

Protocol CIT-02

Strategies to Improve Long Term Islet Graft Survival

Version 7.0 (12/Nov/2012)

BB-IND 9336

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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INVESTIGATOR SIGNATURE PAGE**Protocol**

CIT-02

Version/Date:

Version 7.0, November 12, 2012

IND:

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Protocol Chair:

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Short Title:

Islet graft survival

Study Sponsor:

National Institute of Allergy and Infectious Diseases (NIAID) And 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 (DCC).

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-02: Strategies to Improve Long Term Islet Graft Survival**” **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

Glossary of Abbreviations

ACE	American College of Endocrinology
AE	Adverse Event
AIDS	Acquired Immunodeficiency Syndrome
AIR_{glu}	Acute Insulin Response to Glucose
ALS	Antilymphocyte Serum
APC	Antigen Presenting Cell
ATG	Anti-thymocyte Globulin
BG	Blood Glucose
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
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
EU	European Union
FDA	Food and Drug Administration
FSIGT	Frequently Sampled Intravenous Glucose Tolerance
G-CSF	Granulocyte Colony Stimulating Factor
GFR	Glomerular Filtration Rate
GGT	Gamma Glutamyl Transpeptidase
HbA1c	Glycosylated Hemoglobin
HFS	Hypoglycemic Fear Survey
HIV	Human immunodeficiency Virus
HLA	Histocompatibility Antigen
HSA	Human Serum Albumin
HSV	Herpes Simplex Virus
HTLV1	Human T-cell Lymphotropic Virus Type 1
IBMIR	Instant Blood Mediated Inflammatory Reaction
ICH	International Conference on Harmonization
IE	Islet Equivalents
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
LSF	Lisofylline
MAGE	Mean Amplitude of Glycemic Excursion
MMTT	Mixed-Meal Tolerance Test
NCI	National Cancer Institute
NIAID	National Institute of Allergy and Infectious Diseases
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
PCR	Polymerase Chain Reaction
PI	Principal Investigator
pit-hGH	Pituitary Growth Hormone
PLT	Platelet Count
PNF	Primary Non-function
PRA	Panel Reactive Antibodies
PSA	Prostate specific antigen
PTLD	Post-transplant Lymphoproliferative Disorder
PT	Prothrombin time
PTT	Partial Thromboplastin Time
QOL	Quality of Life
RNA	Ribonucleic Acid
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SGOT	Serum Glutamic-oxaloacetic Transaminase
SGPT	Serum Glutamate Pyruvate Transaminase
SOP	Standard Operating Procedure

T1D	Type 1 Diabetes
TAT	Thrombin-antithrombin
TB	Tuberculosis
TCAE	Terminology Criteria for Adverse Events
TNF	Tumor Necrosis Factor
ULN	Upper Limit of Normal
UM	University of Miami
UNOS	United Network for Organ Sharing Disorders
WHO	World Health Organization
WOCBP	Women of Child Bearing Potential

Protocol Synopsis

Title: Strategies to Improve Long Term Islet Graft Survival

Clinical Phase: Phase 2

Short title: Islet Graft Survival

IND Sponsor: DAIT/NIAID/NIH

IND Number: BB-IND 9336

Activation Date: December 2006

Accrual Objective: 12 patients

Accrual Period: Two years

Study Duration: 24 months after final transplant

Treatment Description: This protocol will focus on treating islets pre-transplant, and subjects (during and post-transplant), with Lisofylline an agent with a unique spectrum of anti-inflammatory, anti-apoptotic and β cell enhancing properties, to improve β cell function and viability, maximizing pre-transplant functional islet mass, islet engraftment and long-term function, in subjects with T1D.

Primary Objective: The primary objective is to determine the proportion of subjects who are insulin independent after a single islet transplant at 75 ± 5 days post-transplant in subjects treated with Lisofylline added to a standard islet transplant regimen who enroll at the same sites as the CIT-02 subjects.

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

Study Design: This is an open label, randomized prospective, single-arm, phase 2, multi-centered trial.

Objectives of this study include gaining experience with the experimental treatment regimen as well as to determine the proportion of subjects with insulin independence.

There is a larger parallel study with additional centers and more subjects (CIT-07) where subjects are treated with the standard islet transplant regimen. Subjects will be randomized to receive either the standard immunosuppression regimen under CIT-07 or the experimental immunosuppression in this study (CIT-02) at the time that the islets are available for transplantation and have met the criteria for number and viability before culture. Subjects will be randomized 2:1 into CIT-07 and CIT-02 respectively. The randomization will be performed centrally. All islets will be processed and recipients (N=12) will be recruited and transplanted at two sites, the University of Miami (UM) and University of Illinois at Chicago (UIC). Randomization will be stratified by the two sites (UM and UIC).

Primary Endpoint: The primary endpoint will be insulin-independence at 75 ± 5 days following a single islet transplant.

Primary Analysis: The primary analysis will provide an estimate of the proportion of subjects with insulin independence and a corresponding 95% confidence interval.

Secondary Endpoints:

The key secondary endpoints is the proportion of subjects with HbA1c < 7.0% AND free of severe hypoglycemic events from Day 28 to Day 365, inclusive, after the first islet transplant.

The other secondary endpoint is the proportion of subjects with HbA1c < 7.0% AND free of severe hypoglycemic events from Day 28 to Day 365 inclusive, after the final islet transplant.

Secondary Efficacy Endpoints

At 75 ± 5 days following the first islet transplant and following each subsequent islet transplant(s):

- 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 (QOL)

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 and final islet transplant:

- 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
- QOL
- 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® (CGMS)
- The proportion of subjects receiving a second islet transplant
- The proportion of subjects receiving a third islet transplant
- Rate of favorable outcome at each center preparing islets (rate of subjects with an HbA1c < 7.0% and free of severe hypoglycemic events).

Secondary efficacy endpoints measured at 365 ± 14 days following the final islet transplant will include the change in the above measures from the results obtained at 75 ± 5 days following the final islet transplant.

At two years (730 ± 14 days) following the final islet transplant:

- The percent change from baseline insulin requirements.
- The number of severe hypoglycemic events from 28 days to two years.
- HbA1c
- Clarke Score
- Basal (fasting) and 90-min glucose and c-peptide (MMTT).
- B-score
- C-peptide: (glucose • creatinine) ratio
- CGMS
- QOL

Secondary Safety Endpoints

- Safety, including incidence of post-transplant infections, malignancies, morbidity, and other AEs (e.g., increased body weight and hypertension) associated with conventional immunosuppression.
- Renal function as measured by serum creatinine, GFR and other relevant laboratory parameters.
- Lipid profiles (triglycerides, total cholesterol, LDL cholesterol, HDL cholesterol) over time.

At 75 ± 5 following each transplant and 365 ± 14 days following the first and final islet transplant, and at two years following the 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 of normal [ULN])
- The incidence and severity of AEs related to the immunosuppression including: allergy; reduction in GFR; addition or intensification of anti-hyperlipidemic therapy;; 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
- The incidence of discontinuation of immunosuppression

At 365 ± 14 days following the first islet transplant:

- The incidence of worsening retinopathy as assessed by change in retinal photography from pre-transplant. If pupil dilation is not possible, then a manual ophthalmologic evaluation can be substituted.

Secondary Analyses: The secondary analyses will compare the secondary endpoints in this study to the corresponding outcomes in the CIT-07 study of subjects who enroll at the same sites as the CIT-02 subjects. Estimates and 95% confidence intervals will also be calculated.

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 subject age and insulin-dependent diabetes duration of ≥ 28 .
5. Absent stimulated c-peptide ($<0.3\text{ng/mL}$) 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 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 within the last 6 months prior to randomization;

OR

Marked glycemic lability characterized by wide swings in BG despite optimal diabetes therapy and defined by a glycemic lability index (LI) score greater than or equal to the 90th percentile ($433\text{ mmol/L}^2/\text{h}\cdot\text{wk}^{-1}$) 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 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) $>30\text{ kg/m}^2$ or patient weight $\leq 50\text{kg}$.
2. Insulin requirement of $>1.0\text{ IU/kg/day}$ or $<15\text{ U/day}$.
3. HbA1c $>10\%$.
4. Untreated proliferative diabetic retinopathy.
5. Blood Pressure: SBP $>160\text{ mmHg}$ or DBP $>100\text{ mmHg}$.
6. Measured glomerular filtration rate (GFR) using iohexol of $<80\text{ mL/min/1.73 m}^2$ (or for subjects with an iodine allergy will be calculated using the subject's measured serum creatinine and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation ¹). Strict vegetarians (vegans) with a calculated GFR <70

mL/min/1.73m² are excluded. The absolute (raw) GFR value will be used for subjects with body surface areas >1.73m².

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. Presence or history of active infection including hepatitis B, hepatitis C, HIV, or tuberculosis (TB) Subjects with laboratory evidence of active infection are excluded even in the absence of clinical evidence of active infection.
11. Negative screen for Epstein-Barr Virus (EBV) by IgG determination.
12. Invasive aspergillus, histoplasmosis, or 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). Participant with lymphopenia are allowed if the investigator determines there is no additional risk and obtains clearance from a hematologist.
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 medications 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.

Study Definitions

Full graft function: Islet transplant recipients will be considered to have full islet graft function if they are insulin independent.

Partial graft function: Islet transplant recipients who do not meet criteria for insulin independence, but have either a basal or stimulated c-peptide level ≥ 0.3 ng/mL (0.1 nmol/L).

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 graft failure do not need to complete the day 75 metabolic assessments.

Primary nonfunction (PNF): Graft failure that occurs between 3 and 7 days post-transplant.

Insulin-independent:

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:

- One HbA1c level, one fasting serum glucose level, and a Mixed Meal Tolerance Test are documented within the visit window (e.g. 70-80 days at Day 75) and 7 consecutive days of blood sugar and insulin readings are documented within ± 7 days of the visit window (e.g. 63 – 87 days at Day 75);
- HbA1c $\leq 7.0\%$ or a $\geq 2.5\%$ decrease from baseline;
- Fasting capillary glucose level should not exceed 140 mg/dL (7.8 mmol/L) more than three times in the 7 consecutive days (fasting is defined as 1st blood sugar reading of the day not noted as post-prandial or bedtime);
- Post-prandial serum glucose ≤ 180 mg/dL (10.0 mmol/L) at 90 minutes during the MMTT;
- 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;
- At least one MMTT fasting or stimulated c-peptide levels ≥ 0.5 ng/mL (0.16 nmol/L).

Insulin-dependent: Islet transplant recipients who do not meet the criteria for insulin independence will be considered insulin-dependent.

Protocol eligible: Participants will be considered “protocol eligible” once all screening assessments required to confirm eligibility in the study have been completed.

Wait List: Protocol eligible participants who have been listed for islet transplant with UNOS or an equivalent transplant network.

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.

Intensive diabetes management: 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.

Women of Child Bearing Potential (WOCBP):

WOCBP includes any female who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) or is not postmenopausal (defined as amenorrhea \geq 12 consecutive months; or women on hormone replacement therapy with documented serum follicle stimulating hormone level $>$ 35 mIU/mL). Even women who are using oral, implanted, or injectable contraceptive hormones or mechanical products such as an intrauterine device or barrier methods (diaphragm, condoms, spermicides) to prevent pregnancy or practicing abstinence or where the partner is sterile (eg, vasectomy), should be considered to be of child bearing potential.

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1 BACKGROUND AND RATIONALE

1.1 Background

The recent improvement in clinical islet transplantation outcome, since the introduction of the Edmonton protocol² and other islet trials³⁻⁶ has paralleled a renewed enthusiasm on the potential for islet transplantation as a viable strategy for restoration of euglycemia in patients with Type 1 Diabetes Mellitus (T1D). It is well known that optimal glycemic control could delay the onset, and slow the progression of retinopathy, nephropathy, and neuropathy in patients with T1D⁷. However, the benefits of islet transplantation must be weighed against current challenges, including the risks associated with the requirement for chronic recipient immunosuppression (IS), and the observed reduction of islet function over time. A reduced islet function after the initial year post-transplant could be associated with either chronic rejection and/or recurrence of autoimmunity due to ineffective IS, exhaustion of the sub-optimal beta cell mass that successfully engrafts post-transplant and/or beta cell directed toxicity of the selected IS drugs⁸. It is now essential to devise strategies to maximize early islet mass/engraftment, while minimizing late loss of islet mass following islet transplantation.

Our central hypothesis is that treatment of islets pre-transplant, and of recipients (before, during and post-transplant), with Lysofylline, an experimental agent with a unique spectrum of anti-inflammatory, anti-apoptotic and beta cell enhancing properties, will improve beta cell function and viability as well as islet engraftment and long-term function, therefore maximizing functional islet mass before and after transplantation in patients with T1D.

This protocol will focus on examining the effect of the novel agent, Lisofylline (LSF) on islet viability and function, engraftment and long-term function.

The following specific aims will determine whether LSF will:

1. Increase the proportion of subjects rendered insulin independent with a single (1-donor) islet transplant at POD 75.
2. Enhance the magnitude of glucose and non-glucose stimulated insulin responses of islet allografts.
3. Achieve long-term insulin independence by preventing the decline in β -cell function.

Initial results from the Edmonton trial^{2,9,10} and subsequent follow-up^{9,10} have proven that insulin independence can consistently be achieved using this protocol, a steroid-free IS regimen including daclizumab induction and administration of sirolimus and tacrolimus. In this trial subjects received sequential islet transplants from two or more donors, which resulted in insulin independence with improved glycemic control and improvements in their lifestyle that are expected to outweigh the risks of long-term IS in this selected subgroup of patients with labile T1D, hypoglycemia unawareness and/or progressive complications.

We have obtained similar results using Edmonton-like IS regimens, with 20 out of 20 completed patients (in Islet Alone and Islet after Kidney Protocols) achieving insulin independence, long-term islet graft function, normalization of HbA1c levels, and immediately after the first islet transplant, elimination of severe hypoglycemic episodes. Even though complete islet graft failure has been rare, worsening of metabolic control over time has been observed in several series^{4,5,9,10}, requiring restoration of limited exogenous insulin treatment. In the 17 subjects described in the Edmonton series^{9,10}, 9 of 15 (60%) and 4 of 6 (67%) were insulin independent at 1 and 2 years respectively after islet transplantation. In the Miami Islet Alone transplantation trial the one year insulin independence rate was 79% (11/14) and all subjects have islet graft function (100%), i.e., we have not had any instances of complete graft failure.

The requirement for multiple donors in the Edmonton protocol represents a limitation, especially in view of the limited number of organ donors each year. None of the subjects in the Edmonton trial^{9,10}, achieved insulin independence with a single transplant; however in the joint Miami-Baylor College of Medicine trials, 6 out of 31 subjects (20%)

achieved insulin independence with the first islet transplant. Careful selection of “ideal” donors and highly insulin sensitive recipients could lead to increased rates of insulin independence in some cases¹¹. Therefore, it is now essential to devise strategies to maximize early islet mass/engraftment, while minimizing late loss of islet mass following islet transplantation. Our preliminary data suggests that a possible reason for the worsening of metabolic control in subjects requiring restoration of some level of exogenous insulin therapy may be immunological destruction of islets¹². Inadequate immunosuppressive levels (when sirolimus is tapered after 3 months) may have accounted for our findings; however, alternative reasons for the observed worsening of metabolic control could include progressive exhaustion of a marginal β -cell mass, β -cell toxicity (tacrolimus) and insulin resistance (tacrolimus). The progressive decline in β -cell function seen in islet allografts parallels the decline observed in patients with type 2 diabetes mellitus¹³. Regulation of the β -cell mass appears to involve a balance of β -cell replication and apoptosis. Disruption of any of these pathways of β -cell formation or increased rates of β -cell death could cause a decrease in β -cell mass¹⁴⁻¹⁶. An inadequate β -cell mass will lead to inappropriate insulin secretion, resulting in mild chronic hyperglycemia, increased levels of fatty acids that ultimately contribute to an inability to sustain adequate insulin secretion, defective β -cell proliferation and/or introduction of measures that increase β -cell apoptosis¹⁴⁻¹⁶. Recent autopsy data in patients with type 2 diabetes mellitus showed that β -cell mass is decreased in type 2 diabetes mellitus and that the mechanism underlying this finding is increased β -cell apoptosis^{15, 16}.

