CLINICAL ISLET TRANSPLANTATION (CIT)
PROTOCOL CIT-07
Islet Transplantation in Type 1 Diabetes
Version 8.0 (20 August 2012)
BB-IND 9336

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

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Title:

Islet Transplantation in Type 1 Diabetes

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INSTRUCTIONS: Please have the Principal Investigator print, sign, and date at the indicated location below. A copy should be kept for your records and the original signature page sent to the Data Coordinating Center.

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

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

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

As the Site Principal Investigator, I agree to conduct protocol CIT-07, "Islet Transplantation in Type 1 Diabetes" according to good clinical practices. I agree to carry out the study by the criteria written in the protocol and understand that no changes can be made to this protocol without written permission of the NIAID and NIDDK.

_____

Site Principal Investigator (Print)

_____　　　_____

Site Principal Investigator (Signature)　　　　　　**Date**

Protocol Synopsis

Title	Islet Transplantation in Type 1 Diabetes
Clinical Phase	Phase 3
IND Sponsor	DAIT/NIAID/NIH
IND Number	BB-IND 9336
Activation Date	October 2006
Accrual Objective	48
Accrual Period	24 months
Follow-up Period	24 months after final transplant
Study Design	A prospective, single-arm, multi-center study in islet transplantation
Treatment Description	Subjects will receive up to 3 separate infusions of islets. Subjects will receive induction and maintenance immunosuppression consisting of ATG (basiliximab instead of ATG for the 2nd and 3rd transplants, if applicable), sirolimus and low-dose tacrolimus. In addition, subjects will receive etanercept for anti-inflammatory therapy.
Primary Endpoint	The proportion of subjects with an HbA1c <7.0% at Day 365 AND free of severe hypoglycemic events from Day 28 to Day 365 inclusive following the first islet transplant, with the day of transplant designated Day 0.

Secondary Endpoints　The key secondary endpoints are the following:

1) The proportion of subjects with an HbA1c <7.0% AND free of severe hypoglycemic events from Day 28 to Day 730, inclusive, after the first islet transplant.

2) The proportion of subjects with HbA1c ≤ 6.5% at one year after the first islet transplant AND free of severe hypoglycemic events from Day 28 to Day 365 after the first islet transplant.

3) The proportion of subjects with HbA1c ≤ 6.5% at two years after the first islet transplant AND free of severe hypoglycemic events from Day 28 to Day 730 after the first islet transplant.

4) The proportion of subjects free of severe hypoglycemic events from Day 28 to Day 365 after the first islet transplant.

5) The proportion of subjects free of severe hypoglycemic events from Day 28 to Day 730 after the first islet transplant.

6) The proportion of subjects with HbA1c <7.0% at one year after the first islet transplant.

7) The proportion of subjects with HbA1c <7.0% at two years after the first islet transplant.

8) The proportion of subjects with HbA1c ≤6.5% at one year after the first

islet transplant.

9) The proportion of subjects with HbA1c ≤6.5% at two years after the first islet transplant.

10) The proportion of insulin-independent subjects at one year after the first islet transplant.

11) The proportion of insulin-independent subjects at two years after the first islet transplant.

Other secondary endpoints include the following:

- The proportion of subjects with an HbA1c <7.0% AND free of severe hypoglycemic events from Day 28 to Day 730, inclusive, after the final islet transplant.

- The proportion of subjects with HbA1c ≤ 6.5% at one year after the final islet transplant AND free of severe hypoglycemic events from Day 28 to Day 365 after the final islet transplant.

- The proportion of subjects with HbA1c ≤ 6.5% at two years after the final islet transplant AND free of severe hypoglycemic events from Day 28 to Day 730 after the final transplant.

- The proportion of subjects free of severe hypoglycemic events from Day 28 to Day 365 after the final islet transplant.

- The proportion of subjects free of severe hypoglycemic events from Day 28 to Day 730 after the final islet transplant.

- The proportion of subjects with HbA1c <7.0% at one year after the final islet transplant.

- The proportion of subjects with HbA1c <7.0% at two years after the final islet transplant.

- The proportion of subjects with HbA1c ≤6.5% at one year after the final islet transplant.

- The proportion of subjects with HbA1c ≤6.5% at two years after the final islet transplant.

- The proportion of insulin-independent subjects at one year after the final islet transplant.

- The proportion of insulin-independent subjects at two years after the final islet transplant.

Efficacy Endpoints

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

- The percent reduction in insulin requirements
- HbA1c
- MAGE[1]
- LI[2]
- Ryan hypoglycemia severity (HYPO) score[2]
- Basal (fasting) and 90-min glucose and c-peptide derived from the mixed-meal tolerance test (MMTT)
- β-score[3]

- C-peptide: (glucose · creatinine) ratio
- Acute insulin response to glucose (AIR_{glu}), insulin sensitivity, and disposition index derived from the insulin-modified frequently-sampled IV glucose tolerance (FSIGT) test[4,5]
- Glucose variability[6] and hypoglycemia duration[7] derived from the CGMS
- Quality of life (QOL) measures

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

At 365 ± 14 days following the first 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• creatinine) ratio
- AIRglu, insulin sensitivity, and disposition index derived from the FSIGT test[4,5]
- 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)

At two years 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)
- β-score
- C-peptide: (glucose• creatinine) ratio
- CGMS
- QOL

<u>Safety Endpoints</u>

At 75 ± 5 days following <u>each</u> transplant and 365 ± 14 days following the <u>first</u> and <u>final</u> 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; increase in urinary albumin excretion; addition or intensification of anti-hypertensive therapy; addition or intensification of anti-hyperlipidemic therapy; oral ulcers; lower extremity edema; gastrointestinal toxicity; neutropenia, anemia, or thrombocytopenia; viral, bacterial, or fungal infections; and benign or malignant neoplasms

- The incidence of a change in the immunosuppression drug regimen

- The incidence of immune sensitization defined by presence of anti-HLA antibodies absent prior to transplantation

- The incidence of discontinuation of immunosuppression

At 365 ± 14 days following the <u>first</u> 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.

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 patient age and insulin dependent diabetes duration of ≥ 28.
5. Absent stimulated c-peptide (<0.3ng/mL) in response to a mixed meal tolerance test (MMTT;Boost® 6 mL/kg body weight to a maximum of 360 mL; another product with equivalent caloric and nutrient content may be substituted for Boost) measured at 60 and 90 min after the start of consumption.
6. Involvement in intensive diabetes management defined as self monitoring of glucose values no less than a mean of three times each day averaged over each week and by the administration of three or more insulin injections each day or insulin pump therapy. Such management must be under the direction of an endocrinologist, diabetologist, or diabetes specialist with at least 3 clinical evaluations during the 12 months prior to study enrollment.

7. At least one episode of severe hypoglycemia in the 12 months prior to study enrollment.

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

   OR

   Marked glycemic lability characterized by wide swings in blood glucose despite optimal diabetes therapy and defined by an LI score greater than or equal to the 90th percentile (433 mmol/L^2/h·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.

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

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

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

3. HbA1c >10%.

4. Untreated proliferative diabetic retinopathy.

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

6. Measured glomerular filtration rate (using iohexol) of <80 mL/min/1.73m^2 (or for subjects with an iodine allergy, calculated using the subject's measured serum creatinine and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation).[8] Strict vegetarians (vegans) with a calculated GFR <70 mL/min/1.73m^2 are excluded. The absolute (raw) GFR value will be used for subjects with body surface areas >1.73 m^2.

7. Presence or history of macroalbuminuria (>300 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, 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). Participants with lymphopenia are allowed if the investigator determines there is no additional risk and obtains clearance from an independent hematologist.

16. A history of Factor V deficiency.

17. Any coagulopathy or medical condition requiring long-term anticoagulant therapy (*e.g.*, warfarin) after islet transplantation (low-dose aspirin treatment is allowed) or patients with an international normalized ratio (INR) >1.5. The use of Plavix is allowed only when portal vein access is obtained using a mini-laparotomy procedure at the time of islet transplant.

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

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

20. Symptomatic cholecystolithiasis.

21. Acute or chronic pancreatitis.

22. Symptomatic peptic ulcer disease.

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

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

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

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

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

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

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

30. Treatment with any immunosuppressive regimen at the time of enrollment.
31. A previous islet transplant.
32. A previous pancreas transplant, unless the graft failed within the first week due to thrombosis, followed by pancreatectomy and the transplant occurred more than 6 months prior to enrollment.

