collect 3 months after immunosuppressive medication is stopped following graft failure.				
CENTRAL LABORATORY/METABOLIC ASSESSMENTS						
HbA1c[7]		X[2]				X
Fasting serum gluc/c-pep & serum creatinine[7]		X[2]				X
MMTT[7]		X[2]				X
CARDIOVASCULAR ASSESSMENTS						
Carotid intimal thickness (IMT)		Collect at 5 years post-initial-islet transplant visit in subjects who completed the baseline carotid IMT assessment in their CIT parent study.[8]				
IMMUNOSUPPRESSION LEVELS						
Blood Trough Levels (if applicable)[5]		X[2]	X	X	X	X
Visit schedule based on anniversary of last islet transplant in CIT parent study; visit schedule repeats each year.	**Timing**	**SC**	Local Visits[9]			On-Site Visit
			3 month	6 month	9 month	**Anniversary of Final Islet Transplant**
	Visit #	**0**	See MOP for Table of Visit Numbers			
Visit Window (specified in days)		**- 90**	±14	±14	±14	**± 30**
MECHANISTIC ASSAYS – University of Pennsylvania Sub-Study[10] Only						
Autoantibody[7]				X		X
Immunophenotyping[7]				X		X
Cytokine profiling[7]				X		X
Glucose-potentiated arginine[7]						X
ARCHIVED SAMPLES						
Serum						X

[1] These visits are performed locally but can be done on site if preferred.

[2] Only collect if screening is ≥ 90 days since final parent study visit. Otherwise, results from final parent study visit should be used for screening (Visit 0).

[3] Also collect AE assessment at time of premature study termination, if applicable.

[4] Subjects must record insulin usage for 7 consecutive days within the visit window.

[5] Also collect as clinically indicated.

[6] Chemistry includes: Sodium, albumin, magnesium, chloride, potassium, alk phosphatase, total bilirubin, CO2, creatinine, ALT (SGPT), BUN, gamma GT, glucose, AST (SGOT), calcium, phosphorus

[7] Do not collect after confirmed graft failure.

[8] For those subjects who missed CIMT collection at the 5-year time point, collect at the next available opportunity.

[9] These visits are performed locally but can be done on site if preferred.

[10] Please refer to CIT07 Protocol Appendix 6 for details on the University of Pennsylvania Sub-Study.

Appendix 2. Study Contacts

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