Improving Outcomes of Islet Transplantation

There is significant potential for increasing the number of surviving islets pre and post-transplantation, through interventions: 1) during islet processing and subsequent pre-transplant in-vitro culture, 2) before, during and after islet transplant (i.e., in the early engraftment period), and 3) over the long-term post-transplantation.

In this proposal we hypothesize that LSF should preserve β -cell mass (both *in vitro* and *in vivo*) and result in enhanced islet engraftment, insulin secretory dynamics, and metabolic control

1.2 Preclinical and Clinical Experience

1.2.1 Preclinical Studies

LSF has been studied in multiple animal models. Recent data with human islets¹⁷ have shown that LSF not only preserves beta-cell insulin secretion in the presence of cytokines, but also enhances glucose-stimulated insulin secretion significantly, as compared to the controls; LSF alone reduced the basal rate of apoptosis and blocked the increase of apoptosis caused by cytokines. In transplantation experiments of human islets into nude mice, fewer numbers of LSF-treated islets led to insulin independence in the recipients as compared to those receiving untreated control islets¹⁷. These results suggest that LSF treatment enhances beta cell function, and provides the ability to use fewer numbers of islets to reverse diabetes.

LSF blocked differentiation of T_H0 to T_H1 cells by blocking IL-12 signaling in a murine model of human multiple sclerosis¹⁸. LSF also protects rodent and human islet cells from both the toxicity of and the loss of ability to secrete insulin after multiple pro-inflammatory cytokine exposure. In a low dose streptozotocin model of T_H1 disease, LSF-treated mice maintained significantly lower BG levels and higher blood insulin levels than did control mice, apparently by preventing T_H1 -mediated secondary injury to the islet β cells. LSF effectively suppressed T cell activation and differentiation via inhibition of the STAT4-mediated IL-12 signaling. The effect of LSF on T cells is responsible for its therapeutic function in prevention of autoimmune diabetes onset and protection of syngeneic islets grafts from autoimmune destruction in NOD mice. The action in inflammatory blockade renders LSF a beneficial effect to preserve and improve human islet function and viability.

1.2.2 Clinical Studies

More than 1000 patients and 47 healthy subjects have been enrolled in clinical trials with LSF; this includes more than 650 patients treated with active compound (see Diakine's Investigator Brochure for LSF for details). In healthy volunteer studies, LSF was well tolerated, and most AEs were mild in severity and were resolved promptly without intervention. No subject in a healthy volunteer study has experienced a serious adverse event (SAE). The incidence of AEs was higher in subjects receiving LSF ≥ 6 mg/kg, and 7 mg/kg over 30 minutes was deemed the maximum tolerated dose (MTD) due to the increased frequency of events (mild and moderate) that occurred at 8 mg/kg over either 30 or 60 minutes. A dose of 6 mg/kg provides blood levels in the therapeutic range. Doses of 5 mg/kg/Q6H for 28 days have been well tolerated in subjects following bone marrow transplantation, and 7 mg/kg over a 30 minute infusion was determined to be the MTD in healthy volunteers. Additionally, 235 subjects were enrolled in a randomized, placebo controlled trial of LSF for treatment of acute lung injury and acute respiratory distress syndrome²⁰. In studies with LSF in oncology indications, more than 430 subjects have received LSF²⁰. LSF has been well tolerated in these studies compared with the expected background level of AEs reported in the patient population. The most frequently reported AEs in oncological subjects were nausea and/or vomiting, stomatitis, diarrhea, and chills. Similar percentages of subjects receiving LSF at 3 mg/kg or placebo experienced SAEs. The majority of AEs reported were expected events following the chemo- and radiation therapy administered. No hypoglycemia has been reported in any of the LSF trials and LSF has been administered safely in subjects receiving cyclosporine A, systemic corticosteroids, acyclovir, gancyclovir, fluconazole, trimethoprim sulfamethoxazole and high dose IL-2 among other chemo therapeutic agents. The excellent safety profile taken together with the pre-clinical efficacy data, provides the rationale for use of LSF in islet transplantation.

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, morbidity³². Iatrogenic hypoglycemia is a consequence of 3 compromised defense mechanisms, whose pathophysiology was thoroughly reviewed by Cryer³²⁻³⁵.

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 onset³⁶. Third, epinephrine response to falling glucose levels is compromised, in terms of the magnitude of the response and the threshold for the response³⁷, 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^{38, 39}. 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 Diabetes Control and Complications Trial (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 administration⁴⁰. The DCCT Research Group definition replaced the more stringent 1980s definition of severe hypoglycemia based on loss of consciousness⁴¹⁻⁴³.

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^{38, 44, 45}. Hypoglycemia-associated autonomic failure is triggered by recurrent episodes of hypoglycemia, which reduce the magnitude of hormonal counter-regulation and reduce symptomatic responses to a given degree of subsequent hypoglycemia^{34, 46}, 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 hypoglycemia⁴⁷. 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^{38, 39}. The patient characteristic that most strongly predicted severe hypoglycemia in the DCCT was a history of prior severe hypoglycemic events⁴⁸.

Hypoglycemia is said to be a major concern of prospective employers⁴⁹. Neuroglycopenia can cause social embarrassment, and even lead to ostracism or be mistaken for disorderly or unlawful behavior (1). The more distressing the severe hypoglycemic episode, the greater the psychological fear of hypoglycemia⁵⁰. 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^{32, 51}. 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 failure⁵².

In patients with long-term (*i.e.* >15 years) T1D, scrupulous avoidance of hypoglycemia fails to restore normal glycemic thresholds or magnitudes of hormonal counter-regulation 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 counter-regulation (*i.e.*, deficient glucagon and epinephrine responses to hypoglycemia)⁵³⁻⁵⁷. 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 appreciable⁵⁸. Thus, it appears that, while hypoglycemia unawareness is reversible by meticulous prevention of hypoglycemia, defective glucose counter-regulation may not be reversible⁵⁹.

A successful pancreas transplant restores epinephrine responses and symptom recognition during hypoglycemia in patients with longstanding T1D and autonomic neuropathy⁶⁰. In type 1 diabetic islet transplant recipients with documented pretransplant hypoglycemia unawareness and defective hormonal counter-regulatory 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 hypoglycemia⁶¹. 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 recipients⁶². Ryan *et al.* documented the absence of episodes of severe hypoglycemia in 12 successful islet transplant recipients (median follow-up, 10.2 months)⁹ 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 sympathoadrenal 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 counter-regulation 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 trial.

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”^{63, 64}. Brittle diabetes in addition to lability has the added connotation that there may be associated frequent admissions to hospital ^{65, 66}. 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 SC insulin degradation ⁶⁷ but now the most severe cases are recognized to usually have a factitious origin⁶⁸. 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 ^{65, 66} but these are subject to the vagaries of local practice. More quantitative measures have been the mean amplitude of glycemic excursions (MAGE) ⁶⁹ and the M value of Schlichtkrull⁷⁰. 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 Schlichtkrull⁷⁰ but this logarithmic expression of the glucose deviation from a standard glucose level has not been validated. More recently⁷¹ 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 levels⁷².

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)⁷³. 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 under 300 mmol/L²/hr·wk⁻¹ with a median of 223 (25 – 75th percentiles 130 – 329 mmol/L²/h·wk⁻¹). An LI \geq 433 mmol/L²/h·wk⁻¹ (90th 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 Agents: Allogeneic Islets

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 BG 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 DCCT established that these microvascular complications of diabetes can be prevented by maintaining near-normal glucose control using multiple daily injections of insulin or insulin “pump” therapy in patients with T1D.⁷⁴ However, this degree of control can be impossible to achieve in many patients despite modern insulin analogs and delivery systems,⁷⁵ and also leads to life threatening episodes of insulin-induced hypoglycemia.⁷⁴

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 BG in diabetic recipients, with resultant stabilization and even reversal of microvascular complications.⁷⁶ However, despite the ability of whole organ pancreas transplantation to correct glucose homeostasis in T1D, the procedure requires major surgery and is not without risk.

According to the United Network for Organ Sharing (UNOS) pancreas registry data, almost 10% of whole organ pancreas grafts fail early due to technical complications and require an additional laparotomy for graft removal. 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 peri-operative myocardial infarction), this procedure is generally reserved in most centers 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. While whole pancreas transplantation has been performed in non-uremic T1D patients experiencing severe problems with metabolic control, long-term pancreatic graft function and survival is inferior when compared to simultaneous pancreas-kidney transplantation, primarily due to immunologic graft loss. 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 with less procedural related morbidity. 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 whole 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. Accordingly, the islet transplant group from Edmonton initiated a protocol where islets isolated from two or more donor pancreata 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.² 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 rapamycin. The efficacy of the Edmonton approach has now been confirmed by several other centers, including reports where single donor transplant recipients enjoyed a high rate of initial insulin-independence.⁷⁷ Unfortunately, loss of graft function occurs over time, and insulin-independence rates at Edmonton have declined from 72% at one year to 28% by three years⁷⁸. Similarly, insulin independence rates at Miami have declined from 79% at one year to 20% by three years.⁷⁹ Recent data demonstrate a functionally low engrafted β -cell mass in insulin-independent transplant recipients under Edmonton immunosuppression that likely declines over time,⁸⁰ suggesting that the eventual recurrence of diabetes and return to insulin therapy may result from both early (engraftment) and late (survival) immunologic graft loss. Novel strategies aimed at promoting the engraftment or survival of transplanted islets may lead to improved long-term graft function and more sustained insulin-independence for T1D patients.

1.4.2 Investigational Agent: LSF

Lisofylline (LSF)

1-(5-R-hydroxyhexyl)-3,7-dimethylxanthine, is a novel anti-inflammatory compound that also has a unique spectrum of activity to improve beta cell function and viability. LSF is a synthetic, modified methylxanthine, with a powerful

effect to reduce production of inflammatory cytokines, including $\text{TNF-}\alpha$, $\text{IFN-}\gamma$, IL-6 and IL-1 β , selectively suppressing neutrophil and leukocyte adhesion and phagocytic activity, and decreasing neutrophil migration and degranulation⁸⁸⁻⁹⁰. We hypothesize that LSF will reduce the apoptosis rate of islets and preserve beta cell function during the islet isolation steps; in the immediate post transplant period, it is possible that LSF could prevent the nearly 50% loss of islets by suppressing reactions in the hepatic microenvironment.

LSF was originally designed and tested as an agent to reduce mortality during serious infections associated to cancer chemotherapy. It is not currently a licensed product (see Section 3 for LSF dose details).

1) Rationale for the Proposed Strategy to Enhance Islet Survival during In-Vitro Culture

In this proposal, we provide a rationale for the proposed supplementations of our islet culture medium with LSF which has been shown to provide islet cyto-protection and prevent of islet apoptosis:

Lisofylline (LSF): LSF allows retention of beta-cell insulin secretory function after inflammatory cytokine insult and regulates immune cellular function to prevent autoimmune diabetes⁹⁴⁻⁹⁷. LSF also exhibits the ability to ameliorate hemorrhage-induced tissue injury^{98,99}, damage from endotoxic shock and to preserve tissue function during periods of decreased blood flow or under poorly ventilated conditions¹⁰⁰. LSF blocks interleukin-12 (IL-12) induced T helper (Th1) cell differentiation¹⁸, and also directly protects isolated beta cells from Th1 cytokine-induced dysfunction and apoptosis. LSF action also acts by maintaining normal mitochondrial function and this may be a potential mechanism for its ability to improve the insulin secretory function of isolated beta cells and islets during exposure to Th1 cytokines^{94,101}. This unique spectrum of activity suggests that agents such as LSF could have clinical utility by preventing beta cell damage after islet transplantation, and/or in the setting of recurrent autoimmunity^{96,102-104}. This hypothesis is supported by recently published studies that show LSF can significantly reduce both spontaneous T1D mellitus development in the NOD mouse and diabetes recurrence after islet transplantation in the NOD model¹⁰²⁻¹⁰⁴. Our hypothesis is that anti-inflammatory agents that have the dual effects of: 1) maintaining mitochondrial function and preventing Th1 cytokine damage in isolated beta cells and 2) blocking IL-12 action will be valuable agents to provide in vivo protection of islets from early non-specific inflammatory events, therefore enhancing engraftment and preventing Th1- mediated autoimmunity (auto and potentially, allo).

LSF inhibits phosphatidic acid formation to prevent oxidant-mediated capillary leak, thus reducing capillary barrier damage caused by oxidative stress¹⁰⁵. All of these conditions, including inflammatory cytokine release, low blood flow, and poor oxygen supply in tissue, are present during islet isolation. Therefore, LSF may be able to improve these unfavorable conditions and to preserve beta-cell function prior to and after islet transplantation. The cytoprotective effects result from the ability of LSF to stabilize epithelial cell barrier integrity to oxidative injury and prevent differentiation of inflammatory (Th1) lymphocytes, resulting in decreased pro-inflammatory and cellular injury responses.

We hypothesize that LSF will reduce the apoptosis rate of islets and preserve beta cell function during the islet isolation steps; in the immediate post transplant period, it is possible that LSF could prevent the nearly 50% loss of islets by suppressing reactions in the hepatic microenvironment.

2) Rationale for the Proposed Strategy to Enhance Post-Transplant Islet Engraftment

The unique spectrum of activity of LSF should prevent beta cell damage after islet transplantation. The postulated mechanisms are: reduction of inflammatory cytokine production (including $\text{TNF-}\alpha$, $\text{IFN-}\gamma$, IL-6 and IL-1 β), suppression of neutrophil and leukocyte adhesion and phagocytic activity, and a decrease in neutrophil migration and degranulation. In addition it will protect islets from Th1 cytokine-induced dysfunction and apoptosis. LSF should also prevent islet damage by autoimmunity.

The use of LSF should preserve β -cell function. In addition to its anti-apoptotic action, LSF enhances insulin secretion which should also be beneficial on the functioning of a marginal β -cell mass.

3) Rationale for the Proposed Strategy to Enhance Long Term Islet Survival and Function

Even with the Edmonton immunosuppressive regimen, surviving islet mass remains marginal in many recipients, despite the use of two or more donors. This may result in suboptimal glycemic control at times of stress or intercurrent infection, leaves no room for any islet loss, and may contribute to long term islet exhaustion. In addition, tacrolimus-induced β -cell toxicity has been demonstrated repeatedly in solid organ transplantation with ¹⁰⁶ even small maintenance doses ¹⁰⁷. In this proposal we hypothesize that LSF should preserve β -cell mass (both *in vitro* and *in vivo*) and result in enhanced islet engraftment, insulin secretory dynamics, and metabolic control.

1.4.3 Immunosuppressive Medications for Initial 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 allorecjection, avoidance of the use of 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 ^{113, 114}. 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 antibodies¹¹⁵. 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 ^{113, 115-118}. 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 death¹¹⁹.

The resistance of islet-directed autoimmune responses to conventional immunosuppressive drugs¹²⁰⁻¹²³ 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 cells¹¹⁷. 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^{124, 125}. Clinical evidence also indicates that destructive anti-islet autoimmunity persists for decades after manifestation of T1D^{121, 126, 127} 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 antilymphocyte serum (ALS) after development of overt autoimmune diabetes leads to long-lasting abrogation of autoimmunity¹²⁸. 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 group¹²⁹. 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 transplant¹³⁰. 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%)¹³¹. 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 well¹³². 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. Louis¹³³. 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 days¹¹³. 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 recipients¹³⁰. 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. 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 group². 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 cancers¹³⁴.

1.4.3.2 MAINTENANCE IMMUNOSUPPRESSION WITH SIROLIMUS AND LOW-DOSE TACROLIMUS

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 recipients¹³⁵. To maintain normoglycemia, immunosuppressed non-diabetic kidney transplant recipients must increase insulin secretion 2.5 times¹³⁶. 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 drugs¹³⁷. 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^{2, 136, 138, 139}.

The Edmonton group therefore developed a steroid-free maintenance immunosuppressive protocol based on the combination of sirolimus and low-dose tacrolimus². This strategy was designed to provide potent synergistic immunosuppression, thus avoiding diabetogenic impact on a limited beta cell transplant reserve¹³⁷⁻¹³⁹.

Sirolimus is a promising agent for maintenance immunosuppression of islet allograft recipients, mainly because of its efficacy in the absence of diabetogenic side effects¹⁴⁰. Sirolimus is as effective as cyclosporine A in preventing renal graft loss due to rejection while maintaining superior graft function¹⁴¹. 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 withdrawal¹⁴². 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 steroids²⁰. 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. The data from the Edmonton group suggest that sirolimus combined with low-dose tacrolimus without the addition of steroids may represent a safe and very effective maintenance immunosuppressive regimen².