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Glossary of Abbreviations

ACE	American College of Endocrinology
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

FDR	False Discovery Rate
FSIGT	Frequently Sampled Intravenous Glucose Tolerance
FWER	Family-Wise Error Rate
G-CSF	Granulocyte Colony Stimulating Factor
GFR	Glomerular Filtration Rate
HbA1c	Glycosylated hemoglobin
HFS	Hypoglycemic Fear Survey
HIV	Human Immunodeficiency Virus
HLA	Histocompatability Antigen
HSA	Human Serum Albumin
HSV	Herpes Simplex Virus
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
MAGE	Mean Amplitude of Glycemic Excursions
MMTT	Mixed-Meal Tolerance Test
NCI	National Cancer Institute
NIAID	National Institute of Allergy and Infectious Disease
NIDDK	National Institute of Diabetes and Digestive and 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
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
SC	Subcutaneous
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
TNFR	Tumor Necrosis Factor Receptor
ULN	Upper Limit of Normal
UNOS	United Network for Organ Sharing
WHO	World Health Organization

Study Definitions

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

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.

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 <7.0% or a ≥ 2.5% decrease from baseline;

- Fasting capillary glucose level should not exceed 140 mg/dL (7.8 mmol/L) more than three times in the 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 ≥ 0.5 ng/ml.

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

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.

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).

Protocol eligible: Participants will be considered 'protocol eligible' once all screening assessments <u>required to confirm eligibility in the study</u> have been completed.

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

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).

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

1.   BACKGROUND AND RATIONALE

1.1   Background

Type 1 diabetes (T1D) afflicts nearly 2 million people in the United States, most of them children or young adults. Untreated, it is a fatal disease. Exogenous insulin, administered by multiple injections or by a continuous subcutaneous (SC) infusion from a wearable pump, allow long term survival in those who develop the disease, and most who are treated in this way will have a very good health-related quality of life. However, insulin therapy does not provide normal glycemic control, and long term survivors commonly develop vascular complications such as diabetic retinopathy (the most common cause of adult blindness) and diabetic nephropathy (the most common indication for adult kidney transplantation). The Diabetes Control and Complications Trial (DCCT) established that these microvascular complications of diabetes can be prevented by maintaining near-normal glucose control in patients with T1D[9]. However, this degree of control is not always achievable despite modern insulin analogs and delivery systems[10], and when achieved, it is invariably associated with episodes of insulin-induced hypoglycemia [11] that can be life-threatening.   A small minority of individuals with T1D develop hypoglycemia unawareness, a condition that is life-threatening, is associated with severe deterioration in quality of life and activity restriction, and is not amenable to medical therapy.

The hope of achieving near-normal glucose control without hypoglycemia in T1D has provided the impetus for developing effective strategies for β-cell replacement via pancreas or isolated islet transplantation. When successful, pancreas transplantation can normalize blood glucose (BG) in diabetic recipients, with associated stabilization and even reversal of microvascular complications[12]. However, the risks of the procedure (an almost 10% early failure rate due to technical complications, anastomotic leak, bleeding, and infection) and the need for lifelong immunosuppression have in most centers limited the target population of this therapy to diabetics <50 years of age with minimal or no coronary artery disease, and at some centers pancreas transplant is offered only with concomitant kidney transplant. As a result, T1D patients in need of β-cell replacement are often excluded from whole pancreas transplantation. Islet transplantation, in contrast, is accomplished by a much simpler procedure in which the islets are infused into the portal vein. While this procedure is not without risk, the procedural morbidity is much less than that of whole pancreas transplantation.

On the other hand, whereas about 80% of whole pancreas transplant recipients will be insulin independent at one year after their transplant, <10% of 447 islet recipients transplanted between 1990 and 1999 achieved one year insulin independence. This was attributed to low engrafted islet mass combined with high metabolic demand imposed by glucocorticoids used to prevent rejection. In the year 2000, the group from Edmonton reported a series of 7 consecutive islet transplant recipients treated with islets from multiple donors and a glucocorticoid-free immunosuppressive regimen[13]. These islet recipients were insulin free at follow-up ranging from 4.5 to 15 months. All of the recipients had experienced severe hypoglycemic episodes prior to transplant, and afterwards, none did. The efficacy of the Edmonton approach has now been confirmed by several other centers, and represents a major breakthrough in the field. However, it has also become clear that, in most islet recipients, there is loss of graft function over time; in Edmonton, insulin-independence rates have declined from 72% at one year to 28%

by three years[14].   A multicenter trial using the Edmonton protocol has both confirmed the results of the initial experience and raised questions relating to the expansion of the procedure to multiple centers, the toxicities of the immunosuppressive regimen, and the evaluation of the islet product.

1.2   Preclinical and Clinical Experience

1.2.1   Preclinical Studies

The first report of a systematic analysis of the efficacy of anti-lymphocyte serum (ALS) to prevent rejection of islet allograft was reported by Barker, *et al.* in 1973, demonstrating prolonged survival of allogeneic rat islets in recipients treated with rabbit anti-rat lymphocyte serum[15]. Further confirmation of the tolerogenic efficacy of ALS was confirmed by the permanent survival of rat islet allografts implanted into the thymus or the intraportal site following treatment of the recipients with a single injection of ALS[16,17]. The efficacy of ALS in large animal models was reported by Hirschberg, *et al.* [18] in a cynomolgus monkey model of islet transplantation including induction with high dose (20mg/kg) of Thymoglobulin® followed by sirolimus monotherapy as a maintenance immunosuppression. Thymoglobulin® resulted in marked lymphocyte depletion that gradually recovered in approximately one month after initiation of the treatment. In this study, the majority of the cynomolgus monkeys suffered from toxicities that were attributed to rapamycin; however, the surviving animals remained insulin independent for 169 days after reduction of rapamycin dose.

1.2.2   Clinical Studies

The rationale for utilization of Thymoglobulin® as an induction immunosuppression has been based on a number of basic studies demonstrating the beneficial effect of this agent on prevention of the recurrent autoimmune disease in transplanted islets, mediated by deletion of autoreactive memory cells or induction of regulatory T cells. As indicated below (please see section 1.4.2.1), two polyclonal anti-thymocyte antibody preparation are available in the US, Thymoglobulin® and ATGAM®. In randomized double blind clinical trials, Thymoglobulin® was noted to be more efficacious than ATGAM® for induction immunosuppression for the treatment of acute renal allograft rejection in adult renal transplant recipients. The repertoire of antibodies present in Thymoglobulin® includes a variety of anti-adhesion molecules that have been reported to interfere with leukocyte responses to chemotactic signals inhibiting expression of integrins required for cellular adhesions and mobility. This later effect may be the basis for the effectiveness of Thymoglobulin® in reducing the non specific inflammation during the reperfusion injury of the renal allografts. The clinical experience utilizing Thymoglobulin® as a component of induction therapy in renal transplantation or islet after kidney transplantation has been reviewed in section 1.4.2.1 (please see below). The most relevant experience utilizing induction with Thymoglobulin® in conjunction with maintenance with Rapamune® and Tacrolimus has been reported by Hering, *et al.* in eight type 1 diabetic recipients of islet transplants [19]. The objective of the study was to assess the efficacy and safety of a single donor islet transplant utilizing induction immunotherapy with Thymoglobulin® with the secondary objective of assessing the proportion of islet transplant recipients who achieve insulin

independence in the first year after islet transplantation.   There were no serious, unexpected procedure or immunosuppression related adverse events (AEs), and all recipients achieved insulin independence and freedom from hypoglycemia. This clinical experience may be related to improved islet engraftment secondary to pretransplant induction therapy with Thymoglobulin® and anti-inflammatory agent etanercept.

1.3   Rationale for Selection of Study Population

Iatrogenic hypoglycemia is a major unresolved problem for many patients with T1D. It is the limiting factor in the management of T1D, causing some deaths as well as recurrent physical, and recurrent (or even persistent) psychosocial, morbidity [20]. Iatrogenic hypoglycemia is a consequence of 3 compromised defense mechanisms, whose pathophysiology was thoroughly reviewed by Cryer[20-23].

First and perhaps most important, glucose-regulated insulin levels are not present in c-peptide-negative type 1 diabetic patients. The second defense mechanism, glucagon secretion in response to developing hypoglycemia, is lost in virtually all patients with T1D within 5 to 10 years after its onset [24]. Third, epinephrine response to falling glucose levels is compromised, in terms of the magnitude of the response and the threshold for the response [25], 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[26,27]. Type 1 diabetic patients with impaired epinephrine responses also have the clinical syndrome of hypoglycemia unawareness, which refers to the absence of adequate autonomic warning symptoms of developing hypoglycemia.