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.

1.4.5 Immunosuppressive/Anti-inflammatory Therapy: Etanercept (Enbrel[®])

Etanercept is a dimeric fusion protein consisting of the extracellular ligand-binding portion of the human 75 kilodalton (p75) tumor necrosis factor receptor (TNFR) linked to the Fc portion of human IgG1. Etanercept inhibits binding of both tumor necrosis factor (TNF)- α and TNF- β to cell surface TNFRs, rendering TNF biologically inactive.

The basic premise is that peri-transplant administration of etanercept will interfere with the biological activity of TNF- α released early post-transplant as part of the activation of the innate immune response. Blockade of TNF- α in the early

post-transplant period is expected to lessen early islet loss and promote a milieu favoring the induction of immunologic tolerance. It is well recognized that TNF- α and TNF- β play multiple roles in the development and function of the immune system and have pleomorphic regulatory effects on the development and expression of autoimmunity¹⁴³. Blockade of TNF in the neonatal period results in a dramatic increase in the levels of CD4⁺CD25⁺ regulatory T cells in NOD mice^{143, 144}. Such an effect of etanercept on CD4⁺CD25⁺ regulatory T cells in islet transplant recipients could prove critical for protection of transplanted islets from alloimmunity and recurrent autoimmunity.

In a clinical trial at the University of Minnesota, insulin independence and normoglycemia were restored in 8 of 8 recipients of $7,271 \pm 1,035$ islet equivalents (IEQ)/kg from a single-donor pancreas¹⁴⁵. These subjects received ATG and etanercept for induction immunotherapy. The available information suggests that restoration of insulin independence with a lower islet mass prepared from a single-donor pancreas can in part be ascribed to the administration of etanercept in the peri-transplant period. The following sections therefore describe in more detail the experimental findings and clinical observations that form the basis for administering etanercept in the peri-transplant period.

Experimental findings: Increasing evidence suggests that blocking TNF- α in the early post-transplant period will diminish nonspecific islet beta cell loss, maximize engraftment and functional survival of transplanted islets, and thus increase the proportion of islet allograft recipients who become insulin independent following single-donor islet allotransplantation. We propose to administer the soluble receptor for TNF (sTNFR-Fc), etanercept (Enbrel®) in the early post-transplant period.

TNF- α is known to be cytotoxic to human islet beta cells¹⁴⁶. In murine models, selective inhibition of TNF- α in the peri-transplant period has promoted reversal of diabetes after marginal-mass islet isografts¹⁴⁷. Peri-transplant administration of etanercept has subsequently been studied in a mouse islet allograft model by Farney *et al.* (unpublished). Streptozotocin-diabetic C57BL/6 mice received 150 allogeneic B10.BR islets and either etanercept (100 μ g at -24hrs, 50 μ g at +24, +72, +120, and +168 hrs post-transplant) or saline. The proportion of euglycemic recipient animals was significantly higher in the etanercept group (4/7 versus 0/11). These findings demonstrate that specific TNF- α inhibition improves the functional outcome of a marginal mass islet allograft, again confirming that islets are sensitive to nonspecific inflammation in the peri-transplant period.

Clinical observations: Temporary etanercept administration has previously been studied in globally immunosuppressed kidney¹⁴⁸⁻¹⁵¹ and bone marrow transplant recipients^{152, 153}. In renal transplant recipients, etanercept was combined with depleting T cell antibodies (OKT3 or ATG). These studies demonstrated that etanercept is well tolerated and may limit the severity of the acute cytokine release syndrome associated with OKT3 and ATG administration. The most significant observation of one study¹⁴⁹ was a more rapid improvement in renal function in the etanercept-treated patients. Another study in renal transplant recipients found a higher incidence of infection in treated patients compared to controls in the 3 months after transplant. The etiology of this difference was unclear and the overall conclusion of this study was that etanercept is well tolerated by renal transplant patients receiving OKT3 induction therapy. Recent studies in bone marrow transplant recipients^{152, 153} provide preliminary evidence of the safety and efficacy of etanercept administration for the treatment of chronic graft-versus-host disease. In summary, in renal and bone marrow transplant recipients, SAEs related to the administration of etanercept were not communicated, suggesting that transient etanercept administration does not pose significant risks to globally immunosuppressed patients.

Nineteen islet transplant recipients have received etanercept in the peri-transplant period for the purpose of enhancing engraftment and functional survival of transplanted human islets at the University of Minnesota, University of California San Francisco, and the University of Miami. Etanercept was administered as follows: 50 mg IV at 1 hr prior to transplant, 25 mg SC on days +3, +7, and +10 post-transplant. The treatment schedule (an intravenous loading dose of 50 mg followed by three subcutaneous injections of 25 mg) is based on the results of a safety trial in healthy volunteers¹⁵⁴, a bioavailability study in healthy volunteers, and a toxicity and dose finding trial in refractory rheumatoid arthritis.¹⁵⁵ The time to maximum concentration after subcutaneous and intravenous etanercept administration were found to be 66 and

0.8 hrs, respectively.¹⁵⁶ An IV loading dose administered 1 hr prior to transplant is given to ensure therapeutic etanercept levels at the time of islet infusion. In 10 patients transplanted at the University of Minnesota, etanercept was combined with ATG, and in 2 patients at the University of California San Francisco, etanercept was combined with hOKT3γ1 (Ala-Ala) for induction immunotherapy. At the University of Miami etanercept was combined with daclizumab (n=4) or Campath® (n=3) induction immunotherapy. No AEs related to etanercept were encountered in these 19 patients.

The early post-transplant islet function of the last 2 Minnesota and the 2 UCSF patients is very promising. All four patients have received islets from 1 donor pancreas, one patient is insulin-independent and the other three have achieved markedly improved glycemic control on substantially reduced exogenous insulin doses. At the University of Miami, one of the three subjects who received Campath® and etanercept is off insulin after receiving islets from one donor. The follow-up on the first 8 Minnesota patients is more complete and will be discussed in more detail below. As described before, insulin independence was achieved in all 8 patients with islets prepared from one pancreas.

Compared with the hOKT3γ1 (Ala-Ala) trial¹⁴⁰ at the University of Minnesota, in which 4 of 6 single-donor islet recipients achieved and maintained insulin independence, the 8 single-donor islet allograft recipients given peri-transplant ATG plus etanercept trial had a significantly higher acute c-peptide response to arginine (ACR_{Arg}) on days ≥180 post-transplant: 1.07 ± 0.15 ng/mL (vs. 0.74 ± 0.21 ng/mL in hOKT3γ1 (Ala-Ala) trial¹⁴⁰; $p=0.028$). This improvement occurred despite transplantation of fewer islets: $7,271 \pm 1,035$ (vs. $10,302 \pm 2,594$ IE/kg in a previous trial¹⁴⁰; $p=0.01$). To facilitate comparison of the proportion of engrafted islets between studies, the ACR_{Arg} was corrected for implanted IE/kg, and expressed as the engraftment index. The engraftment index in the ATG plus etanercept islet transplant trial was $150 \pm 29 \times 10^{-6}$ ng·kg/mL, as compared with $73 \pm 23 \times 10^{-6}$ ng·kg/mL in the hOKT3γ1 (Ala-Ala) trial¹⁴⁰. Since pancreas procurement, preservation, islet processing, and culture protocols in the 2 studies were all identical, it is assumed that the islet potency was the same and therefore interpret the high efficacy of single-donor, marginal-dose islet transplants in the ATG plus etanercept trial as preliminary evidence of improved engraftment. Many of the effects of anti-thymoglobulin are shared with the anti-CD3 monoclonal antibody, hOKT3γ1 (Ala-Ala). Thus, they may not sufficiently explain the ability of the ATG plus etanercept protocol to facilitate diabetes reversal after single-donor, marginal-dose islet transplants. Therefore, the results are most likely related to the peri-transplant administration of etanercept.

1.5 Known and Potential Risks and Benefits to Human Subjects

1.5.1 Risks of Primary Investigational Agent: Allogeneic Islets

Transplantation of islets is associated with the 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 have been discussed separately in the section entitled "Known and Potential Risks of the Study Procedure."

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 cellular 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 islets¹⁵⁷. Additionally, the University of Minnesota investigators have previously reported on the incidence and significance of contaminated islet preparations in clinical islet auto- and allotransplantation¹⁵⁸. 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 tissues 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 centers¹⁵⁹⁻¹⁶¹. In the ITN-sponsored trial of islet transplantation using the Edmonton 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 immunosuppression¹⁶²⁻¹⁶⁴. 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 kidney¹⁶⁵.

1.5.1.4 ACCELERATION OF RETINOPATHY WITH ACUTE CORRECTION IN GLYCEMIC CONTROL

In the DCCT study⁴⁰, 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.¹⁶⁶ 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 Secondary Investigational Agent: LSF

1.5.2.1 LISOFYLLINE (LSF)

Lisofylline (LSF): In healthy volunteer studies, subjects have received LSF at doses ranging from 1 to 8 mg/kg/dose. LSF was well tolerated in these studies; most AEs have been mild in severity and resolved promptly without intervention (see Diakine's Investigator Brochure for LSF for details). Two subjects experienced severe AEs, both following a 3mg/kg, 10 minute infusion. Both study subjects experienced severe vasovagal reaction with hypotension. It should be noted that there have been no SAEs reported in studies of healthy normal volunteers. The following table summarizes the AEs by four or more subjects across the four trials in healthy subjects, grouped by dose received.

Table 1. Summary of Adverse Events in Trials of Healthy Subjects with LSF alone

LSF Dose mg/kg	Duration of infusion (min)	Number of Subjects Dosed	N (%) with any AE	N with Nausea	N with Dizziness	N with Headache	N with Dyspepsia	N with Abd. Pain	N with Emesis
1	10	12	3(25)	1	0	0	0	0	0
2	10	12	2(17)	0	1	0	1	0	0
3	10	22	17(76)	7	5	6	1	1	0
4	10	4	2(50)	0	1	0	0	0	0
5	10	4	3(75)	1	1	0	0	0	0
6	20	8	4(50)	2	2	1	0	1	1
6	30	4	2(50)		0	1	0	0	0
7	30	4	1(25)	1	0	0	0	0	0
7	60	4	2(50)	1	1	0	0	0	0
8	30	4	4(100)	3	2	1	0	0	0
8	60	4	4(100)	2	3	1	0	0	1
Overall	---	47	32 (68%)	17 (36%)	13 (28%)	10 (21%)	2 (4%)	2 (4%)	2 (4%)

The most common AEs associated with LSF have been nausea, nausea and vomiting, and dizziness. These effects have occurred in both healthy subjects and patients and do not appear to occur with greater frequency in any specific study population which included patients undergoing bone marrow transplant and a diagnosis of acute myeloid leukemia. In these patient studies, LSF was administered at repeated dosing concomitantly with cancer chemotherapeutic agents or radiotherapy.

Similar percentages of patients receiving LSF 3mg/kg or placebo experienced serious AEs. The majority of AEs reported were for events that are not unexpected following the chemo and radiation therapy administered. No hypoglycemia has been reported in any of the LSF trials and LSF has been administered safely in patients receiving cyclosporine A, systemic corticosteroids, acyclovir, ganciclovir, fluconazole, trimethoprim sulfamethoxazole and high dose IL-2 in other chemotherapeutic agents.

1.5.3 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.

1.5.3.1 MONOCLONAL ANTIBODY IL-2 RECEPTOR BLOCKER

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

1.5.3.1.1 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 (see product monograph for details). 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.

1.5.3.1.2 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 studies, 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.

1.5.3.2 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%), 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®⁸¹. 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 hypoglycemia¹⁴⁵. 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 required¹⁴⁵.

1.5.3.3 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 exposure¹⁶⁷. Sirolimus alters the pharmacokinetic profiles of other CNIs (*e.g.* tacrolimus) and may thereby potentiate nephrotoxicity¹⁶⁸. Fervenza *et al* described nephrotoxicity from sirolimus in patients with chronic glomerulopathies that was non-reversible on cessation of therapy¹⁶⁹. 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^{10, 170}.

The majority of islet transplant recipients receiving sirolimus in conjunction with tacrolimus have experienced transient mouth ulceration, lower extremity edema^{2, 170}; perinephric edema and a high incidence of benign ovarian cysts have also been described in islet recipients in association with sirolimus¹¹. Pneumonitis and colitis have also occurred^{5, 171}. The most common (>30%) adverse reactions are: peripheral edema, hypertriglyceridemia, hypertension, hypercholesterolemia, creatinine increased, 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.

1.5.3.4 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 (PLM), 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%], 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.

1.5.3.5 CYCLOSPORINE (NEORAL®)

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

1.5.3.6 MYCOPHENOLATE MOFETIL (CELLCEPT®) AND MYCOPHENALATE 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 lymphoproliferative disease, 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, and pure red cell aplasia have been reported in patients treated with CellCept® or Myfortic®.

1.5.4 Risks of Immunosuppressive/Anti-inflammatory Therapy: Etanercept (ENBREL®)

Etanercept is a dimeric soluble form of the p75 TNFR that blocks TNF binding and reduces inflammation¹⁴⁸⁻¹⁵². It is FDA-approved for use in severe rheumatoid arthritis, juvenile arthritis, ankylosing spondylitis, and psoriatic arthritis. In controlled trials, approximately 37% of patients treated with Enbre® developed injection site reactions (see package insert). All injection site reactions were described as mild to moderate (erythema and or itching, pain or swelling) and generally did not necessitate drug discontinuation. In placebo controlled trials there was no increase in the incidence of serious infections. The observed rates and incidence of malignancies were similar to those expected for the population studied. However, the incidence of TB has been shown to be statistically higher in anti-TNF-alpha-treated patients¹⁷²⁻¹⁷⁴, and based on post-marketing studies warnings have been issued about the following conditions, which have been reported with the use of Enbre®: serious infections of sepsis, including fatalities; an increased risk of lymphoma and other malignancies in children and adolescents; and leukemia which have been reported with the use of Enbre®. Many of the serious infections occurred in patients on concomitant immunosuppressive therapy.

Experience with anti-TNF alpha therapies in clinical and experimental islet transplantation has been limited. Farney *et al.* described a beneficial role of etanercept in promoting engraftment of marginal mass islet grafts in mice¹⁴⁷. Hering *et al.* used etanercept in a recent trial of 8 type 1 diabetic patients receiving single donor islet transplant, and all 8 achieved insulin independence suggesting a beneficial role for anti-TNF therapy in clinical islet transplantation¹⁴⁵.

1.5.5 Risks of the 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.

1.5.5.1 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.

1.5.5.2 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, local vein thrombosis, infection or thrombophlebitis.

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

1.5.5.3 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, pleural effusion, or damage to the gall bladder. 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.

The Department of Radiology at the University of Miami Hospitals and Clinics has done over 600 percutaneous portal catheterizations. Complications (bleeding, portal vein thrombosis) have only been encountered in patients with underlying portal hypertension. In 76 islet transplantation procedures we have experienced 3 hemorrhagic complications that required blood transfusion in one. No thrombosis has been observed. Pleural effusions without clinical consequences have occurred in three cases and have resolved without treatment. We anticipate, therefore, that our transplantation procedure will be relatively benign and that it could be accomplished with minimal discomfort to the patient. Conscious sedation during the transplant procedure involves the risks of respiratory depression or arrest. Subjects will be monitored closely by the radiology nurse and the DRI nurse practitioner and treated as necessary. A possible risk associated with the islet transplant procedure is the elevation of liver enzymes which can be observed in the days following the procedure. This is usually a transient raise in enzymes, which does not

necessitate any therapeutic intervention, and liver enzyme levels return to the normal range within 2 weeks. Subjects' liver enzyme levels (ALT, AST, ALP and GGT) will be monitored 48 hours post-transplant and then weekly for the first month.

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), intra-abdominal bleed (n=2), low hemoglobin (n=1), right hemothorax (n=1), and subcapsular hematoma of the liver¹⁷⁵. 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 necessary¹⁷⁶. 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 Registry¹⁷⁶. Reports on intra-abdominal (n=1)⁵ and intrathoracic bleeding (n=1)¹⁷⁷ 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 series¹⁰. Subsequently, a further increase in risk of bleeding has been observed by the Edmonton program and has been attributed in part to concomitant aspirin therapy¹⁷⁰. The risk has since been ameliorated by avoidance of pretransplant aspirin and more effective measures to seal the catheter tract in the liver¹⁷⁰. 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 bleeding¹⁷⁸. At the University of Minnesota, no bleed-related complications occurred in 20 consecutive subjects when the catheter tract was sealed with combined coils and gelfoam¹⁴⁵.