Hypoglycemia unawareness and the associated inability to respond adequately to falling glucose levels explain the frequent episodes of neuroglycopenia in such patients. Moderate hypoglycemia refers to a hypoglycemic episode complicated by neuroglycopenia in which the patient is still able to overcome the situation without assistance. Severe hypoglycemia refers to a situation in which neurologic impairment is severe enough to prevent self-treatment, placing patients at risk for injury to themselves or others. Accordingly, the DCCT Research Group defined severe hypoglycemia as an event with symptoms consistent with hypoglycemia in which the patient requires the assistance of another person; it is associated with a BG level below 50 mg/dL and with prompt recovery after oral carbohydrate, intravenous (IV) glucose, or glucagon administration[28]. The DCCT Research Group definition replaced the more stringent 1980s definition of severe hypoglycemia based on loss of consciousness[29-31].

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[26,32,33]. Hypoglycemia-associated autonomic failure is triggered by recurrent episodes of hypoglycemia, which reduce the magnitude of hormonal counterregulation and

reduce symptomatic responses to a given degree of subsequent hypoglycemia[21,34], thereby initiating and perpetuating a vicious cycle.

Hypoglycemia-associated autonomic failure is an important risk factor for severe hypoglycemia, which is associated with significant morbidity and mortality. Patients with hypoglycemia unawareness have a nearly 7-fold increased risk of severe hypoglycemia[35]. 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[26,27]. The patient characteristic that most strongly predicted severe hypoglycemia in the DCCT was a history of prior severe hypoglycemic events[36].

Hypoglycemia is said to be a major concern of prospective employers[37]. Neuroglycopenia can cause social embarrassment, and even lead to ostracism or be mistaken for disorderly or unlawful behavior[22]. The more distressing the severe hypoglycemic episode, the greater the psychological fear of hypoglycemia[38]. 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[22,39]. Pramming *et al.* found that their patients were as concerned about the development of severe hypoglycemia as they were about the development of blindness or renal failure[40].

In patients with long-term (*i.e.*, >15 years) T1D, scrupulous avoidance of hypoglycemia fails to restore normal glycemic thresholds or magnitudes of hormonal counterregulation to hypoglycemia. Avoidance of iatrogenic hypoglycemia sufficient to reverse the clinical syndrome of hypoglycemia unawareness does not normalize the key components of the clinical syndrome of defective glucose counterregulation (*i.e.*, deficient glucagon and epinephrine responses to hypoglycemia)[41-45]. In one recent report on patients with autonomic neuropathy and longstanding diabetes, Fanelli *et al.* demonstrated that, after meticulous prevention of hypoglycemia, only the threshold – not the magnitude – of responses of autonomic symptoms was normalized. In addition, the recovery of epinephrine responses to hypoglycemia was barely appreciable[46]. Thus, it appears that, while hypoglycemia unawareness is reversible by meticulous prevention of hypoglycemia, defective glucose counterregulation may not be reversible[47].

A successful pancreas transplant restores epinephrine responses and symptom recognition during hypoglycemia in patients with longstanding T1D and autonomic neuropathy[48]. In type 1 diabetic islet transplant recipients with documented pretransplant hypoglycemia unawareness and defective hormonal counterregulatory responses during hypoglycemia, Meyer *et al.* demonstrated, at 1 month post-transplant, improved glycemic thresholds and/or peak incremental responses of epinephrine, norepinephrine, and cortisol, as well as restoration of autonomic warning symptoms during hypoglycemia[49]. In a more recent study by Paty *et al.*, intrahepatic islet transplantation did not restore hypoglycemic hormonal counterregulation or symptom recognition in type 1 diabetic recipients[50]. Ryan *et al.* documented the absence of episodes of severe hypoglycemia in 12 successful islet transplant recipients (median follow-up, 10.2 months)[51] whose diabetes was complicated by recurrent episodes of severe hypoglycemia pretransplant. This would suggest that hypoglycemia associated autonomic failure associated with defective counterregulation and impaired sympathodrenal responses is not just due to recurrent hypoglycemia. After a sustained period without any hypoglycemia, most patients'

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

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

1.3.1   Glycemic Lability

Defining labile diabetes is a challenge but a working definition of labile diabetes may be: "Very variable glucose control associated with unpredictable responses to insulin". Labile diabetes is akin to the more extreme brittle diabetes which has been defined as describing the patient "whose life is constantly being disrupted by episodes of hypo- or hyperglycemia whatever their cause"[52,53]. Brittle diabetes in addition to lability has the added connotation that there may be associated frequent admissions to hospital[54,55]. 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[56] but now the most severe cases are recognized to usually have a factitious origin[57]. 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[54,55] but these are subject to the vagaries of local practice. More quantitative measures have been the mean amplitude of glycemic excursions (MAGE)[1] and the M value of Schlichtkrull[58]. The MAGE relies on capillary glucose readings over two days (a minimum of seven readings a day) and an amplitude is an excursion of glucose in excess of the standard deviation of the mean values for the day. If the mean of these amplitudes is ≥ 11.1 mmol/L the subject is considered to have labile diabetes. Where the MAGE fails as a measure of lability is with the subject who has a gradual decline in glucose over the day from 22 to 2 mmol/L. Such a profile will give a MAGE of 20 but such a gradual decline need not be considered truly labile.

Also used in the past has been the M value of Schlichtkrull[58], but this logarithmic expression of the glucose deviation from a standard glucose level has not been validated. More recently[6], the advent of continuous glucose monitoring system® (CGMS) has allowed insight into the patterns of glucose. The CGMS® profiles give exquisite details that have been quantified in terms of mean and standard deviation. Determining lability with this process has been more difficult and the suggested method has been the determination of the absolute value of measured glucose minus 5.5 mmol/L. This has the drawback that sustained high glucose levels will result in a high value but the profile may not necessarily be labile. In addition, the technique is currently limited to three days of monitoring and may be less accurate at low glucose levels[59].

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)[2]. 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/L^2/h ·wk^{-1} with a median of 223 (25 – 75th percentiles 130 – 329 mmol/L^2/h ·wk^{-1}). An LI ≥ 433 mmol/L^2/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   Investigational Product: 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[9]. However, this degree of control can be impossible to achieve in many patients despite modern insulin analogs and delivery systems[10], and also leads to life threatening episodes of insulin-induced hypoglycemia[11].

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[12]. 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[13]. 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[60]. 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 years[14]. Similarly insulin-independence rates at Miami have declined from 79% at one year to 20% by three years[61]. Recent data demonstrate a functionally low engrafted β-cell mass in insulin-independent transplant recipients under Edmonton immunosuppression that likely declines over time[62], 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 Immunosuppressive Medications for Initial Transplant

1.4.2.1 ANTI-THYMOCYTE GLOBULIN

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

Two polyclonal anti-thymocyte antibody preparations have been marketed in the United States, Thymoglobulin® and ATGAM®. Two randomized double-blind clinical trials indicated that Thymoglobulin® is more efficacious than ATGAM® for induction immunosuppressive therapy and for the treatment of acute graft rejection episodes in adult renal transplant recipients[63,64]. Thymoglobulin® induction therapy achieved rejection-free allograft survival in 96% of the patients. The incidence of cytomegalovirus (CMV) disease in the first year was 12.5%, and no patient developed post-transplant lymphoproliferative disease (PTLD). ATG is known to contain a variety of anti-adhesion molecule antibodies[65]. 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[63,65-68]. Recent studies have shown that early administration of a variety of antibodies directed at adhesion molecules reduces graft dysfunction, and acute and chronic rejection associated with ischemia-reperfusion injury and brain death[69].

The resistance of islet-directed autoimmune responses to conventional immunosuppressive drugs [70-74] and the immediate exposure of intraportally transplanted islets to primed autoreactive, islet beta cell-directed T cells provide a strong rationale for pretransplant initiation of ATG, which is known to cause selective depletion of activated T cells and dose-dependent depletion of resting T cells[75]. 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[76,77]. Clinical evidence also indicates that destructive anti-islet autoimmunity persists for decades after manifestation of T1D[71,78,79] and that type 1 diabetic individuals with long disease duration do not spontaneously anergize their autoreactive effector Th1 cells and/or restore Th2 or other regulatory T cell function. Accordingly, reprogramming the recipient's immune system seems to be of paramount importance if autoimmune recurrence in transplanted islets is to be prevented.