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 CITR¹⁷⁵. 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 the 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 surgery¹⁷⁹.

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 procedure¹⁸⁰⁻¹⁸². 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^{183, 184}. Three of the 86 islet transplant recipients reported to CITR have experienced transient elevations of liver enzymes requiring prolongation of post-transplant hospitalization or admission¹⁷⁵. 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 usually resolved within 4 weeks¹⁸³. No correlation between the increase in liver function tests (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^{77, 185} and appears to be due to a paracrine action of insulin secreted from intrahepatic islets. More subjects with steatosis required supplementary exogenous insulin than not¹⁸⁵, 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 case¹⁷⁶. 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 later¹⁸⁶. 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 (18mmHg) than first or second transplants (12 mmHg)¹⁸⁷. The baseline pressures were normal in all cases, suggesting absence of chronic portal hypertension¹⁸⁷. At the University of Miami, we have not observed an increase in portal pressures with subsequent transplants. Higher tissue volumes have caused a greater rise in portal venous pressure, evident at volumes over 3 cc ($r^2 = 0.63$, $p < 0.001$).¹⁷⁸

Portal Vein Thrombosis

Transplanted islets release tissue factor and exhibit prothrombotic properties when infused to an intravascular site such as the portal vein¹⁸⁸. A partial portal vein thrombosis has been reported in one of six patients transplanted at the intramural National Institutes of Health (NIH) program⁵. In the Edmonton single-center experience, the risk of partial vein thrombosis was 3% in more than 100 intraportal islet transplants¹⁷⁰. The management of partial vein thrombosis includes anticoagulation therapy which may lead to intra-abdominal hemorrhage requiring transfusion and surgical intervention¹⁸⁹. There is one published report of complete thrombosis of the portal vein after transplantation of partially purified pancreatic islets in a combined islet/liver allograft, which necessitated emergency re-transplantation of the liver¹⁹⁰. 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. At the University of Miami, we have not had any case of portal vein thrombosis⁷⁹.

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 Registry¹⁷⁶. 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 CITR¹⁷⁵. Gall bladder hematoma (n=1) and gall bladder opacification (n=2) have been observed as well.

1.5.5.4 FOLLOW-UP PROCEDURES:

Glomerular Filtration Rate (GFR)

Risks associated with this GFR 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 ¹⁹¹.

1.5.6 Benefits

Our potential recipients are informed that the best treatment for insulin-dependent diabetes is a prescribed diet, carefully balanced with insulin administration and exercise, and associated with frequent self-monitoring of BG. A successful islet transplant will not necessarily change all of these requirements and, in fact, is unlikely to do so within the context of the requirements of the study.

The DCCT ⁴⁸ revealed that optimal glycemic control could delay the onset, and slow the progression of, long term microvascular and neurologic complications in patients with T1D Mellitus. The near normalization of glycemic control seen in islet allograft recipients that are insulin independent has similar potential to prevent the onset and progression of the clinical complications of diabetes.

1.5.6.1 BENEFITS OF ALLOGENEIC ISLET TRANSPLANTATION

Successful islet transplantation alleviates T1D patients from life-threatening hypoglycemia and psychosocially debilitating glycemic lability.⁷³ 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 years⁷⁸. Furthermore, as long as graft function is maintained, fear of hypoglycemia and anxiety are significantly lower after islet transplantation.¹⁹² Indeed, T1D subjects in the DCCT who had persistent c-peptide production had a significantly reduced risk of severe hypoglycemia despite intensive insulin therapy.¹⁹³ 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.

1.5.6.2 BENEFITS OF STUDY REGIMEN

The expected benefits from **LSF** are that it will protect islets from the pro-inflammatory cytokines IL-1 β , interferon γ and TNF α , and from oxygen radicals. In addition, LSF will reduce inflammatory cytokine production, decrease neutrophil and macrophage migration and reduce free fatty acid levels. LSF can also improve glucose induced insulin secretion. The overall effect will be to reduce islet apoptosis, increase β -cell resistance to inflammatory cytokines and preserve islet function. All of these effects will improve hopefully islet graft engraftment and survival, which will translate in an increase percentage of insulin independent patients, for a longer duration of time.

2 OBJECTIVES

2.1 Primary Objective

The primary objective is to determine the proportion of subjects who are insulin independent at 75 ± 5 days post-transplant after one islet transplant among subjects treated with LSF in addition to the standard islet transplant regimen used in the CIT-07 protocol.

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

Objectives of this study include gaining experience with the experimental treatment regimen as well as to determine the proportion of subjects with insulin independence. There is a larger study with additional centers and more subjects (CIT-07) where subjects are treated with the standard islet transplant regimen. When a pancreas becomes available subjects will be randomized 2:1 into CIT-07 and CIT-02 respectively. .

Our Central Hypothesis is that treating islets pre-transplant, and recipients (before, during and post-transplant), with selected agents with a unique spectrum of anti-inflammatory, anti-apoptotic and beta cell enhancing properties, will be critically important to improve beta cell function and viability as well as islet engraftment and long-term function, therefore we expect that the treatment with Lisofylline will increase the proportion of subjects who are insulin independent (see definition on Section 3.1.1) after one infusion at 75 ± 5 days post-transplant

2.2 Secondary Objective(s)

Our secondary objectives are to:

1. Increase the proportion of patients rendered insulin independent with a single (1- donor) islet transplant at 1 year by preservation of β -cell mass and enhancement of β -cell function.
2. Enhance the magnitude of glucose and non-glucose stimulated insulin responses of islet allografts.
3. Achieve long-term insulin independence by preventing the decline in β -cell function.

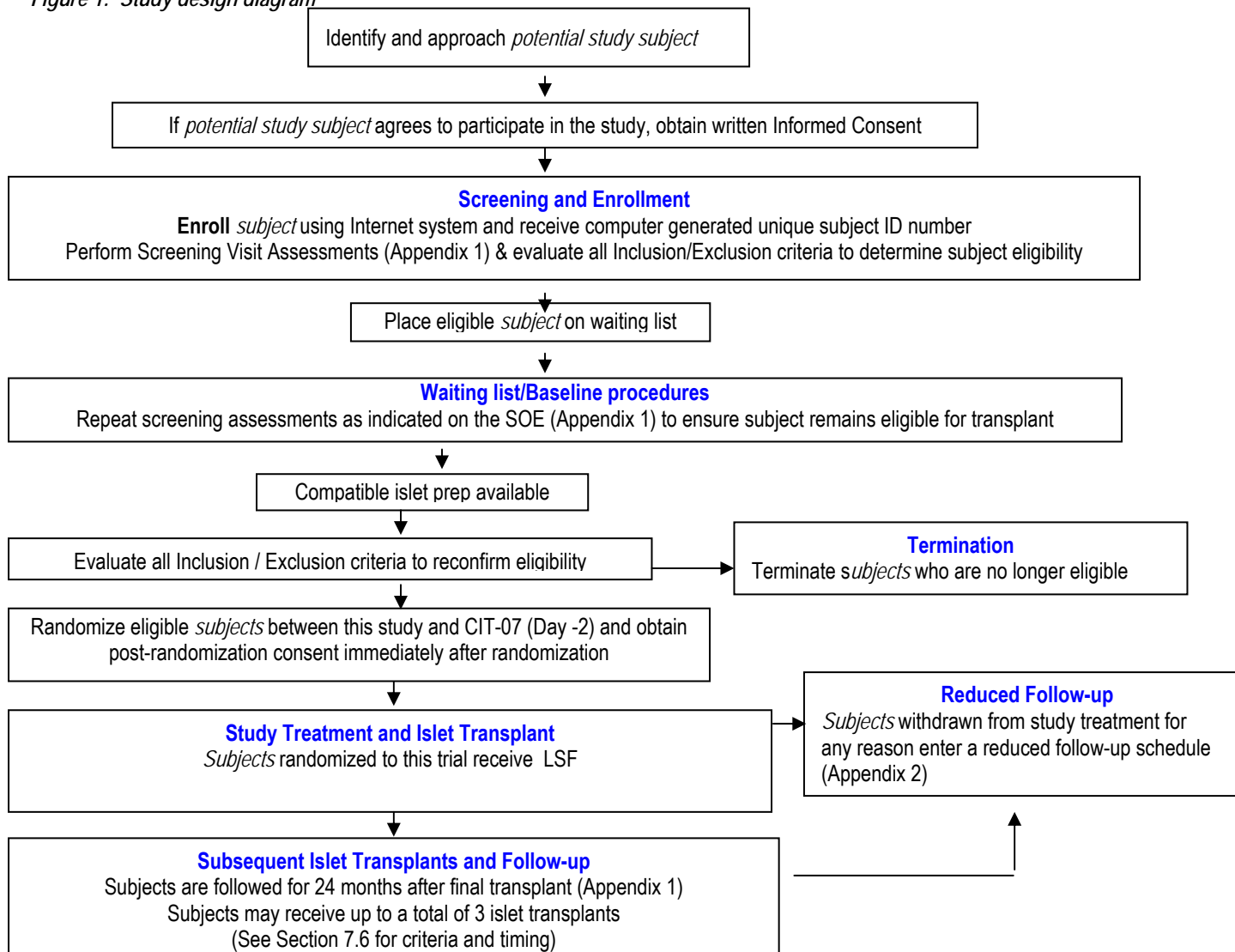
3 STUDY DESIGN

CIT-07 and CIT-02

This trial is an open label, randomized prospective, single-arm, phase 2, multi-centered trial. Some of the centers participating in this phase 2 study (CIT-02) will also undertake a separate, phase 3 study (CIT-07) in islet transplantation, using a standard manufacturing and immunosuppressive regimen. The phase 3 trial (CIT-07) will have inclusion/exclusion criteria and endpoint measures that are identical to those in this phase 2 trial (CIT-02). In order to avoid bias in selection of subjects at centers participating in both studies, eligible subjects will be randomized, prior to transplantation, to participate either in the CIT-02 or the CIT-07 study, with probabilities 1/3 and 2/3 respectively.

Subjects who meet the general inclusion/exclusion criteria will be approached regarding their participation. Subjects who sign the informed consent will be enrolled and assigned a unique subject identification number. Subjects will then be formally evaluated for eligibility through the performance of screening visit procedures. More than one visit may be necessary to complete all of the screening procedures. Subjects [N=24 (CIT-07) and N=12 (CIT-02)] will be recruited at the UM and UIC.

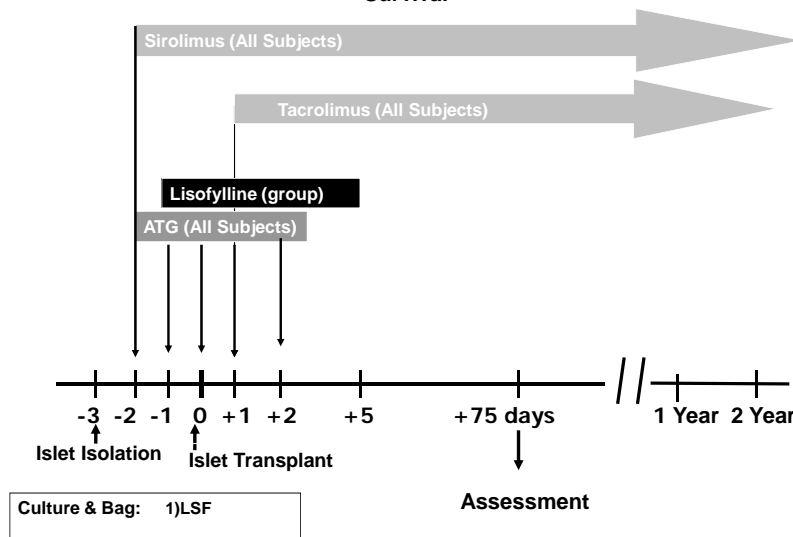
Figure 1: Study design diagram



CIT-02

In this proposal we will be examining LSF (and its effect on engraftment and β -cell function). The randomization will be performed centrally and stratified by center (UM and UIC). The central hypothesis of this proposal is that treatment of islets *in vitro* and *in vivo* with LSF will result in better islet engraftment and therefore higher rates of insulin independence and better metabolic outcome.

Figure 2. Study Flow Chart: Strategies To Improve Long Term Islet Graft Survival

**The LSF group design is as follows:**

Islets cultured *in vitro* for 36 - 72 hours in transplant media with LSF (100 $\mu\text{mol/L}$).

LSF in the infusion bag (same concentration as in culture medium).

Treatment of the recipient with LSF (6 mg/kg intravenously over 30 minutes four times daily with a reduction to 3 mg/kg if patient experiences nausea or other side effects) will start on Day -1 (patients should receive a minimum of 3 doses before transplant) and will continue until a total of 28 doses have been administered (typically, Day 5).

The expected outcomes of this trial are as follows:

The LSF Group will evaluate the effect of LSF treatment on islets *in vitro* and *in vivo* for 1 week. The hypothesis for this group is that the novel anti-inflammatory agent LSF will protect islets from the pro-inflammatory cytokines IL-1 β , IFN- γ and TNF α , and from oxygen radicals. In addition, LSF will reduce inflammatory cytokine production, decrease neutrophil and macrophage migration and reduce free fatty acid levels. LSF can also improve glucose induced insulin secretion. The overall effect will be to reduce islet apoptosis, increase β -cell resistance to inflammatory cytokines and preserve islet function, thereby increasing the rate of insulin independence.

3.1 Study Endpoints

3.1.1 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:

- One HbA1c level, one fasting serum glucose level, and a Mixed Meal Tolerance Test are documented within the visit window (e.g. 70-80 days at Day 75) and 7 consecutive days of blood sugar and insulin readings are documented within ± 7 days of the visit window (e.g. 63 – 87 days at Day 75);
- HbA1c $\leq 7.0\%$ or a $\geq 2.5\%$ decrease from baseline;
- Fasting capillary glucose level should not exceed 140 mg/dL (7.8 mmol/L) more than three times in the 7 consecutive days (fasting is defined as 1st blood sugar reading of the day not noted as post-prandial or bedtime);
- Post-prandial serum glucose ≤ 180 mg/dL (10.0 mmol/L) at 90 minutes during the MMTT;
- 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;
- At least one MMTT fasting or stimulated c-peptide levels ≥ 0.5 ng/mL (0.16 nmol/L).

3.1.2 Secondary Endpoints

The key secondary endpoints is the proportion of subjects with HbA1c $< 7.0\%$ AND free of severe hypoglycemic events from Day 28 to Day 365, inclusive, after the first islet transplant.

The other secondary endpoint is the proportion of subjects with an HbA1c $< 7.0\%$ AND free of severe hypoglycemic events from Day 28 to Day 365, inclusive, after the final islet transplant.

Efficacy Endpoints

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

- The percent reduction in insulin requirements
- HbA1c
- MAGE⁶⁹
- LI⁷³
- Ryan hypoglycemia severity (HYPO) score⁷³
- Basal (fasting) and 90-min glucose and c-peptide derived from the mixed-meal tolerance test (MMTT)
- β -score¹⁹⁴
- C-peptide: (glucose \cdot X creatinine) ratio

- Acute insulin response to glucose ($AI R_{glu}$), insulin sensitivity, and disposition index derived from the insulin-modified frequently-sampled intravenous glucose tolerance (FSIGT) test^{94, 200}
- Glucose variability⁷¹ and hypoglycemia duration¹⁹⁵ derived from the continuous glucose monitoring system® (CGMS)
- Quality of life (QOL) measures (DQOL, HSQ 2.0, Hypoglycemia Fear Survey-HFS)

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 and final islet transplant:

- 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
- $AI R_{glu}$ insulin sensitivity, and disposition index derived from the insulin-modified frequently-sampled intravenous glucose tolerance (FSIGT) test^{94, 200}
- Glucose variability⁷¹ and hypoglycemia duration¹⁹⁵ derived from the continuous glucose monitoring system® (CGMS)
- QOL
- The proportion of subjects receiving a second islet transplant
- The proportion of subjects receiving a third islet transplant
- Rate of favorable outcome at each center preparing islets (rate of subjects with an HbA1c < 7.0% and free of severe hypoglycemic events).