Maki *et al*. demonstrated that immunotherapy of non-obese diabetic (NOD) mice with ALS after development of overt autoimmune diabetes leads to long-lasting abrogation of autoimmunity[80]. ALS given within 14 days of disease onset gradually reversed hyperglycemia with a 76% cumulative incidence of remission. Diabetic NOD mice that failed to respond to ALS treatment accepted subsequent islet isografts for a prolonged period (mostly >100 days), indicating that autoimmunity was abrogated in the latter animals in which extensive irreversible beta cell destruction had already occurred by the time of ALS treatment. These experimental findings are corroborated by clinical observations reported by the Brussels group[81]. Of 7 islet-after-kidney recipients treated at Brussels, only the 3 patients who had received ATG as induction immunosuppressive therapy during the first 10 days following their previous kidney transplant showed long-term islet graft survival. Furthermore, according to an analysis performed by the International Islet Transplant Registry on all 50 insulin-independent, type 1 diabetic islet allograft recipients transplanted through 1999, 23 had received single-donor islet transplants, and 19 of those 23 had received anti-thymocyte or anti-lymphocyte globulin for induction immunosuppression and 1 had received ATG at the time of a previous pancreas transplant[82]. 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%)[83]. 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 97.0%, 97.0% and 75%, respectively. CMV infection was diagnosed in eight patients. All but one patient tolerated the ATG course well[84]. These two studies indicate that ATG can be used safely and effectively without concomitant steroid administration.

The total ATG dose to be administered is 6 mg/kg. This dose is based on studies performed at Washington University in St. Louis[85]. This reduced total dose of ATG has been found to be equally effective for induction immunosuppression in kidney transplantation when compared to historical controls that had received 1.5 mg/kg per day for at least seven days[63]. The proposed ATG dose escalation strategy has been pioneered by James Russell in Calgary, Alberta, in more than 70 bone marrow transplant recipients (presented at the European Bone Marrow Transplant Meeting in Innsbruck, Austria, April, 2000). The University of Minnesota has reported their preliminary experience with this regimen of ATG administration in 8 type 1 diabetic islet transplant recipients[82]. ATG was found to be effective in preventing rejection and autoimmune recurrence. All eight recipients have achieved insulin independence. The medication was well tolerated in all subjects; unexpected acute complications were not encountered. Serious adverse events (SAEs) were not encountered secondary to ATG.

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

Induction immunotherapy with anti-interleukin-2 receptor antibody is a critical component of the steroid-free immunosuppressive protocol recently developed for islet transplantation by the Edmonton group[13]. The safety and efficacy of daclizumab and basiliximab have previously been documented in multi-center trials in renal transplantation. When added to therapy with cyclosporine, azathioprine, and prednisone, daclizumab reduced the frequency of acute rejection and improved short-term graft survival in renal transplant recipients, and basiliximab reduced the frequency of acute rejection and did not affect graft or patient survival. At six months, there were no significant differences between the daclizumab or basiliximab and the placebo group with respect to infectious complications or cancers[86].

1.4.2.2 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 recipients[87]. To maintain normoglycemia, immunosuppressed non-diabetic kidney transplant recipients must increase insulin secretion 2.5 times[88]. Even when systemic drug levels are carefully controlled, intraportally transplanted

islets bathed in portal blood are exposed to higher and probably toxic local concentrations of orally administered immunosuppressive drugs[89]. 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[13,88,90,91].

The Edmonton group therefore developed a steroid-free maintenance immunosuppressive protocol based on the combination of sirolimus and low-dose tacrolimus[13]. This strategy was designed to provide potent synergistic immunosuppression, thus avoiding diabetogenic impact on a limited beta cell transplant reserve[89-91].

Sirolimus is a promising agent for maintenance immunosuppression of islet allograft recipients, mainly because of its efficacy in the absence of diabetogenic side effects[92]. Sirolimus is as effective as cyclosporine A in preventing renal graft loss due to rejection while maintaining superior graft function[93]. Sirolimus combined with the concentration-controlled regimen of cyclosporine presents a promising synergistic regimen, which reduces the incidence of acute rejection episodes among recipients of kidney grafts markedly, permits profound cyclosporine dose reduction, and facilitates corticosteroid avoidance or withdrawal[94]. A recent pilot study in 32 organ transplant recipients (liver, kidney, and pancreas) demonstrated the safety and efficacy of a regimen combining sirolimus with a low dose (33% of the recommendation) of tacrolimus and steroids[95]. 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 regimen[13].

1.4.3   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®. Basiliximab will be used instead of Thymoglobulin® for all subsequent islet transplants.

1.4.4   Immunosuppressive / Anti-inflammatory Therapy: Etanercept

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 autoimmunity[96]. Blockade of TNF in the neonatal period results in a dramatic increase in the levels of CD4+CD25+ regulatory T cells in NOD mice[96,97]. 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±1,035 islet equivalents (IEQ)/kg from a single-donor pancreas[19]. 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 cells[98]. In murine models, selective inhibition of TNF-α in the peri-transplant period has promoted reversal of diabetes after marginal-mass islet isografts[99]. 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 µg at –24hrs, 50 µg 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 kidney[100-103] and bone marrow transplant recipients[104,105]. 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 study[100] 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[104,105] 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 volunteers[106], a bioavailability study in healthy volunteers[107] and a toxicity and dose finding trial in refractory rheumatoid arthritis[108]. The time to maximum concentration after subcutaneous and intravenous etanercept administration were found to be 66 and 0.8 hrs, respectively[107]. 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 3 subjects who received Campath® and etanercept is off insulin after receiving islets from 1 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) trial[109] 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 (ACRArg) on days ≥180 post-transplant: 1.07±0.15 ng/mL (vs. 0.74±0.21 ng/mL in hOKT3γ1 (Ala-Ala) trial[109]; p=0.028). This improvement occurred despite transplantation of fewer islets: 7,271±1,035 (vs. 10,302±2,594 IE/kg in a previous trial[109]; p=0.01). To facilitate comparison of the proportion of engrafted islets between studies, the ACRArg was corrected for implanted IE/kg, and expressed as the engraftment index. The engraftment index in the ATG plus etanercept islet transplant trial was $150\pm29 \times 10^{-6}$ ng•kg/mL, as compared with $73\pm23 \times 10^{-6}$ ng•kg/mL in the hOKT3γ1 (Ala-Ala) trial[109]. 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 Use of Investigational Agent: Transplant of 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 are discussed separately in Section 1.5.4.

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, , 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 islets[110]. Additionally, the University of Minnesota investigators have previously reported on the incidence and significance of contaminated islet preparations in clinical islet auto- and allotransplantation[111]. Positive cultures from islet tissue preparations were identified in 11 of 29 patients (38%) receiving autologous islets. The occurrence of serious infection morbidity (as defined as positive blood cultures, abscesses, or intra-abdominal infections) did not differ significantly between the positive and negative culture groups (p=0.99). In the allogeneic islet transplant group, 7 of 33 patients (21%) received tissue that retrospectively were determined to be contaminated. None of these patients developed serious infectious complications (despite broad-spectrum immunosuppression). Despite the occurrence of contaminated grafts, there

was no serious increase in infectious morbidity. Presumably the inocula were kept low by the multiple washing steps allowing the recipients to clear the organisms without serious sequelae.

Of the islet allotransplants performed at the University of Minnesota between 1993 and 1999, 3 of 20 patients (15%) received tissue that was retrospectively determined to be contaminated. The species isolated included *Candida krusei, Enterococcus faecium*, and two strains of coagulase-negative Staphylococcus. None of these patients have had SAEs related to the contamination of the transplanted islet tissue.

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

1.5.1.3 SENSITIZATION OF THE RECIPIENT TO DONOR ANTIGENS

As with any allogeneic transplant, islet transplant recipients may become sensitized to islet-donor histocompatibility antigens (HLA), leading to development of panel reactive alloantibodies (PRA). These alloantibodies may develop while the recipients demonstrate full or partial islet function on maintenance immunosuppression. Furthermore, donor specific alloantibodies may develop after loss of the islet transplant function and discontinuation of the immunosuppressant drug. Data on the development of cytotoxic antibodies against donor HLA in islet allotransplant recipients with failing grafts have been reported from several islet transplant centers[112-115] 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 immunsuppression[116-118]. Importantly, the incidence of elevated PRA (>20%) in recipients who had lost their islet transplant function and discontinued their immunosuppression rose to approximately 84%.

The available information suggests that there is a strong correlation between islet allograft failure and a rise in anti-donor HLA sensitization as detected by PRA testing. A potential consequence of high PRA levels in type 1 diabetic recipients with failed islet transplants is that if these individuals develop diabetic nephropathy in the future, it may increase their time waiting on a transplant list to qualify for a suitable kidney[119].

1.5.1.4 ACCELERATION OF RETINOPATHY WITH ACUTE CORRECTION IN
GLYCEMIC CONTROL

In the DCCT study[28], 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[120]. 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   Risk of Induction and Maintenance Immunosuppressive Therapies

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

1.5.2.1 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.

1.5.2.2 RABBIT ANTITHYMOCYTE GLOBULIN (THYMOGLOBULIN®)

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

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

Published results of the use of Thymoglobulin® in clinical and experimental islet transplantation are limited to relative small cohorts. Hirshberg *et al.* described the successful role of rabbit ATG and sirolimus in reducing rejection of islet allografts in primates, with no evidence of direct islet toxicity from Thymoglobulin®[18]. Hering *et al.* described a beneficial role of Thymoglobulin® induction (6mg/kg) in 8 patients with T1D receiving single donor islet grafts, all of whom achieved insulin independence and were protected against recurrence of hypoglycemia[19]. Acute islet rejection was described in patients receiving calcineurin-free immunosuppression when sirolimus levels fell below 9ng/mL. The use of higher doses of sirolimus exacerbated the neutropenic side effects of Thymoglobulin®, but these could be managed safely without risk of opportunistic infections when appropriate dose reduction and/or administration of Granulocyte Colony Stimulating Factor (G-CSF; Neupogen®) if required[19].

1.5.2.3 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 exposure[121]. Sirolimus alters the pharmacokinetic profiles of other CNIs (*e.g.*, tacrolimus) and may thereby potentiate nephrotoxicity[122]. Fervenza *et al.* described nephrotoxicity from sirolimus in patients with chronic glomerulopathies that was non-reversible on cessation of therapy[123]. 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[124,125].

The majority of islet transplant recipients receiving sirolimus in conjunction with tacrolimus have experienced transient mouth ulceration, lower extremity edema[13,125]; perinephric edema and a high incidence of benign ovarian cysts have also been described in islet recipients in association with sirolimus[126]. Pneumonitis and colitis have also occurred[127,128]. 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.

1.5.2.4   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 (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.

1.5.2.5 CYCLOSPORINE (NEORAL®)

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

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

CellCept® and Myfortic® are associated with: diarrhea, leucopenia, vomiting, and evidence of higher frequency of certain types of infections. CellCept® and Myfortic® may increase the risk of developing 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®.

1.5.3   Risks of Immunosuppressive / Anti-inflammatory Therapy: Etanercept (Enbrel®)

Etanercept is a dimeric soluble form of the p75 TNFR that blocks TNF binding and reduces inflammation[100-104]. 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 Enbrel® 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 patients[129-131], and based on post-marketing studies warnings have been issued about the following conditions, which have been reported with the use of Enbrel®: serious infections of sepsis, including fatalities; an increased risk of lymphoma and other malignancies in children and adolescents; and leukemia. 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 mice[99]. 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 transplantation[19].

1.5.4   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, 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.

1.5.4.1 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.

1.5.4.2 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.

1.5.4.3 THE PROCEDURAL RISKS OF ISLET TRANSPLANTATION

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

Open Surgical Approach

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

Percutaneous Transhepatic Approach

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

Risk of Bleeding after Percutaneous Islet Transplantation

In the 158 islet transplant procedures submitted to the Collaborative Islet Transplant Registry (CITR), the reported SAEs associated with bleeding include hemoperitoneum (n=1), intraabdominal bleed (n=2), low hemoglobin (n=1), right hemothorax (n=1), and subcapsular hematoma (n=1) of the liver[132]. Subcapsular hematoma of the liver following percutaneous transhepatic injection of islets into the portal vein in two cases has also been reported to the international Islet Transplant Registry. No surgical intervention was necessary [133]. One instance of injury to hepatic artery leading to death during percutaneous transhepatic catheterization of the portal vein has been reported previously to the Islet Transplant Registry[133]. Reports on intra-abdominal (n=1) [128] and intrathoracic bleeding (n=1)[134] have been published. The risk of significant hemorrhage after percutaneous islet transplantation defined as a drop in hemoglobin of more than 25 g/L or the need for transfusion or surgery was 9% in the Edmonton series[124]. Subsequently, a further increase in risk of bleeding has been observed by the Edmonton program and has been attributed in part to concomitant aspirin therapy[125]. The risk has since been ameliorated by avoidance of pre-transplant aspirin and more effective measures to seal the catheter tract in the liver[125]. When effective methods are used to ablate the transhepatic portal catheter tract, bleeding can be avoided completely; at the University of Miami, D-Stat thrombostatic agent has been used to seal the catheter tract and has avoided risk of bleeding [135]. At the University of Minnesota, no bleed-related complications occurred in 20 consecutive subjects when the catheter tract was sealed with combined coils and gelfoam [19].

Hypoglycemia

Severe hypoglycemia is a risk associated with the infusion of islets. Iatrogenic hypoglycemia in the immediate post-transplant period is a rare event. Frequent blood glucose monitoring immediately following islet transplantation is recommended to avoid severe unrecognized hypoglycemia in the early post-transplant period. In longer-term follow-up, life-threatening hypoglycemia (Grade 4) occurred in six of the 236 SAEs reported to CITR[132]. 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 surgery [136].

Disseminated Intravascular Coagulation (DIC)

DIC has been documented after autologous islet transplantation of dispersed pancreatic islet tissue in 3 out of about 400 patients expected to have undergone this procedure[137-139]. 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[140,141]. Three of the 86 islet transplant recipients reported to CITR have experienced transient elevations of liver enzymes requiring prolongation of post-transplant hospitalization or admission[132]. 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 weeks[140]. 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[142,143] and appears to be due to a paracrine action of insulin secreted from intrahepatic islets. More subjects with steatosis required supplementary exogenous insulin than not [142], suggesting that steatosis may be associated with insulin resistance and graft dysfunction. The clinical relevance of steatosis associated with intrahepatic islet transplantation remains questionable. To the best of our knowledge, there is no evidence of clinically significant, persistent liver dysfunction following intraportal islet transplantation.

Portal Hypertension

Portal hypertension following intraportal infusion of unpurified allogeneic islet tissue resulted in a tear of the splenic capsule requiring splenectomy in one case[133]. The elevation in portal pressure following intraportal islet transplantation is temporary in most instances. In 1981, Cameron *et al*. reported on 4 patients with chronic pancreatitis who developed portal

hypertension during intraportal infusion of only partially-purified auto-islet preparations, and in whom direct or indirect measurements of portal pressure were performed 3 to 12 months later[144]. In all patients, the portal pressure had returned to normal and portal venograms were normal. Casey *et al.* reported on changes in portal pressure following sequential islet transplants at the University of Alberta, and found that third islet transplants were associated with significantly greater final portal pressures (18 mmHg) than first or second transplants (12 mmHg)[145]. The baseline pressures were normal in all cases, suggesting absence of chronic portal hypertension[145].

Portal Vein Thrombosis

Transplanted islets release tissue factor and exhibit prothrombotic properties when infused to an intravascular site such as the portal vein[146]. A partial portal vein thrombosis has been reported in one of six patients transplanted at the intramural National Institutes of Health (NIH) program[128]. In the Edmonton single-center experience, the risk of partial vein thrombosis was 3% in more than 100 intraportal islet transplants[147]. The management of partial vein thrombosis includes anticoagulation therapy which may lead to intra-abdominal hemorrhage requiring transfusion and surgical intervention[148]. There is one published report of complete thrombosis of the portal vein thrombosis after transplantation of partially purified pancreatic islets in a combined islet/liver allograft, which necessitated emergency re-transplantation of the liver[149]. This complication probably related to the transplantation of partially purified islet tissue derived from 4 donors into a freshly transplanted liver. A right upper quadrant ultrasound including Doppler examination of the portal vein is performed on islet transplant recipients on days 1 and 7 post-transplant. Early diagnosis and prompt management of branch vein portal occlusion with systemic heparinization may prevent clot propagation. Repeated intraportal islet transplants are generally contraindicated in patients that have experienced prior portal thrombus.