Secondary efficacy endpoints measured at 365 ± 14 days following the final islet transplant will include the change in the above measures from the results obtained at 75 ± 5 days following the final islet transplant:

At two years (730 ± 14 days) following the final islet transplant:

- The percent change from baseline insulin requirements.
- The number of severe hypoglycemic events.
- HbA1c
- Clarke Score
- Basal (fasting) and 90-min glucose and c-peptide (MMTT).
- B-score
- C-peptide: (glucose • creatinine) ratio
- CGMS
- QOL

Secondary Safety Endpoints

- Safety, including incidence of post-transplant infections, malignancies, morbidity, and other AEs (e.g., increased body weight and hypertension) associated with conventional immunosuppression.
- Renal function as measured by serum creatinine, GFR and other relevant laboratory parameters.
- Lipid profiles (triglycerides, total cholesterol, LDL cholesterol, HDL cholesterol) over time.

At 75 ± 5 days following each transplant and at 365 ± 14 days following the first and final islet transplant, and at two years following the 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 and protein 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
- The incidence of discontinuation of immunosuppression

At 365 ± 14 days following the first islet transplant:

- The incidence of worsening retinopathy as assessed by change in retinal photography. If pupil dilation is not possible, then a manual ophthalmologic evaluation can be substituted.

3.1.3 Additional Exploratory Analyses

Additional endpoints will be determined for exploratory data analyses to generate metabolic and mechanistic hypotheses for further investigation. Each of the following tests allows for the quantification of several metabolic parameters explained in the mechanistic assays section.

Identification of clinical strategies that limit early islet loss and enhance long-term islet survival and function are important to this protocol. In order to clearly ascertain the effect of LSF, on transplant outcome (CIT-02), it is essential to address issues related to metabolic function and immunological alterations (auto/allo reactivity). Is metabolic function enhanced/maintained and do immune alterations occur that are different than those observed in controls (thymoglobulin, etanercept, sirolimus, tacrolimus; CIT-07) that do not receive LSF and that can explain improved islet survival? Based on our historical studies, we have identified metabolic tests and immunologic assessments that can provide information. For this reason, we are undertaking additional priority 2 testing in CIT-02 that will be most informative if the data can be compared to that obtained from control subjects in CIT-07.

For metabolic purposes we will perform a 5 hour (300 min) MMTT, that will allow us to determine informative measurements, such as the peak c-peptide, glucose, glucagon, amylin and proinsulin, the time for c-peptide, glucose, glucagon, amylin and proinsulin to peak, and the Mixed Meal Stimulation Index (MMSI) ($MMSI = AUC \text{ c-peptide} / AUC \text{ glucose}$). These variables will help assess if metabolic function more closely approximates normal in patients treated with LSF as compared to patients that receive control immune suppression alone and if this function can be maintained over time.

In addition, the CGMS will be performed quarterly, as we have observed that early perturbations (6 and 9 months) in glucose variability and percentage hyperglycemia time (%HGT, defined as the percentage of time BG levels are above 140 mg/dL) detected with CGMS can serve as an early indicator of graft dysfunction. Therefore, we will also monitor %HGT at all the timepoints of CGMS monitoring.

For immunological purposes, both cellular and molecular assays that are indicative of immune activation will be performed (Priority 2 Mechanistic Assays), including: bioplex assessment of soluble mediators in the early post-transplant period, RT-PCR based analysis of granzyme B gene expression, microarray, cell surface and intracellular flow cytometric analyses, T and B cell assays for allo and autoantigen specific reactivity.

4 SELECTION AND WITHDRAWAL 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, insulin-dependence for > 5 years at the time of enrollment, and a sum of subject age and insulin-dependent diabetes duration of ≥ 28 .
5. Absent stimulated c-peptide (<0.3ng/mL) in response to a 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 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 BG despite optimal diabetes therapy and defined by an LI score greater than or equal to the 90th percentile ($433 \text{ mmol/L}^2/\text{h} \cdot \text{wk}^{-1}$) 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 a 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) $>30 \text{ kg/m}^2$ or patient weight $\leq 50 \text{ kg}$.
2. Insulin requirement of $>1.0 \text{ IU/kg/day}$ or $<15 \text{ U/day}$.
3. HbA1c $>10\%$.
4. Untreated proliferative diabetic retinopathy.
5. Blood Pressure: SBP $>160 \text{ mmHg}$ or DBP $>100 \text{ mmHg}$.

6. Measured glomerular filtration rate (using iohexol of) $<80 \text{ mL/min/1.73 m}^2$ (or for subjects with an iodine allergy, calculated using the subject's measured serum creatinine and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation¹). Modification of Diet in Renal Disease [MDRD] study estimation formula). Strict vegetarians (vegans) with a calculated GFR $<70 \text{ mL/min/1.73m}^2$ are excluded. The absolute (raw) GFR value will be used for subjects with body surface areas $>1.73\text{m}^2$
7. Presence or history of macroalbuminuria ($>300\text{mg/g}$ 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. Presence or history of active infection including hepatitis B, hepatitis C, HIV, or tuberculosis (TB). Subjects with laboratory evidence of active infection are excluded even in the absence of clinical evidence of active infection.
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 ($<1,000/\mu\text{L}$), neutropenia ($<1,500/\mu\text{L}$), or thrombocytopenia (platelets $<100,000/\mu\text{L}$). Participants with lymphopenia are allowed if the investigator determines there is no additional risk and obtains clearance from a hematologist.
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 $>130 \text{ mg/dL}$, treated or untreated; and/or fasting triglycerides $>200 \text{ mg/dL}$).

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 medications 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 the 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.

5 STUDY TREATMENT REGIMEN

5.1 Investigational Agents:

- Allogeneic Islets
- Lisofylline (DiaKine Therapeutics Incorporated)

Please refer to section 1. 5, and to applicable product labeling for known and potential risks to human subjects associated with the study medication.

5.1.1 Investigational Agent: Allogeneic Islets

5.1.1.1 FORMULATION, DOSAGE, AND ADMINISTRATION

The final product is a 200 mL sterile suspension of $\geq 70\%$ viable, $\geq 30\%$ pure, allogeneic human purified CMRL 1066 Transplant Media for administration by intraportal infusion. The final product is supplied in up to three 200 mL Ricordi® bags, containing a dose of $\geq 5,000$ IEQ/kg recipient body weight (BW) for the first transplant, and $\geq 4,000$ IE/kg recipient BW for subsequent transplants.

5.1.1.2 FINAL PRODUCT FORMULATION:

Table 2: Composition of Final Drug Product Containing Lisofylline
[Product Code: PHPI-L-01]

Component	Quantity per Batch
Purified Human Pancreatic Islets	$\geq 4.0 \times 10^3$ IEQ ¹ /kg recipient BW ²
Albumin Human, USP	2.5%
Lisofylline	100 μ M
CMRL 1066 Transplant Media, contains HEPES and without Sodium Bicarbonate	q.s to 200 mL per bag ³

¹ IEQ = Islet Equivalents

² BW = Body Weight

³ 600 mL maximum total volume in up to three Ricordi® infusion bags

Administration: The islet mixture is delivered slowly via gravity drainage from a bag attached to the catheter in the portal vein or portal 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 the 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 guidelines and subject safety.

5.1.2 Other Investigational Agent: LSF

5.1.2.1 FORMULATION, PACKAGING, AND LABELING

LSF drug product (Lisofylline Injection, 60 mg/mL) is provided in 5 mL sterile glass ampules (pharmacy bulk package) containing an aqueous hypotonic (140 mOsm/L) solution that is to be mixed with saline before administration. The drug product contains 60 mg/mL lisofylline, with 10 mM citric acid as a buffer. Ampules of LSF drug product must be stored at controlled room temperature (20 to 25°C). Clinical supplies have a shelf life of at least 24 months under these conditions.

5.1.2.2 PREPARATION, ADMINISTRATION, AND DOSAGE

Lisofylline Injection, 60 mg/mL, is supplied as a pharmacy bulk package. Before administration, the drug product is to be diluted with 0.9% sodium chloride for intravenous infusion, using sterile technique. The necessary volume of drug product should be drawn into a sterile syringe through a sterile needle, and then injected through a syringe filter into a polyvinyl IV bag containing a volume of 0.9% saline solution appropriate to the planned intravenous infusion regimen. The diluted LSF solution should be administered using sterile technique, via a central or peripheral intravenous access line, over 30 minutes. Care to avoid extravasation of LSF should be taken. Solutions of LSF should not be co-administered or mixed with any solution other than 0.9% sodium chloride. Intravenous tubing should be flushed with 5 mL sterile 0.9% sodium chloride before and after administration of LSF. No preservative is included in the formulation; therefore, the diluted drug should be administered within 48 to 72 hours after preparation. The drug product is intended only as a single use ampule. Solutions that turn cloudy must be discarded.

Lisofylline will be given IV at a dose of 6 mg/kg over 30 minutes, four times daily from day -1 (subject should receive a minimum of 3 doses before transplant) until a total of 28 doses have been administered (typically, day 5). The dose should be reduced to 3 mg/kg if the patient experiences severe or intolerable nausea, other severe or intolerable side effects, or if in the opinion of the investigator it is necessary for the safety or well being of the subject.

5.1.3 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. Unused drugs will be destroyed.

5.2 Immunosuppression Medications

The immunosuppressive agents used in this study are being administered for an indication other than the one for which they are currently approved and for use in combinations, which have not been formally studied. These medications have only been used for a short period of time and in a small number of subjects. In the islet transplant setting, they have been used in several Islet Alone and Islet After Kidney Transplant Trials. A number of known side effects can be experienced when taking these drugs.

5.2.1 Immunosuppression Regimen

Immunosuppression will consist of a five day, 12 hour/day continuous IV infusion of rabbit Anti-thymocyte Globulin (ATG, Thymoglobulin®), starting two days prior to the islet transplant. Tacrolimus will be commenced on post-operative day 1 (POD +1), dose adjusted to attain trough levels of 3-6ng/mL, and sirolimus commenced on POD -2, dose adjusted to trough levels of 10-15 ng/mL for the first three months post infusion then 8-12 ng/mL thereafter. Etanercept will be given IV before the islet transplant (50 mg), and then at 25 mg (subcutaneously) on POD +3, +7 and +10. In case that subsequent islet transplants (three months post 1st transplant) are required, daclizumab (2 mg/kg IV) will be given the day of transplant followed by a four dose course of daclizumab (1 mg/kg) at two week intervals. Mycophenolate mofetil (MMF) will replace sirolimus or tacrolimus in case of suspected drug specific toxicity that warrants the discontinuation of that drug. MMF will replace tacrolimus in case of tacrolimus induced nephrotoxicity (please refer to section 5.7.2.5 for details).

5.2.2 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.

5.2.2.1 RABBIT ANTI-THYMOCYTE GLOBULIN (ATG, THYMOGLOBULIN®)

A total of 6 mg/kg will be given as an IV infusion 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 doses will be administered as directed on the package insert and the Manual of Procedures.

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 as needed during 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.

5.2.2.2 SIROLIMUS (RAPAMUNE®)

Sirolimus will be administered at an initial dose of 0.05 – 0.2 mg/kg PO on day -2 relative to islet transplant, followed by 0.05 - 0.1 mg/kg QD. The daily dose will be adjusted to the whole blood 24-hr trough to target, as tolerated, 10-15 ng/mL for the first 3 months and 8-12 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.

5.2.2.3 TACROLIMUS (PROGRAF®)

Tacrolimus will be administered at an initial dose 0.015 mg/kg PO BID on day +1, whole blood 12-hr trough adjusted to 3-6 ng/mL. For subjects who have converted to MMF, tacrolimus will be administered to target whole blood trough levels of 10-12 ng/ml for the first 3 months post-transplant, 8-10 ng/ml from 3-6 months post-transplant, and 6-8 ng/ml thereafter.

Should subjects experience a decrease in their GFR of $\geq 33\%$ 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.

5.2.2.4 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.

5.2.2.5 MYCOPHENOLATE MOFETIL (CELLCEPT®)

Mycophenolate mofetil may be used at a dose of 500 to 1500 mg PO BID as a replacement for tacrolimus or sirolimus. Subjects must practice two methods of contraception while taking MMF. If a subject experiences severe neutropenia (absolute neutrophil count $<1 \times 10^9/L$) 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.

5.2.2.6 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®.

5.2.3 Subsequent Allogeneic Islet Transplants and Second Attempts at Initial Transplants

The immunosuppressive regimen for subsequent islet transplants and second attempts at initial transplants that were cancelled due to intolerance to thymoglobulin (see section 5.7.2) will be identical to the regimen for the initial islet transplant with the following exceptions:

5.2.3.1 MONOCLONAL ANTIBODY IL-2 RECEPTOR BLOCKER

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

5.2.3.1.1 DACLIZUMAB (ZENAPAX®)

Daclizumab (Zenapax®), will be used instead of Thymoglobulin® for all subsequent islet transplants. 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 IV doses of daclizumab will be given with all subsequent islet transplants and second attempts at initial 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 7.6 for indications for subsequent transplants), all five doses of daclizumab will be repeated.

5.2.3.1.2 BASILIXIMAB (SIMULECT®)

Two IV doses of basiliximab will be given with all subsequent islet transplants and second attempts at initial 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 7.6 for indications for subsequent transplants), both doses of basiliximab will be repeated.

5.2.3.2 TACROLIMUS (PROGRAF®) AND SIROLIMUS (RAPAMUNE®)

Tacrolimus and sirolimus will be administered for subsequent transplants as described for the initial transplant.

5.3 Concomitant Medications

Concomitant medications may be administered as per standard clinical practice. A detailed list will be kept of all medications subjects took from the time of admission for transplantation. However, a number of drug interactions with the three immunosuppressive agents are known to exist. Concomitant administration of any drug known to interact with the study drugs should be carefully considered and monitored as appropriate. Please refer to package inserts and investigational brochures of these drugs and the Manual of Procedures. It is suggested that the participant refrain from using any herbal remedies.

5.3.1 Immunosuppressive / Anti-Inflammatory Therapy

Etanercept (Enbrel®) will be administered at a dose of 50 mg IV on day 0 (1 hr prior to transplant), and 25 mg SC on days +3, +7, and +10 post-transplant.

5.3.2 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.

5.3.2.1 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.

5.3.2.2 CLOTRIMAZOLE (MYCELEX TROCHE®)

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

5.3.2.3 VALGANCICLOVIR (VALCYTE®)

Valganciclovir will be administered starting on day -2 for initial transplants, Day -1 for subsequent transplants, at a dose of 450 mg PO QD, increasing to 900 mg QD by day 12 and continuing for 14 weeks post-transplantation. If the CMV status of the donor and recipient are both negative, then valganciclovir administration can be adjusted or eliminated.

5.3.3 Anticoagulation Prophylaxis / Hematological Agents

5.3.3.1 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 3U/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.

5.3.3.2 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). The dose can be modified or extended at the discretion of the investigator.

5.3.3.3 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.

5.3.3.4 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 posttransplant (Day 7). Pentoxifylline will not be given to the LSF arm, due to lack of safety data available using this combination.

5.3.4 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 postprandial levels below 180 mg/dL after insulin discontinuation will be considered insulin independent.

5.3.5 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)
- any medications in the macrolide antibiotic class other than Zithromax.
- 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. Any aberration of trough levels of immunosuppressive agents that could indicate non-adherence, lack of compliance that poses a significant clinical risk and or derangement of protocol data collection will be documented. Please refer to Section 5.7.3 for a description of possible indications for premature discontinuation of study treatment.

5.7 Modification or Discontinuation of Study Treatment

5.7.1 Modification of Protocol Specific Drug(s)

In the event that the protocol-specific medication, LSF, is not tolerated, the doses will be decreased to the maximum tolerated dose. If still not tolerated, the drug will be discontinued but the subject will continue taking the immunosuppressive therapy in order to protect the islet graft.

5.7.2 Modification of Consensus Immunosuppression Regimen

Should an islet product become unsuitable for transplantation subsequent to recipient randomization and treatment with induction immunosuppression, maintenance immunosuppression will be discontinued. . An emergency request will be placed through UNOS that the next available pancreas for islet transplantation is directed to the selected manufacturing site. When an organ becomes available, investigators should refer to the CIT MOP to determine the amount and type of induction immunosuppression that will be administered at the time of the islet transplant. 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.

If a subject demonstrates an allergic reaction to thymoglobulin that results in cancellation of the initial transplant, then investigators should follow the steps outlined in the above paragraph. Once another organ becomes available, the subject will receive the alternate immunosuppressive regimen outlined in section 5.2.3.