Injuries to Other Structures

One instance of gall bladder perforation during percutaneous transhepatic catheterization of the portal vein requiring laparoscopic cholecystectomy has been reported to the Islet Transplant Registry[133]. Acute cholecystitis, possibly related to percutaneous transhepatic catheterization of the portal vein, has been noted in 2 of the 86 islet allograft recipients reported to CITR[132]. Gall bladder hematoma (n=1) and gall bladder opacification (n=2) have been observed as well.

1.5.4.4 FOLLOW-UP PROCEDURES

Glomerular Filtration Rate (GFR)

Risks associated with the 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[150].

1.5.5   <u>Benefits</u>

Successful islet transplantation alleviates T1D patients from life-threatening hypoglycemia and psychosocially debilitating glycemic lability[2]. While the long-term durability of these responses is at present uncertain, they persist for as long as some graft function is maintained, despite the eventual return to insulin therapy in the majority of recipients. This partial function, as indicated by continued c-peptide production, may be present in as many as 80% of recipients after 5 years[14]. Furthermore, as long as graft function is maintained, fear of hypoglycemia and anxiety are significantly lower after islet transplantation[151]. Indeed, T1D subjects in the DCCT who had persistent c-peptide production had a significantly reduced risk of severe hypoglycemia despite intensive insulin therapy[152]. Additionally, while most transplant recipients experience only a temporary reprieve from exogenous insulin therapy, a few have maintained insulin-independent graft function for more than 3 years. Novel strategies aimed at promoting the engraftment or survival of transplanted islets may lead to improved long-term graft function and further the duration of insulin-independence after transplantation, and hopefully lead to reductions in the secondary complications of T1D.

2.   OBJECTIVES

2.1   Primary Objective

The primary objective is to 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.

2.2   Secondary Objective

To establish islet release criteria that accurately characterize the islet product and are predictive of clinical transplant outcomes.

3.   SELECTION OF SUBJECTS

3.1   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 patient age and insulin dependent diabetes duration of ≥ 28.

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

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

7. At least one episode of **severe hypoglycemia** in the 12 months prior to study enrollment.

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

    OR

    Marked glycemic lability characterized by wide swings in blood glucose despite optimal diabetes therapy and defined by an LI score greater than or equal to the 90th percentile (433 mmol/L^2/h ·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.

3.2   Exclusion Criteria

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

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

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

3. HbA1c >10%.

4. Untreated proliferative diabetic retinopathy.

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

6. Measured glomerular filtration rate (using iohexol) of <80 mL/min/1.73m^2 (or for subjects with an iodine allergy, calculated using the subject's measured serum creatinine and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation[153]). Strict vegetarians (vegans) with a calculated GFR <70 mL/min/1.73m^2 are excluded. The absolute (raw) GFR value will be used for subjects with body surface areas >1.73 m^2.

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

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

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

10. 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). Participants with lymphopenia are allowed if the investigator determines there is no additional risk and obtains clearance from an independent hematologist.[8]

16. A history of Factor V deficiency.

17. Any coagulopathy or medical condition requiring long-term anticoagulant therapy (*e.g.*, warfarin) after islet transplantation (low-dose aspirin treatment is allowed) or patients

with an international normalized ratio (INR) >1.5. The use of Plavix is allowed only when portal vein access is obtained using a mini-laparotomy procedure at the time of islet transplant.

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

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

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

    c) left ventricular ejection fraction <30%.

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

20. Symptomatic cholecystolithiasis.

21. Acute or chronic pancreatitis.

22. Symptomatic peptic ulcer disease.

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

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

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

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

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

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

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

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

31. A previous islet transplant.

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

4. STUDY DESIGN

This is a prospective, single-arm, multi-center study in islet transplantation. The centers participating in this phase 3 study will also undertake separate, phase 2 studies in islet transplantation, using innovative manufacturing and/or immunosuppressive regimens. These phase 2 trials will have inclusion/exclusion criteria and endpoint measures that are identical to those in the phase 3 trial. In order to avoid bias in selection of subjects for these studies, eligible subjects will be randomized, prior to transplantation, to participate either in the phase 3 or a site-specific phase 2 study.

Subjects who meet the general inclusion/exclusion criteria will be approached regarding their participation. Subjects who sign 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. The participating centers will accrue subjects over a 24 month period and will treat a total of 48 study subjects.

Identify and approach *potential study subject*

If *potential study subject* agrees to participate, obtain Written Informed Consent

Screening and Enrollment
Enroll *subject* using Internet system and receive computer generated unique subject ID number.
Perform Screening Assessments (Appendix 1) & evaluate all Inclusion/Exclusion criteria to determine subject eligibility

Place eligible *subject* on waiting list

Waiting List / Baseline procedures
Repeat Screening Assessments as indicated on the SOE (Appendix 1) to ensure *subject* remains eligible for transplant

Compatible islet prep available

Evaluate all Inclusion/Exclusion criteria to reconfirm eligibility

If applicable, randomize eligible *subjects* between this study and site-specific Phase 2 trial (Day -2). Obtain post-randomization consent immediately after randomization.

Islet Transplant
Eligible *subjects* receive IS regimen and initial islet transplant

Follow-up and Subsequent Islet Transplants
Subjects are followed for 24 months post last transplant
Subjects may receive up to a total 3 islet transplants (See Section 7.6 for criteria and timing)

Figure 1: Study design diagram

4.1 Study Endpoints

4.1.1 Primary Endpoint

The primary endpoint for this study is the proportion of subjects with an HbA1c <7.0% at Day 365 AND free of **severe hypoglycemic events** from Day 28 to Day 365 inclusive following the first islet transplant, with the day of transplant designated Day 0.

4.1.2 Secondary Endpoints

Because there are a large number of secondary endpoints, it is impractical to account for all multiple comparisons. However, a few secondary endpoints have been identified as **key** secondary endpoints.

Key Secondary Endpoints

The target level for HbA1c chosen for this study is 7.0%. This value was chosen because it is the level recommended by the American Diabetes Association and is considered to be the clinically relevant goal for subjects with T1D. A HbA1c level of 6.5% is the goal recommended by the American College of Endocrinology. We have included achieving a HbA1c level of 6.5%, alone and as a composite with freedom from **severe hypoglycemic events** at 1 year after the first islet transplant, as **key** secondary endpoints because they correspond to the ACE recommendations and will be of interest to the medical community. The key secondary endpoints are the following:

1) The proportion of subjects with an HbA1c <7.0% AND free of severe hypoglycemic events from Day 28 to Day 730, inclusive, after the first islet transplant.

2) The proportion of subjects with HbA1c ≤ 6.5% at one year after the first islet transplant AND free of severe hypoglycemic events from Day 28 to Day 365 after the first islet transplant.

3) The proportion of subjects with HbA1c ≤ 6.5% at two years after the first islet transplant AND free of severe hypoglycemic events from Day 28 to Day 730 after the first islet transplant.

4) The proportion of subjects free of severe hypoglycemic events from Day 28 to Day 365 after the first islet transplant.

5) The proportion of subjects free of severe hypoglycemic events from Day 28 to Day 730 after the first islet transplant.

6) The proportion of subjects with HbA1c <7.0% at one year after the first islet transplant.

7) The proportion of subjects with HbA1c <7.0% at two years after the first islet transplant.

8) The proportion of subjects with HbA1c ≤6.5% at one year after the first islet transplant.

9) The proportion of subjects with HbA1c ≤6.5% at two years after the first islet transplant.

10) The proportion of insulin-independent subjects at one year after the first islet transplant.

11) The proportion of insulin-independent subjects at two years after the first islet transplant.

Other secondary endpoints include the following:

- The proportion of subjects with an HbA1c <7.0% AND free of severe hypoglycemic events from Day 28 to Day 730, inclusive, after the final islet transplant.

- The proportion of subjects with HbA1c ≤ 6.5% at one year after the final islet transplant AND free of severe hypoglycemic events from Day 28 to Day 365 after the final islet transplant.

- The proportion of subjects with HbA1c ≤ 6.5% at two years after the final islet transplant AND free of severe hypoglycemic events from Day 28 to Day 730 after the final islet transplant.

- The proportion of subjects free of severe hypoglycemic events from Day 28 to Day 365 after the final islet transplant.

- The proportion of subjects free of severe hypoglycemic events from Day 28 to Day 730 after the final islet transplant.

- The proportion of subjects with HbA1c <7.0% at one year after the final islet transplant.

- The proportion of subjects with HbA1c <7.0% at two years after the final islet transplant.

- The proportion of subjects with HbA1c ≤6.5% at one year after the final islet transplant.

- The proportion of subjects with HbA1c ≤6.5% at two years after the final islet transplant.

- The proportion of insulin-independent subjects at one year after the final islet transplant.
- The proportion of insulin-independent subjects at two years after the final islet transplant.

Efficacy Endpoints

At 75 ± 5 days following the first and subsequent transplant(s):
- The percent reduction in insulin requirements
- HbA1c
- MAGE[1]
- LI[2]
- Ryan hypoglycemia severity (HYPO) score[2]
- Basal (fasting) and 90-min glucose and c-peptide derived from the mixed-meal tolerance test (MMTT)
- β-score[3]
- C-peptide: (glucose · creatinine) ratio
- Acute insulin response to glucose (AIR_{glu}), insulin sensitivity, and disposition index derived from the insulin-modified frequently-sampled IV glucose tolerance (FSIGT) test[4,5]
- Glucose variability[6] and hypoglycemia duration[7] derived from the CGMS
- Quality of life (QOL) measures

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

At 365 ± 14 days following the <u>first</u> and <u>final</u> 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 · creatinine) ratio
- AIR_{glu}, insulin sensitivity, and disposition index derived from the FSIGT test[4,5]
- 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)

At two years following the <u>final</u> 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)
- β-score
- C-peptide: (glucose• creatinine) ratio
- CGMS
- QOL

Safety Endpoints

At 75 ± 5 days following <u>each</u> transplant, at 365 ± 14 days following the <u>first</u> and <u>final</u> 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; increase in urinary albumin excretion; addition or intensification of anti-hypertensive therapy; addition or intensification of anti-hyperlipidemic therapy; oral ulcers; lower extremity edema; gastrointestinal toxicity; neutropenia, anemia, or thrombocytopenia; viral, bacterial, or fungal infections; and benign or malignant neoplasms

- The incidence of a change in the immunosuppression drug regimen
- The incidence of immune sensitization defined by presence of anti-HLA antibodies absent prior to transplantation
- The incidence of discontinuation of immunosuppression

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 <u>first</u> 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.

5.   STUDY TREATMENT REGIMEN

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

	Days Relative to Transplant															
	-2	-1	0	1	2	3	4	5	6	7	8	9	10	14	28	42	56
Islet Transplant			X														
ATG *(initial transplant only)*	◆———————●																
Basiliximab *(only subsequent transplants or 2nd attempts at initial transplants)*			X				X										
Sirolimus	◆——————————————————————————————————▶																
Etanercept			X			X				X			X				
Tacrolimus					◆——————————————————————————————▶												
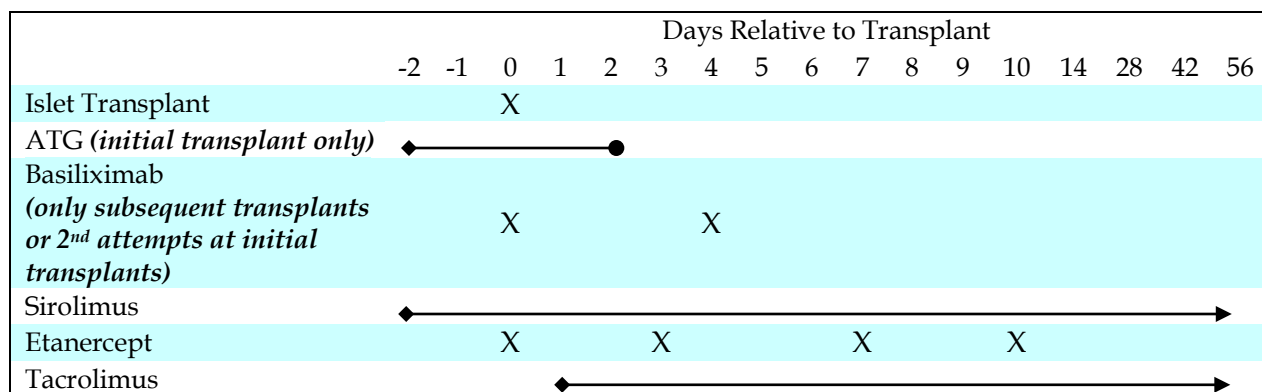
Figure 2: Islet transplant and immunosuppression regimen

5.1   Investigational Agent: Allogeneic Islets

5.1.1   Formulation, Dosage, and Administration

The final product is a 200 mL sterile suspension of ≥70% viable, ≥30% pure, allogeneic human purified islets in 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 ≥5,000 IEQ/kg recipient body weight (BW) for the first transplant, and ≥4,000 IE/kg recipient BW for subsequent transplants.

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

Component	Quantity per Batch
Purified Human Pancreatic Islets	≥4.0 x 10^3 IEQ/kg recipient BW (total IEQ/infusion)
CMRL 1066 Transplant Media, with HEPES and without sodium bicarbonate	q.s. to 200 mL per bag
Human Serum Albumin (HSA), USP	2.5%

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 rare circumstance that percutaneous access cannot be achieved).

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

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

5.1.2   Drug Accountability

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

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

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

5.2   Immunosuppression Medications

5.2.1   Initial Allogeneic Islet Transplant

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

5.2.1.1   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.

5.2.1.2 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.

5.2.1.3 TACROLIMUS (PROGRAF®)

Tacrolimus will be administered at an initial dose of 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 ≥33% compared with baseline, a nephrology consult will be obtained, and tacrolimus target trough levels will be reduced by 25% should CNI toxicity be suspected as the primary cause for the decline in renal function.

5.2.1.4 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.

5.2.1.5 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 <1x10^9/L) while taking mycophenolate mofetil, mycophenolate mofetil exposure will be reviewed and

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

5.2.1.6 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®.

5.2.2 Subsequent Allogeneic Islet Transplants and Second Attempts at Initial Transplants

The immunosuppressive regimen for subsequent islet transplants and second attempts at initial transplants (see section 5.7.1) will be identical to the regimen for the initial islet transplant with the following exceptions.

5.2.2.1.1 BASILIXIMAB (SIMULECT®)

Two IV doses of basiliximab, a monoclonal antibody IL-2 receptor blocker, 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.

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

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

5.3 Concomitant Medications

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 6 months after islet transplant. 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-transplant. If the CMV status of the donor and recipient are both negative, then valgancyclovir 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. If a site does not use PTT to titrate heparin, a comparable site-specific method and value should be used.

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 post-transplant (Day 7).

5.3.4   **Updated Vaccinations**

Subjects will remain up to date on CDC-recommended adult vaccinations; please refer to the MOP for guidance.

5.3.5   **Insulin Therapy**

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

5.3.6   **Other Standard Therapies**

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

5.4   Rescue Medications

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

5.5   Prohibited Medications

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

- steroid medication (save topicals and prednisone at a dose of ≤ 5 mg daily, or an equivalent dose of hydrocortisone, for physiological replacement only)
- any medications in the macrolide antibiotic class other 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 nonadherence, lack of compliance that poses a significant clinical risk and or derangement of protocol data collection will be documented. Please refer to Section 5.7.2 for a description of possible indications for premature discontinuation of study treatment.

5.7   Modification or Discontinuation of Study Treatment

5.7.1   Modification of Consensus Immunosuppression Regimen

5.7.1.1 ISLETS ARE UNSUITABLE

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.

5.7.1.2 GRAFT FAILURE

Subjects who experience graft failure will be maintained on their current immunosuppressive regimen as long as a subsequent transplant is possible. If/when it is determined that a subject will not receive a subsequent transplant, then immunosuppression will be stopped and the subject will move to the reduced follow-up schedule (see section 5.7.2).

5.7.1.3 ALLERGIC REACTION TO ATG

If a subject demonstrates an allergic reaction to thymoglobulin that results in cancellation of the initial transplant and the investigators feel that future use of the drug in that subject is contraindicated, the steps outlined in section 5.7.1.1 should be followed. Once another organ becomes available, the subject will receive the alternate immunosuppressive regimen outlined in section 5.2.2.

5.7.1.4 INTOLERANCE OF PROTOCOL MEDICATIONS

In the event that protocol-regulated concomitant medications are not tolerated, the subject will continue taking the immunosuppressive therapy in order to protect the islet graft. In the event that the immunosuppression regimen is not tolerated, the Site principal investigator (PI) may elect to prescribe an alternative immunosuppression regimen. The intent would be for the alternative regimen to be temporary in nature where possible. Any non-protocol directed study treatment modification that the site PI determines is necessary should be reported as a protocol deviation.