5.7.2.1 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) SC 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.

5.7.2.2 RABBIT ANTI-THYMOCYTE GLOBULIN-INDUCED CYTOKINE RELEASE

Thymoglobulin® infusion 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.

5.7.2.3 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/ μ L and the subject is afebrile, then the following will be done:

- Reduce rabbit ATG by 50%.
- Reduce the prophylactic use of valganciclovir from 900 mg 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 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, subject symptoms, and measured temperatures.

If a subject's absolute neutrophil count is less than 1000 cells/ μ L 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 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/ μ L 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 $>12\text{ng/mL}$.
- If subject is using mycophenolate mofetil or mycophenolate sodium in lieu of sirolimus consider holding dose.
- Obtain CMV antigenemia or PCR for CMV.
- Consider fluoroquinolones in afebrile subjects.
- 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/ μ L 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 $>12\text{ng/mL}$.
- If subject is using mycophenolate mofetil or mycophenolate sodium in lieu of sirolimus consider holding dose.
- Obtain CMV antigenemia or PCR for CMV.
- Administer G-CSF.

5.7.2.4 THROMBOCYTOPENIA

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

5.7.2.5 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 choose ONE of the therapeutic alternatives shown in the following table:

Table 3 Response to nephrotoxicity

Allowable therapeutic responses to CNI-induced nephrotoxicity	Rationale
Discontinue sirolimus, and replace it with mycophenolate mofetil or mycophenolate sodium	The nephrotoxic effect of CNIs is increased by concomitant administration of sirolimus ^{168, 196}
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.

5.7.3 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: (see study definitions)
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. 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 choose 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 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 (PNF) occurs in 3 or more consecutive subjects at 2 or more participating clinical sites.
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 hours 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 10.5.

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

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, subject 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 Screening, Enrollment and Waiting List

This is an open label, phase 2, prospective, randomized, multi-centered trial. All islets will be processed and recipients recruited and transplanted (N=8) at two sites at the UM and UIC. Each of the two centers aims to enroll and transplant at least six subjects in one year..

Patients who meet the general inclusion criteria for this study will be approached regarding their participation. 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 and assigned a unique subject identification number. Subject eligibility will be confirmed through the performance of the screening visit procedures detailed in Appendix 1, Schedule of Events. More than one visit will be necessary to complete all of the screening procedures. Patients who enroll in this trial may have had some of the required screening tests done prior to signing the enrollment consent document as part of their routine diabetes care or a previous assessment for standard islet and/or pancreas transplantation at the participating site. Results from assessments completed prior to signing informed consent, must be current within the windows stated in the table below.

Table 4: Timeframes for screening assessments

Screening Assessments	Allowable timeframe prior to the date of consent
EBV IgG	No limit. Positive result required for eligibility
Retinopathy evaluation; Physical exam; CXR; Abdominal US; ECG; Cardiac Stress Test or Angiogram; PPD; TSH; Serology; Coagulation; CMV IgG/IgM (if neg)	Within one year
CBC; Chemistry; Lipids	Within 6 months

The screening pregnancy test, first morning spot urine, and blood draws for all central laboratory assessments must be done at the study site after informed consent has been signed. Pregnancy and blood transfusion history will be collected and provided to the central lab for PRA analysis.

In addition to the protocol required screening assessments, subjects should meet site-specific requirements for transplant.

During the period when subjects are awaiting their first transplant, screening/baseline assessments will be repeated at pre-defined intervals as detailed in Appendix 1. As in any other transplant situation, medical conditions that arise (e.g., new serious infection, malignancy, compliance issues, etc.) will automatically trigger a re-evaluation to determine if the subject remains qualified for the protocol. Only qualified subjects may proceed to donor organ matching and transplant.

7.1.1 Screening Visits

The screening evaluation will allow us to determine if there are any conditions that will preclude subjects from being included in the protocol or if they have any condition that requires medical attention before being considered for an islet transplant. The screening evaluation will also provide baseline data for the assessment of the effect of

successful islet transplantation on the progression of diabetic complications. (See detailed schedule of tests in Appendix 1).

Candidates must present recent reports of the following tests done by their physician:

- eye examination
- gynecological exam
- dental exam for infection
- tuberculosis skin test (PPD) every year

The following tests/labs will be done at Screening:

- history and physical examination
- chest X-ray
- abdominal and pelvic ultrasound with careful attention to liver parenchyma and gallbladder
- EKG
- cardiac stress test or angiogram (if older than 35)
- serology for hepatitis B and C, HTLV I and II, EBV, and HIV
- CBC (WBC with Diff, & Plat), chemistry panel, magnesium, lipid profile, HbA1c
- fasting serum glucose/c-peptide and serum creatinine
- Coagulation (PT, PTT, INR)
- thyroid function (TSH)
- serum pregnancy test (females)
- panel reactive antigen (PRA)
- 90 min mixed meal tolerance test (if C-peptide negative)
- first morning spot urine for microalbumin, protein and creatinine
- GFR by iohexol

Metabolic Assessments:

- LI and HYPO score (patients will have to provide BG measurements and insulin requirements for a month to calculate these scores)
- The Clarke questionnaire will be administered to calculate the Clarke score
- blood sugar records (BSR) and 7-Day Diary Log eCRFs

General Assessments:

- Nutritional assessment
- Psychological evaluation

7.1.2 Enrollment and Waiting List

Candidates will be required to provide documentation that their immunizations are up to date as per current CDC guidelines for the general adult population (diphtheria and tetanus; mumps, measles, and varicella if no previous history of the disease). In addition, they will be required to bring proof of prior immunization against hepatitis B, pneumococcus and influenza. If candidates do not have the above immunizations, they must complete the missing immunizations before being activated in the transplant waiting list, except for the flu vaccine, which can be given later if it's not available due to the season. If they require live or attenuated vaccines such as mumps, measles or varicella, they will have to wait at least 8 weeks before they can be placed on the active waiting list.

. After completion of the screening assessments required to confirm eligibility for the study, he/she will be listed for an islet transplant. During this period when subjects are awaiting their first transplant, the remaining screening

assessments – FSIGT, CGMS, and retinal photos- should be completed as time allows. If retinal photos cannot be obtained at WL/BL, they should not be collected post-randomization

Subjects whose screening test results meet the inclusion criteria will be registered in UNOS. They will be informed that they must carry a pager or cell phone and be available 24/7 (on-call) for a possible transplant unless otherwise notified by the islet transplant center. When placed on the waiting list, subjects will be instructed to stop taking aspirin or similar non-steroidal preparations that may have an effect on platelet function, thereby increasing the risk of bleeding from the procedure.

As time on the waiting list is unpredictable and may be long, continued assessment of physical and psychological status will be performed.

Candidates must provide annual reports of the following tests done by their physician:

- gynecological exam, including Pap smear
- dental exam for infection
- tuberculosis skin test (PPD) (every year)

The following tests/labs will be done during Waiting List/Baseline (See detailed schedule of tests in Appendix 1).

- physical examination (every year)
- eye examination (every year – if not obtained at WL/BL, do not collect post-randomization)
- chest X-ray (every year)
- abdominal and pelvic ultrasound with careful attention to liver parenchyma and gallbladder (every year)
- EKG (every year)
- serology for hepatitis B and C, HTLV I and II, CMV, and HIV (every year)
- CMV and EBV by PCR
- HbA1c (every 3 months)
- fasting serum glucose/c-peptide and serum creatinine
- CBC (WBC with Diff, & Plat), chemistry panel, magnesium, lipid profile (every six months)
- Coagulation (PT, PTT, INR) (every year)
- Blood type and HLA
- Crossmatch (before every islet transplant)
- PRA (every 3 months)
- thyroid function (TSH) (every year)
- pregnancy test (females) (before every islet transplant)
- first morning spot urine for microalbumin, protein and creatinine (every six months)

Metabolic Assessments:

- MAGE (every 6 months)
- LI, HYPO, Clarke score (every 6 months)
- The β -score
- Continuous glucose monitoring system (CGMS) (every year)
- Insulin modified FSIGT (every year)
- Atherogenic Profile
- blood sugar records (BSR) and 7-Day Diary Log eCRFs (every 6 months)

General Assessments:

- Nutritional assessment (every year)
- Quality of Life (QOL) Questionnaires (every 3 months)

- Psychological evaluation (every year)
- AE/Hypoglycemic Events/Toxicity Assessment

Mechanistic Assays:

- Alloantibody, Autoantibody, TAT & c-peptide

Specific Mechanistic Assays:

- Soluble mediators
- Granzyme B and related studies
- Phenotype
- RNA for microarray
- T and B cell assay

Archived samples

- Serum, PBMC and plasma, and RNA

7.2 Randomization and Islet Transplantation

7.2.1 Randomization

The DCC will maintain a central web-based randomization system. When a subject has been determined to be eligible, the local center will access the study web page. At that time the system will verify that the subject is indeed eligible. If the subject is eligible, he or she will be enrolled on the combined waiting list for study CIT-07 and this study (CIT-02).

At the time that islets are available for transplantation and have met the criteria for number and viability before culture, the islets will be randomized 2:1 to CIT-07 and CIT-02 respectively. At the completion of enrollment in CIT-07 an interim analysis will be performed of CIT-02. Should CIT-02 continue to enroll, all subjects at UM and UIC after this time will be enrolled in CIT-02. A suitable recipient will be found by ABO blood type, cross match, weight, and time in the waiting list. The suitable recipient will then be assigned the same treatment given to the islets, those assigned to CIT-02 will be included in this study at the time of transplantation. The date and time of randomization will be recorded in the database, as will the date and time of the transplantation.

This is an open label study. No treatment codes are required for the assignment of study drug and no procedures for un-blinding are required.

7.2.2 Hospital Admission

When donor islet cells become available a suitable match will be selected from the list of candidates for the trial. The potential recipient will be notified by pager or cell phone and asked to come to the hospital as soon as possible. The subject will be admitted approximately 48 hours before transplant (POD -2).

The following tests/labs will be done at time of admission:

- History and physical examination
- chest x-ray
- EKG

- serology for hepatitis B and C, HTLV I and II, CMV, and HIV
- CMV and EBV by PCR
- CBC (WBC with Diff, & Plat), chemistry panel, magnesium, , lipids, HbA1c
- coagulation tests (PT, PTT and INR)
- crossmatch
- pregnancy test (females)

The following tests will be done once as baseline at Day -2 (before transplant and prior to the start of immunosuppression):

Mechanistic Assays:

- Alloantibody
- TAT & c-peptide

Specific Mechanistic Assays:

- Soluble mediators, Granzyme B and related studies, Phenotype, RNA for microarray, T and B cell assays

Immunosuppression Monitoring:

- Sirolimus Levels (at Day 0)

Central venous access will be obtained. This may be through placement of a tunneled venous catheter, a central line, a peripherally inserted central (PICC) line or simply a peripheral venous access (although this is the least desirable). The placement of a PICC line will be done by the PICC line service, no guidance is used and a chest x-ray will be obtained following completion of the procedure to confirm placement. Alternatively, the placement of a tunneled central venous catheter or a non-tunneled central line can be done the Radiology Department by Special Procedures under sonographic and fluoroscopic guidance. Finally, if central venous access cannot be obtained, a peripheral line will be used and ATG infusions will be begun immediately.

7.2.3 Islet Isolation and Culture

The source of islets will be deceased donors who are between 15 and 65 years of age. The minimum donor recipient matching will be negative serum cross-match for T cells and ABO compatibility.

Pancreatic islets will be isolated, and processed by common consortium methodologies described in a Drug Master File submitted by NIAID. LSF will be added to the culture media and transplantation bag according to the randomization arm (see Section 3 for details).

7.2.4 Islet Transplantation Procedure

On the third day of admission (POD 0), the islet transplant will be performed. . The subjects will require LSF IV infusions every 6 hours starting at day -1 (subject should receive a minimum of 3 doses before islet transplant) and until a total of 28 doses have been administered (typically, day 5), which is approximately a total of 7 days in the hospital. Pharmacokinetic values for LSF will be obtained as follows:

- On the 1st Infusion at Day -1 (0, 30, 90 minutes, and 6 hours after IV infusion started), and
- On Infusion 27 at typically Day 5 post transplant (6 hours after IV infusion started)

The transplant will be done by a radiologist in the Special Procedures section of the Radiology department, under local anesthesia and conscious sedation or by laparoscopic surgery if indicated. Cefuroxime, 1.5 gm IV will be given at the time of the procedure unless the subject is allergic to penicillin, in which case levofloxacin 500 mg IV will be given. Islets will be infused under sterile conditions over 20-30 min. by gravity drainage¹⁹⁷ through a percutaneous catheter positioned in the main portal vein. A percutaneous trans-hepatic approach will be used and the catheter will be passed into the portal vein under fluoroscopic and ultrasound guidance. Gel foam plugs and/or collagen/thrombin paste will be used to embolize the entire peripheral catheter tract immediately before the catheter is withdrawn to reduce the chances of bleeding¹⁷⁸. A minimum of 5,000 IEQ/kg will be transplanted to maximize possibility of insulin independence. TAT & c-peptide should be repeated before transplant and at 15, 60, and 180 minutes post-transplant. An IV heparin drip will be given for the next 48 hours to keep the PTT between 50 and 60 seconds, to prevent portal vein thrombosis. Forty eight hours later, the IV heparin will be discontinued and enoxaparin sodium (Lovenox®) 30 mg twice a day will be given, by subcutaneous injection, for 5 days, to prevent portal vein thrombosis.

A 2nd islet transplant may be given after the 75 ± 5 days visit and metabolic assessments are completed, if the subject has not achieved insulin independence by this time under the same intervention as the first transplant, except for daclizumab instead of ATG induction. In that case the subject may be admitted on POD -1 or 0.

The University of Miami (DRI) will obtain the pancreata from their respective organ procurement organization (OPO). All the pancreata will be shipped to Miami for isolation, and then transferred back to the original center for infusion.

7.3 Study Treatment Visits: See Appendix 1 for detailed information

Post Transplant Insulin management and Metabolic Control

Following the first islet transplant, the subject will be kept on low dose insulin for one month to avoid stressing the islets during the time of engraftment. Initially, finger stick glucose will be measured every hour for the first 6 hours immediately post-transplant, and then at least every 2 hours until the subject starts eating. Subsequently, finger stick glucose will be measured fasting, before and 2 hours after each meal, at bedtime, and at 3 AM (if clinically necessary) while in the hospital. Upon discharge, all subjects will be instructed to do self monitored BG (SMBG) determinations five times per day (AM fasting, before lunch, 2 hours after lunch, before supper, and at bedtime during the first three months. After the first three months the subjects will be instructed to do SMBG at least four times a day (before each meal and at bedtime). Two weeks before the quarterly visit the subjects will be instructed to check SMBG fasting, before and 2 hours after each meal and at bedtime. Insulin will be adjusted in order to maintain the fasting and preprandial 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) and avoid hypoglycemia.

SC insulin will be administered by multiple injections or insulin pump. At the end of one month exogenous insulin will be weaned off over seven days, as long as glucose levels are maintained on target (see definition of insulin independence in section 3.1.1).

Post Transplant Evaluation

Metabolic control

Glucometer Downloading: Subjects will be required to bring the glucometer provided by the islet transplant center with them to all follow up visits so that it can be downloaded, erased and quality controlled.

Clinical Monitoring

After discharge from the hospital the subjects will be followed up in the Transplant Center on POD 7, day when the liver ultrasound with Doppler is generally performed. If there are no contraindications, the subject will return home. For subsequent islet transplants the subject will be followed up in the Transplant Center three times a week for the

first week. Following his or her return home, each subject will have the clinical monitoring performed as detailed below. Subjects may come to the islet transplant center for testing or use a local laboratory (for months 7, 8, 10 and 11) and have the specimens shipped to the appropriate destinations. Subjects will be responsible for finding a laboratory that can fulfill all the necessary requirements.

Subjects will be required to bring all medications provided by the islet transplant center with them to each follow up visit at the islet transplant center so that a pill count can be performed. This will help to ensure the subject is complying with treatment and taking the correct dose.

7.4 Follow-up Visits

Follow-up Visits to Islet Transplant Center

Subjects will come to the islet transplant center for visits after transplant at 1, 2.5, 4, 5, 6, 9, 12, 18 and 24 months post transplant. The subject that was randomized to Exenatide under CIT-02 protocol version 3.0 will be followed using the CIT-02 version 5.0 follow-up schedule. Please refer to the Schedule of Events (Appendices 1 and 3) for clinical timepoints and specific follow-up study procedures, including which visits can be done at the subject's local laboratory. The timing of all follow-up assessments will "reset" with additional transplants; *i.e.*, the day of the 2nd transplant becomes day 0 and subsequent assessments are conducted in relation to this day.

Subjects who have completed their day 365 visit following their initial transplant (primary endpoint assessment) and are thus unable to obtain a subsequent transplant in CIT are allowed to concurrently enroll in a non-CIT islet transplant study. Subjects will be expected to follow the CIT reduced follow-up schedule until 24 months after their final CIT islet transplant.

The follow-up assessments will include a history and physical exam, as well as metabolic evaluations and other testing as explained below.

Study subjects must provide annual reports of the following tests done by their physician:

- gynecological exam
- dental exam for infection
- mammogram if applicable (all females with family history of breast cancer and/or age 35 or older)
- eye exam
- immunizations
- tuberculosis skin test (PPD)

The following tests/labs will be done during the follow-up period:

- physical examination (at Week 1, 2, , Month 1, 2, 2.5, 4, 5, 6, 9, 12, Y1 post initial transplant, 18 and 24)
- Telephone Consult (at Week 3, Month 15, and 21,)
- eye examination (at Y1 post initial transplant)
- chest X-ray (at Month 12)
- abdominal and pelvic ultrasound with careful attention to liver parenchyma and gallbladder (at Week 1 and Month 12)
- EKG (at Month 12)
- serology for hepatitis B and C, HTLV I and II, and HIV (at Y1 post initial transplant)
- CMV by PCR (at Month 2.5 and M6)
- HbA1c (at Month 2.5, 6, 9, 12, Y1 post initial transplant, 18, 24, and Y2)
- fasting serum glucose/c-peptide and serum creatinine (at Day 3, Week 1, 2, 3, 4, 5, 6, 7, Month 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 9, 10, 11, 12, Y1 post initial transplant, M18, M24, and Y2)

- CBC (WBC with Diff, & Plat) (at Week 1, 2, 3, Month 1, 2, 2.5, 4, 5, 6, 9, 12, 15, 18, 21, and 24)
- chemistry panel, magnesium (at Week 1, 2, 3, Month 1, 2, 2.5, 4, 5, 6, 9, 12, 15, 18, 21, and 24)
- lipid profile (at Month 2.5, 6, 9, 12, 18, and 24)
- fasting and post-prandial c-peptide (at Day 3 and Week 1)
- first morning spot urine for microalbumin, protein and creatinine (at Month 1, 2.5, 12, Y1 post initial transplant, 18, and 24)

Metabolic Assessments:

- MAGE, LI, HYPO, and β -score (at Month 2.5, 6, 9, 12, and Y1 post initial transplant)
- Clarke score (at Month 6, 12, Y1 post initial transplant, 18 and 24)
- Mixed meal tolerance test (at Month 2.5, 6, 9, 12, Y1 post initial transplant, 18, 24 and Y2)
- Continuous glucose monitoring system (CGMS) (at Month 2.5, 6, 9, 12, Y1 post initial transplant, 24, Y2)
- Insulin modified FSIGT (at Month 2.5 and 12, and Y1 post initial transplant)
- Atherogenic Profile (at Y1 and Y2)
- blood sugar records (BSR) (at Month 2.5, 6, 9, 12, and Y1 post initial transplant)
- GFR by iohexol (at Month 1, 2.5, 12, Y1 post initial transplant, and 24)

General Assessments:

- Nutritional assessment (at Month 2.5, Month 6, 12, and Y1 post initial transplant and Y2)
- Quality of Life (QOL) Questionnaires (at Month 2.5, 6, 12, Y1 post initial transplant, 24 and Y2)
- Psychological evaluation (at Y1 post initial transplant)
- AE/Hypoglycemic Events/Toxicity Assessment (at Day 3, Week 1, 2, 3, Month 1, 2, 2.5, 4, 5, 6, 9, 12, 15, 18, 21, 24, and Y2)

Mechanistic Assays:

- Alloantibody, Autoantibody (at Month 2.5, 6, 9, 12, Y1 post initial transplant, 18 and 24)

Specific Mechanistic Assays:

- Soluble mediators (at Day 1, 2, 4, Week 1, 2, 3, 4)
- Granzyme B and related studies (at Day 3, Week 1, 2, 3, 4, 6, Month 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 7, 8, 9, 10, 11, 12, Y1 post initial transplant, M15, 18, 21, 24, and Y2)
- Phenotype (at Week 1, 4, Month 2.5, 6, 9, 12, 18, 24, and Y2)
- RNA for microarray, T and B cell assay (at Month 2.5, 6, 9, 12, 18, 24, and Y2)

Archived samples

- Serum, PBMC and plasma, RNA (at Month 2.5, 6, 9, 12, and Y1 post initial transplant)

Immunosuppression Monitoring:

- Sirolimus Levels (at Day 3, Week 1, 2, 3, Month 1, 2, 2.5, 4, 5, 6, 9, 12, Y1 post initial transplant, 15, 18, 21, and 24)
- Tacrolimus Levels (at Day 3, Week 1, 2, 3, Month 1, 2, 2.5, 4, 5, 6, 9, 12, Y1 post initial transplant, 15, 18, 21, and 24)

After the second transplant (and subsequent transplants if applicable), the schedule of testing will reset to the date of the last transplant for the following testing only: history and physical, nutritional evaluation, glucose readings and insulin requirements, CGMS, c-peptide, HbA1c, metabolic testing (please refer to mechanistic section and schedule of events for detailed information regarding the metabolic tests and the schedule), serology with HIV, EBV, CMV and HSV 1 and 2 serology and EBV and CMV PCR, doppler ultrasound of the liver, immunological testing. Drug levels

(sirolimus, MPA or tacrolimus if applicable) may increase in frequency, if dosages are being adjusted. Chemistry panel and CBC will also need to be intensified (performed every week) in the first 4 weeks after each subsequent transplant. They will return to their original schedule after 4 weeks if stable.

Toxicity Assessment/Adverse Event Assessment:

Toxicity assessment/ AE assessment which includes a health update, the assessment of any symptomatology to determine its likely relation to immunosuppression and assessment for any hypoglycemic episodes will be performed at each visit to the islet transplant center and monthly by telephone assessment otherwise. AEs will be graded according to *Terminology Criteria for AEs in Trials of Adult Pancreatic Islet Transplantation AEs*. The relationship to immunosuppression will also be determined. SAEs will be reported to the FDA and IRB as set forth in data safety and monitoring plan.

Immunosuppression Dose Check:

The immunosuppression dose the subject is currently taking will be checked at each follow up telephone assessment to confirm the subject is taking the correct dose. Dose adjustments will be made with patients verbally on the telephone as appropriate.

Metabolic Parameters

Evaluation of Islet Allograft Function

A battery of different tests is needed in order to assess islet function and insulin sensitivity before and after transplantation and its comparison to normal pancreatic islet function. Please refer to Metabolic Section in Mechanistic and to the Schedule of Events for details.

Continuous Glucose Monitoring System (CGMS)

(See mechanistic)

Immunological Parameters

(See mechanistic)

Quality of Life Assessment

(See mechanistic)

Nutritional Assessment

Marked nutritional changes have been observed in islet transplant recipients. This is a result of both the commencement of immunosuppression and by the subject's dietary adjustment in order to maximize graft function. We will assess the nutritional profile of patients through a questionnaire and interviews prior to, and at selected intervals after, islet transplantation.

The rationale for including the nutritional survey is multifold: First, there is no information on feeding habits in subjects that are accepted on an islet transplantation waiting list. Second, and more importantly, there has been no attempt to elucidate changes in feeding habits pre- and post- transplant, and whether these changes, if they occur, may bear influence on the metabolic outcomes observed after transplantation.

The nutritional assessment will evaluate dietary changes in patients pre- and post- islet transplant and how these relate to weight changes, obesity levels, body composition and if these changes have any effect on graft function.

The subject will also have a physical assessment of body fat using bio-impedance (which measures the ability of the body to conduct a minimal amount of electric current), a measurement of the thickness of a fold of skin at specific

body sites, and measurement of the waist and hip circumferences. These will be done at the same times as the QOL questionnaires.

7.5 Visit Windows

Please see Appendix 1 for visits and follow up laboratory testing.

Study visits should take place within the time limits below:

At 7 days (± 3 days) post transplant:

Subjects remain in the hospital post transplant for approximately 5 days in order to receive the IV LSF.

At 2 weeks (± 3 days) post-transplant

At 3 weeks (± 3 days) post-transplant

At 1 month (± 3 days) post-transplant

At 2 months (± 7 days) post-transplant

At 75 days (± 5 days) post-transplant

At 4 months (± 7 days) post-transplant

At 5 months (± 7 days) post-transplant

At 6 months (± 7 days) post-transplant

Months 9 and 12 (± 14 days) post-transplant

Yearly follow-up visits (± 14 days) post-transplant

At 15 months (± 14 days) post-transplant

At 18 months (± 14 days) post-transplant

At 21 months (± 14 days) post-transplant

At 24 months (± 14 days) post-transplant

At Y2 (± 90 days) post-transplant

7.6 Criteria and Timing for Subsequent Islet Transplants

Subjects who do not meet criteria for a subsequent transplant will enter reduced follow-up schedule (Appendix 2).

7.6.1 Second Islet Transplant

Islet transplant recipients with partial islet graft function (see Study Definitions) can be considered for a second transplant in the interim between the 75 ± 5 days/metabolic assessment visit and 8 months post-initial infusion. Islet transplant recipients with graft failure will be considered for a second islet transplant before 8 months post-initial infusion. In addition to meeting the criteria outlined below, approval from the Steering Committee must be obtained in advance. Please refer to the MOP for details on this process, which includes review of the potency testing from the first transplant product and post-transplant clinical data.

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

1. Subject received $\geq 5,000$ 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 μ mol/L).
5. No evidence of hypersensitization, allergic responses, or other potentially serious drug reactions to medications required by the protocol.
6. PRA $\leq 50\%$ 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).
7. Any medical condition that, in the opinion of the investigator, will interfere with a safe and successful second islet transplant.

If graft failure occurs after the second islet transplant, these recipients will be considered treatment **failures** and immunosuppression will be withdrawn.

7.6.2 Third Islet Transplant

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 μ mol/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 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).

The third transplant must occur prior to 8 months post-first islet transplant.

8 SAFETY MONITORING

AEs that are classified as serious according to the definition set forth by the health authorities must be reported promptly to NIDDK/NIAID, 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)* 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 study-related treatment whether considered related to the treatment 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. 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.
6. Congenital anomaly or birth defect.
7. Other conditions specified in the protocol.

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

Regardless of the relatedness of the AE to study drug, the event must be reported 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 for 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. If a subject enrolls in a non-CIT islet transplant study, adverse events will no longer be collected in CIT starting at the time of the non-CIT study intervention. All adverse event reporting from that point on will be done through the non-CIT 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:

1. Observing the subject.
2. Questioning the subject, which should be done in an objective manner.
3. Receiving an unsolicited complaint from the subject.
4. 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 Grading and Attribution

8.2.3.1 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 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:

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, <i>e.g.</i> , 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.

Table 5 Terminology Criteria for Adverse Events

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

8.2.3.2 DEFINITION OF ATTRIBUTION

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

The relatedness, or attribution, of an AE to islet transplantation, which includes the transplant procedure and/or the islet product, the secondary investigational agent, 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), Lisofylline, or immunosuppression/infection prophylaxis will be defined by using the description provided below.

Table 6. 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.

If a subject enrolls in a non-CIT islet transplant study, serious adverse events will no longer be collected in CIT starting at the time of the non-CIT study intervention. All serious adverse event reporting from that point on will be done through the non-CIT study.

The sponsor will request copies of serious adverse events that occur in the non-CIT study from the Principal Investigator for informational purposes.

8.3.2 Recording Procedure

SAEs will be recorded on the AE 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 the NIAID/NIDDK 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), 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 other than serious AEs will be reported to the DCC by the sites on at least a monthly bases.

8.3.3.3 REPORTING TIMELINE – FROM THE DCC TO THE SPONSOR AND HEALTH AUTHORITIES

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

8.3.3.4 NOTIFYING THE DATA AND SAFETY MONITORING BOARD

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

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 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 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. A woman who becomes pregnant or wishes to while on the study will be counseled as to her choices and will be encouraged to discuss those choices with her obstetrician. 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(s) 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 the treatment group (LSF) to the control group (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 or an approved glucometer or CGMS unit identified in the MOP 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, and the 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 MAGE⁶⁹ and the LI⁷³.

The MAGE requires 14 – 16 capillary 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 capillary 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/L²/hr·wk⁻¹). A LI greater than or equal to the 90th percentile (433 mmol/L²/hr·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 mmol/L) or prompt recovery after oral carbohydrate IV glucose, or glucagon administration.⁴⁰

In addition, composite indices of hypoglycemia frequency, severity, and symptom recognition will be assessed by both the Clarke survey¹⁹⁸ and the HYPO score⁷³.

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 (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, c-peptide, glucagon, amylin, and proinsulin 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 times = 15, 30, 60, 90, 120, 150, 180, 210, 240, 270 and 300 minutes, stimulated glucose, c-peptide, glucagon, amylin, and proinsulin levels will again be drawn. In patients undergoing this test pre-transplant, blood samples are only required for 60 and 90 minutes.

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. Each blood sample collected for glucagon, amylin, and proinsulin levels will be drawn according to the University of Miami SOP and will be shipped frozen to the University of Miami for analysis (See detailed shipping instructions in MOP).

The data from the MMTT will be used to obtain and calculate the 90 minute c-peptide, glucose, glucagon, amylin, and proinsulin; the peak c-peptide, glucose, glucagon, amylin, and proinsulin; the time for c-peptide glucose, glucagon, amylin, and proinsulin to peak, and the mixed meal stimulation index (MSI=AUC c-peptide/AUC glucose).

9.1.2.6 B-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 al¹⁹⁴. 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.^{199, 200}

The CPGCR is calculated as $[\text{c-peptide (ng/mL)} * 100] / [\text{glucose (mg/dL)} * \text{creatinine (mg/dL)}]$. An index of islet graft function, this measure correlates well with both the 90-minute glucose levels during a MMTT and with the β -score²⁷.

9.1.2.8 INSULIN-MODIFIED FREQUENTLY-SAMPLED INTRAVENOUS GLUCOSE TOLERANCE (FSIGT) TEST

The AIR_{glu} insulin sensitivity, and disposition index (DI) will be determined using the FSIGT test. This assessment provides a composite measure of β -cell function, the disposition index (DI), which relates the effect of insulin sensitivity 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 the improved glycemia that occurs with transplantation²⁰¹. These results require confirmation by longitudinal analysis.

The insulin-modified FSIGT test⁹⁴ involves blood sampling at baseline ($t = -10, -5, \text{ and } -1$ min) and at $t = 1, 2, 3, 4, 5, 7, 10, 12, 14, 16, 18, 20, 22, 25, 30, 40, 50, 70, 100, 140, \text{ \& } 180$ minutes post-injection of glucose at $t = -30$ seconds with an injection of insulin at $t = 20$ min. Each pre-transplant blood sample will be used for insulin and glucose determination. Each post-transplant blood sample will be used for insulin and glucose determination; in addition, the baseline ($t = -10, -5, \text{ and } -1$ min) and $t = 1, 2, 3, 4, 5, 7, \text{ and } 10$ minutes post-glucose injection samples will be used for c-peptide determination.

All samples 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 insulin sensitivity, 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 ($\text{DI} = \text{AIR}_{\text{glu}} \cdot \text{SI}$).

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.^{71, 195}

We will also perform CGMS monitoring at 6 and 9 months after the last transplant and quarterly thereafter as we have observed that early perturbations (6 and 9 months) in glucose variability and percentage hyperglycemia time (%HGT, defined as the percentage of time BG levels are above 140 mg/dL) detected with CGMS can serve as an early indicator of graft dysfunction (within 2 to 3 months). Therefore, we will also monitor %HGT at all the timepoints of CGMS monitoring.

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 Hypoglycemia Fear Survey (HFS) is a 23-item self-administered survey for measuring the fear experienced with respect to hypoglycemia. The FHS 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.

9.2 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.

The timing of immune assays will be "reset" with additional transplants; *i.e.*, the day of the 2nd transplant becomes day 0 and subsequent samples for immune assays are drawn in relation to this day.

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. Development of alloantibody specific for 1 or 2 HLA antigens can now be defined using assays that incorporate HLA specific monoclonal antibodies. Malek Kamoun at Penn will provide core lab service for alloantibody assessments.

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

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, and plasma will be archived for future analyses.

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

Plasma: Blood will be collected processed and archived to the NIDDK repository.

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 outcomes in transplanted subjects in whom protocol-directed therapy is high enough to justify exposure to the risks of islet transplantation. All efficacy analyses will be based on the intention-to-treat principle: any subject in whom protocol-directed therapy is initiated will be included in the ITT sample. 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 and as described in this section. Additional 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 3.1.1. The primary analysis will compute an exact binomial estimate and an exact 95% confidence interval for the true rate in each of the two groups.

The primary endpoint should be available for all enrolled 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 ENDPOINTS

As a secondary analysis the proportion of subjects with insulin independence at 75 days after transplant in each of the two study groups will be compared to the proportion of subjects with insulin independence at the UM and at UIC in the CIT-07 study using an exact two-sided test for two proportions. The same proportions will also be compared to all subjects in the CIT-07 study from all four CIT centers (see the Statistical Analysis Plan, SAP). Dichotomous variables such as HbA1c classified as normal ($\leq 7.0\%$) 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 endpoint will be calculated. In addition, the endpoints will be compared to the responses in subjects at UM/UIC 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 two treatment groups, 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 likely 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 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. Numbers and rates of treated subjects with previous history of each condition will be reported.

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 SAP.

10.4 Sample Size

The purpose of this pilot study is to estimate the true rate of insulin independence at 75 days. 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%.

Table 7 Sample Size

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

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.

During the period when CIT is enrolling, CIT 02 will enroll at half the rate. CIT 02 will be continuously monitored with a futility analysis, and a recommendation for stopping new enrollment will be made if the accruing information indicates that the primary endpoint of CIT 02 is unlikely to be meaningfully higher than the historical rate of 15-20%; “meaningfully higher” is taken to be 50%.

As each CIT 02 endpoint is determined an upper bound for the probability of a favorable CIT 02 primary endpoint will be calculated from an exact one-sided 95% confidence interval. A recommendation will be made to stop enrollment in CIT 02 if, and when, the upper bound is below 50%. Operationally the following rule results:

Stopping rule

- Stop at 5 subjects if there are no successes in first 5 subjects.
- Stop at 8 subjects if there is only 1 success in first 8 subjects.
- Stop at 11 subjects if there are 2 or fewer successes in first 11 subjects.
- Otherwise, complete enrollment to 12 subjects.

The rule ensures that should 12 subjects be enrolled, there will be at least 3 successes out of 12 on the primary endpoint.

Should enrollment in CIT 02 be stopped, then long term follow up data will continue to be collected until the end of the study.

An additional interim analysis will also be done for the DSMB at the time that CIT07 enrollment is complete. In this analysis all endpoints of CIT 02 compared to corresponding endpoints of UM and UIC CIT 07 subjects. If the primary endpoint in CIT 02 has a significantly lower rate of favorable outcome, using a one sided test with Type 1 error 0.05, then a recommendation to stop enrollment in CIT 02 will be made.

Additional characteristics are given in detail in the SAP for CIT-02.

TABLE 8: Operating characteristics of stopping rule

Probability of event	Probability stop at 5 subjects	Probability stop at 8 subjects	Probability stop at 11 subjects	Probability reach 12 subjects
0.1	0.59049	0.23915	0.09685	0.07350
0.2	0.32768	0.20972	0.13421	0.32838
0.3	0.16807	0.12353	0.09079	0.61760
0.4	0.07776	0.05599	0.04031	0.82594
0.5	0.03125	0.01953	0.01220	0.93701
0.6	0.01024	0.00492	0.00235	0.98248
0.7	0.00243	0.00077	0.00024	0.99656
0.8	0.00032	0.00005	0.00001	0.99962
0.9	0.00001	0.00000	0.00000	0.99998

Additional characteristics are given in detail in the SAP for CIT-02.

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 SAP. Any changes in these principal features will require a protocol or a SAP amendment, which would be subject to review by the CIT Steering Committee, the independent DSMB, the IND Sponsor, and the Health Authorities. These changes will be described in the Final Study 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 will be maintained at each site in the strictest confidence. However, as a part of the quality assurance and legal responsibilities of an investigation, the investigational site must permit authorized representatives of the sponsor(s), 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 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*²⁰², 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. 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 Time points (specified in Days relative to transplant)	SCR 1	S2	S3	WL/BL ¹	0 ²	3	7	14	21	28 ²⁸	56	75	120	150	180	270	365	365 post initial tx
Visit Number	01	01a	01b	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	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	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	X	X-yrly														
GYN Exam (females):	X			X-yrly														
Retinopathy Evaluation ⁵		X		X-yrly ⁶														X
Physical Exam	X			X-yrly	X		X	X		X	X	X	X	X	X	X	X	X
Telephone Consult									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	
CIT-02-SPECIFIC GENERAL ASSESSMENTS																		
Nutritional Assessment	X			X-yrly								X			X		X	X
LOCAL LABORATORY ASSESSMENTS																		
CBC (WBC + Diff & Plat)	X			X-q6mo	X		X	X	X ¹⁸	X	X	X	X	X	X	X	X	
Chemistry ⁷	X			X-q6mo	X		X	X	X ¹⁸	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														

Appendix 1 Time points (specified in Days relative to transplant)	SCR 1	S2	S3	WL/BL ¹	0 ²	3	7	14	21	28 ²⁸	56	75	120	150	180	270	365	365 post initial tx
Visit Number	01	01a	01b	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	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	N/A	N/A	W1	W2	W3	W4	M2	M2.5	M4	M5	M6	M9	M12	Varies
Serology ⁹ (Hep B, Hep C, HIV, HTLV)	X			X-yrly														X
EBV IgG	X																	
CMV IgG, CMV IgM				X-yrly ¹⁰														X ¹⁰
Coagulation (PT, PTT, INR)	X			X-yrly	X													
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														
CMV by PCR				X								X			X			
EBV by PCR ¹⁷				X														
CIT-02-SPECIFIC LOCAL LABORATORY ASSESSMENTS																		
LSF peak and trough (first infusion) and trough (last infusion) ³²					X ³³		X ³⁴											
CENTRAL LABORATORY METABOLIC ASSESSMENTS																		
First morning spot urine ¹⁶	X			X						X		X					X	X
GFR			X	X-yrly						X		X					X	X
HbA1c	X			X-q3mo								X			X	X	X	X
Fasting serum gluc/c-pep & serum creat	X			X		X	X ²⁴	X ^{24,19}	X ²⁴	X ²⁴	X ²⁵	X ^{25, 36}	X	X	X ¹⁸	X ¹⁹	X	X
90 min c-pep/ glucose (MMTT) ²⁰		X										X ³⁷			X	X	X	X

Appendix 1 Time points (specified in Days relative to transplant)	SCR 1	S2	S3	WL/BL ¹	0 ²	3	7	14	21	28 ²⁸	56	75	120	150	180	270	365	365 post initial tx
Visit Number	01	01a	01b	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	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	N/A	N/A	W1	W2	W3	W4	M2	M2.5	M4	M5	M6	M9	M12	Varies
Insulin modified FSIGT				X-yrly ⁶								X ³⁶					X	X
Artherogenic Profile ³⁶				X														X
LOCAL METABOLIC ASSESSMENTS																		
Glycemic Stability (CGMS)				X-yrly ⁶								X ³⁶				X	X	X
BSR eCRFs ²¹	X			X-q6mo								X ³⁶				X	X	X
CIT-02-SPECIFIC METABOLIC ASSESSMENTS																		
MMTT ²⁰ (Boost Extra, 15, 30, 60, 120, 150, 180, 210, 240, 270, 300 min)												X				X	X	X
CALCULATED METABOLIC ASSESSMENTS																		
MAGE				X-q6mo								X				X	X	X
LI	X			X-q6mo								X				X	X	X
Clarke Score	X			X-q6mo												X		X
HYP0	X			X-q6mo								X				X	X	X
Beta Score				X								X				X	X	X
C-peptide: (glucose X Creatinine) ratio	X			X		X	X ²⁴	X ²⁴	X ^{24,18}	X ²⁴	X ²⁵	X ²⁵	X	X	X ²²	X ²²	X	X
IMMUNOSUPPRESSION LEVELS																		
Sirolimus 24-hour trough Levels					X ³¹	X	X	X	X ¹⁸	X	X	X	X	X	X	X	X	X
Tacrolimus 12-hour trough Levels						X	X	X	X ¹⁸	X	X	X	X	X	X	X	X	X
MECHANISTIC ASSAYS																		
Alloantibody	X			X-q6mo ²³								X				X	X	X
Autoantibody				X								X				X	X	X

Appendix 1 Time points (specified in Days relative to transplant)	SCR 1	S2	S3	WL/BL ¹	0 ²	3	7	14	21	28 ²⁸	56	75	120	150	180	270	365	365 post initial tx
Visit Number	01	01a	01b	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	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	N/A	N/A	W1	W2	W3	W4	M2	M2.5	M4	M5	M6	M9	M12	Varies
CIT-02-SPECIFIC MECHANISTIC ASSAYS																		
Soluble mediators				X ²⁸	X ²⁹	X ³⁰	X	X	X	X								
Granzyme B/etc.				X ²⁸	X	X	X	X	X	X ²⁵	X	X ²⁵	X ²⁵	X ²⁵	X ²⁶	X ²⁶	X ²⁶	X
Phenotype				X ²⁸			X			X		X			X	X	X	
RNA for microarray				X ²⁸								X			X	X	X	
T and B cell assay				X ²⁸								X			X	X	X	
ARCHIVED SAMPLES																		
Serum				X								X			X	X	X	X
Plasma				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-02.

⁴ *Informed Consent #2* includes information specific to CIT-02. IC #2 must be signed immediately after randomization.

⁵ Retinopathy eval includes fundoscopic pictures for WL/BL assessments and Y1. Screening retinopathy evaluation should be done per site-specific standards. If pupils cannot be dilated, then a manual ophthalmologic evaluation can be substituted.

⁶ These can be collected after subject is considered protocol eligible and has been moved to the transplant wait list, as time allows. If retinal photos are not collected pre-randomization, do not collect post-randomization.

⁷ Chemistry includes: Sodium, albumin, magnesium, chloride, potassium, alk phosphatase, total bilirubin, CO₂, creatinine, ALT (SGPT), BUN, gamma GT, glucose, AST (SGOT), calcium, phosphorus

⁸ Complete pregnancy test within 72 hours prior to the initiation of study medication.

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).

¹²Sample 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

¹³C-peptide should be done locally and drawn fasting, and twice between 1-3 hrs post-prandial on Day 3 and Day 7 post-transplant.

¹⁴Finger stick glucose should be done locally and drawn every hour for the first 6 hours immediately post-transplant.

¹⁵Subsequent transplants only. Local result used to determine eligibility for subsequent transplants only.

¹⁶First morning spot urine includes: albumin, protein, and creatinine

¹⁷EBV by PCR should be done post-randomization if reactivation is suspected.

¹⁸Can be performed at a local laboratory.

¹⁹If fasting serum glucose and c-peptide/serum creatinine drawn locally sample should be sent from local lab to study site and then shipped to the Univ of Washington

²⁰MMTT should include samples for c-peptide, glucose, glucagon, amylin, and proinsulin measurements for the screening visit and as necessary when determining graft failure.

²¹Blood Sugar Record (BSR) eCRF is completed using information gathered from subject diary logs, glucometer download data, and insulin requirements.

²²C-peptide glucose creatinine ratio calculated monthly.

²³For 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.

²⁴Once a week

²⁵Every 2 weeks

²⁶Monthly

²⁷If participant receives daclizumab with a subsequent transplant, administration of the day 42 dose will be an unscheduled visit.

²⁸Complete one time while subject is on waiting list and again on Day -2 relative to transplant

²⁹Complete pre-infusion, 1 hr, and 6 hrs post-infusion

³⁰Days 1,2,4

³¹Complete only on Day -0

³²LSF Arm only

³³Complete at Day -1 (at 0, 30, 90 minutes and 6 hours after first Infusion)

³⁴Complete at typically day 5 post-transplant (6 hours after Infusion 27 started)

³⁵Atherogenic profile consisting of fasting lipid panel (TG, TC, HDL, LDL, non-HDL), C reactive protein, serum amyloid A, apolipoprotein A1 and apolipoprotein B. If blood is drawn locally, sample should be sent from local lab to study site and then shipped to the central laboratory (University of Washington).

³⁶Do not collect for participants with graft failure. Results of tests performed at the time of graft failure will be used for day 75 endpoint calculations.

³⁷If participant receives daclizumab with a subsequent transplant, administration of the day 42 dose will be an unscheduled visit.

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 and 2 years 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 two years 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 and 2 years (+/- 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 and 2 years (+/- 7 days) post **last** transplant:

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

Appendix 3: Schedule of Events for 1-Year Additional Follow-up					
Time Point (months [M] relative to final islet transplant; years [Y] relative to initial transplant)	M15	M18	M21	M24	Y2
Visit Number (relative to final islet transplant)	16	17	18	19	
Visit Window (specified in days)	± 14	± 14	± 14	± 14	± 90
GENERAL ASSESSMENTS					
Nutritional Assessment				X	
Physical Exam		X		X	
Telephone Consult	X		X		
QOL				X	X
AE /Hypoglycemic Events/Toxicity Assessment	X	X	X	X	X
LOCAL LABORATORY ASSESSMENTS					
CBC (WBC + Diff & Plat)	X ¹	X	X ¹	X	
Chemistry	X ¹	X	X ¹	X	
Lipids		X		X	
CENTRAL LABORATORY/METABOLIC ASSESSMENTS					
First morning spot urine ²		X		X	
GFR				X	
HbA1c	X ³	X	X ³	X	X
Fasting plasma gluc/c-pep & serum creat		X		X	X
90-min MMTT					X
MMTT (Boost 15, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300 min)		X		X	
Atherogenic Profile ⁴				X	
LOCAL METABOLIC ASSESSMENTS					
Glycemic Stability (CGMS)				X	X
CALCULATED METABOLIC ASSESSMENTS					
Clarke Score		X		X	
IMMUNOSUPPRESSION LEVELS					
Sirolimus Levels	X ¹	X	X ¹	X	
Tacrolimus Levels	X ¹	X	X ¹	X	
MECHANISTIC ASSAYS					
Autoantibody		X		X	
Alloantibody		X		X	
Granzyme B expression	X	X	X	X	
Phenotype		X		X	
RNA for microarray		X		X	
T and B cell assay		X		X	

¹ Can be performed at a local laboratory.

² Central laboratory assessment. First morning spot urine contains albumin, protein, and creatinine.

³ If blood drawn locally, sample should be sent from local lab to the central laboratory.

⁴ Atherogenic profile consisting of fasting lipid panel (TG, TC, HDL, LDL, non-HDL), C reactive protein, serum amyloid A, apolipoprotein A1 and apolipoprotein B. If blood is drawn locally, sample should be sent from local lab to study site and then shipped to the central laboratory (Univ. of Washington).

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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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: <i>Extended Follow-Up after Islet Transplantation in Type 1 Diabetes</i>	
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: <p style="text-align: center;">ATTN: Clinical Trials Statistical & Data Management Center Department of Biostatistics 201 S Clinton St Iowa City, IA 52240-4034</p>	
<p>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.</p> <p>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.</p>	
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>_____</p> <p>Site Principal Investigator (Print)</p> <p>_____</p> <p>Site Principal Investigator (Signature)</p> </div> <div style="width: 45%;"> <p>_____</p> <p>Date</p> </div> </div>	

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 MMIT 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: <ul style="list-style-type: none">• 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	<ol style="list-style-type: none">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	<ol style="list-style-type: none">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	Histocompatibility 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 \geq 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 exposure². Sirolimus alters the pharmacokinetic profiles of other CNIs (*e.g.*, tacrolimus) and may thereby potentiate nephrotoxicity³. Fervenza *et al.* described nephrotoxicity from sirolimus in patients with chronic glomerulopathies that was non-reversible on cessation of therapy⁴. 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 sirolimus⁸. Pneumonitis and colitis have also occurred^{9,10}. The most common (> 30%) adverse reactions are: oral aphthous 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, <i>e.g.</i> , 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.

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