5.7.1.5 RABBIT ANTI-THYMOCYTE GLOBULIN-INDUCED ANAPHYLAXIS

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

5.7.1.6 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.

5.7.1.7 NEUTROPENIA

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

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

- Obtain Infectious Disease Consult.

- Hold rabbit ATG.

- Hold valganciclovir and trimethoprim/sulfamethoxazole.

- Review and obtain current sirolimus trough levels and consider dosage adjustment if trough level are >12ng/mL.

- If subject is using mycophenolate mofetil or mycophenolate sodium in lieu of sirolimus, consider dose reduction.

- Administer G-CSF.

- Monitor temperature BID.

- Follow up within 48-72 hours to obtain: repeat CBC with differential, subject symptoms, and measured temperatures.

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

- Hold rabbit ATG.

- Hold administration of trimethoprim/sulfamethoxazole and/or valganciclovir.

- Review and obtain current sirolimus trough levels and hold dose if trough level are >12ng/mL.

- If subject is using mycophenolate mofetil or mycophenolate sodium in lieu of sirolimus, 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/μL and the subject is febrile, then the following will be done:

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

- Hold rabbit ATG.

- Hold administration of trimethoprim/sulfamethoxazole and/or valganciclovir.

- Review and obtain current sirolimus trough levels and hold dose if trough level are >12ng/mL.

- If subject is using mycophenolate mofetil or mycophenolate sodium in lieu of sirolimus, consider holding dose.

- Obtain CMV antigenemia or PCR for CMV.

- Administer G-CSF.

5.7.1.8 THROMBOCYTOPENIA

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

5.7.1.9 NEPHROTOXICITY

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

Table 2: Response to nephrotoxicity

Allowable therapeutic responses to CNI-induced 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[122,154].
If the trough sirolimus level is maintained at >10 ng/mL without adverse effects, discontinue the CNI and replace it with mycophenolate mofetil or mycophenolate sodium.	CNI should be discontinued only if the subject can tolerate a trough level of sirolimus that will result in adequate immunosuppression.
Decrease the target CNI trough level by 25%	CNI toxicity is dose-related.

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

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

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

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

1. The subject is unwilling or unable to comply with the protocol.

2. The investigator believes that the study treatment is no longer in the best interest of the subject.

3. Graft Failure (see Study Definitions and section 5.7.1.2).

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 scheduled outlined in Appendix 2. Data from these subjects will be used in the intent-to-treat analysis. These subjects are permitted to simultaneously enroll in a CIT or site-specific graft failure follow-up protocol, if available.

6.   CRITERIA FOR PREMATURE TERMINATION OF THE STUDY

6.1   Subject Withdrawal Criteria

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

1. The subject elects to withdraw consent from all future study activities, including follow-up.

2. The subject is "lost to follow-up" (*i.e.*, no further follow-up is possible because attempts to reestablish contact with the subject have failed).

3. The subject dies.

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

6.2   Study Stopping Rules

6.2.1   Protocol Suspension and Review

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

1. The Medical Monitor finds any unexpected fatal or life-threatening AE possibly related to the use of the test therapy;

2. **Primary non-function** occurs in 3 or more consecutive subjects at 2 or more participating clinical sites.

3. There are 6 consecutive study subjects with a c-peptide less than 0.3 ng/mL (on random testing, at baseline and 1-3 hrs post-MMTT) at 75 days post-transplant.

4. Any event(s) which in the opinion of the Medical Monitor or Protocol Chair indicates the need for DSMB review; or

5. The DSMB recommends termination of protocol enrollment and further transplants on a study-wide basis based on a review of the data and finding evidence that such action is necessary. Statistical guidelines for terminating the study based on monitoring guidelines are provided in section 10.

After the protocol is placed on hold, no additional transplants within the trial will be performed at any participating clinical site until the CIT Steering Committee and DSMB meet either in person or by conference call to review in depth the results and circumstances surrounding the

islet functional failure or SAE to determine whether the trial enrollment of new subjects and conduct of additional transplants could be safely resumed.

6.2.2 Site Suspension and Review

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

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

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

3. Two consecutive **primary non-functioning** transplants (see Study Definitions).

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

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

7.   STUDY PROCEDURES

7.1   Enrollment and Screening

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 the Schedule of Events (Appendix 1). More than one visit may 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 sites. Results from assessments completed prior to signing informed consent, must be current within the windows stated in the table below.

Table 3: 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.

7.2   Waiting List/Baseline

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, retinal photos, and carotid intimal thickness (IMT) – should be completed as time allows. Carotid IMT can be collected up to 30 days post-transplant. If retinal photos cannot be obtained at WL/BL, they should not be collected post-randomization. Waitlist assessments will be repeated at pre-defined intervals as detailed in Appendix 1. Results from assessments done closest to the start

of immunosuppression will be used as the subject's baseline values. All one-time waitlist/baseline assessments should be completed on Day -2, whenever possible, but always prior to the start of immunosuppression. 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.

In addition to the specific waitlist/baseline assessments listed in the Schedule of Events (Appendix 1), subject enrolled at the University of Miami and the University of Pennsylvania will have additional assessments performed as outline in the University of Miami Sub-study (Appendix 5) and University of Pennsylvania Sub-study (Appendix 6) respectively.

7.3   Randomization, Islet Transplant, and Study Treatment Visits

Once a compatible islet prep becomes available, subject eligibility will be re-confirmed. At sites with an actively recruiting site-specific Phase 2 trial, eligible subjects will be randomized on Day -2 relative to transplant, between this Phase 3 trial and a site specific Phase 2 trial. Randomizations will occur at a ratio of 2:1, where 2 participants are assigned to CIT07 for every subject assigned to the site-specific Phase 2 trial.   At sites without an actively recruiting site-specific Phase 2 trial, 100% of subjects will be 'randomized' to this Phase 3 trial. Subjects randomized to this Phase 3 trial will receive immunosuppressive therapy beginning on Day -2 (see Section 5 for full description of Study Treatment Regimen). Subjects will receive the initial islet transplant on Day 0 and will continue the immunosuppression regimen detailed in Section 5.

7.4   Follow-up Visits

Subject will undergo a 24-month follow-up period following their last islet transplant. Please refer to the Schedule of Events (Appendices 1 and 4), for the clinical time points of specific follow-up study procedures. 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 followed for adverse events only until 24 months after their final CIT islet transplant.

In addition to the specific follow-up study procedures listed in the Schedule of Events (Appendices 1 and 4), subjects enrolled at the University of Miami will have Nutritional Assessments performed as outlined in the University of Miami Sub-study (Appendix 5).

7.5   Criteria and Timing for Subsequent Islet Transplants

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

7.5.1 <u>Second Islet Transplant</u>

Islet transplant recipients with **partial islet graft function** (see Study Definitions) will be considered for a second islet 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 ≥5,000 IE/kg with the first transplant, but failed to achieve or maintain insulin independence.

2. Subject has been compliant with study monitoring and prescribed immunosuppressive therapy.

3. Subject has no unresolved SAEs.

4. No evidence of progressive renal dysfunction, with blood creatinine rising above 2.0 mg/dL (177 µmol/L).

5. No evidence of hypersensitization, allergic responses, or other potentially serious drug reactions to medications required by the protocol.

6. PRA ≤50% by flow cytometry (assessment performed locally) and the alloantibody specificity not cross-reactive with antigen(s) present in the subsequent islet preparation in order to avoid unacceptable antigen(s).

7. Absence of 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.

7.5.2 <u>Third Islet Transplant</u>

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

1. The subject received greater than 4,000 IE/kg with the second transplant, but remains dependent on insulin for longer than one month after the second transplant.

2. There is evidence of **partial graft function**.

3. The CIT PIs, Site PIs, and the Steering Committee have determined that there were no relevant protocol deviations at the site.

4. The subject has been compliant with study monitoring and prescribed immunosuppressive therapy.

5. No evidence of a serious and life-threatening infection, AE, or other condition that precludes attempting an intraportal injection or continuation of the post-transplant treatment regimen.

6. No evidence of PTLD.

7. No evidence of progressive renal dysfunction, with blood creatinine rising above 2.0 mg/dL (177 μmol/L).

8. No evidence of hypersensitization, allergic responses, or other potentially serious drug reactions to medications required by the protocol.

9. No evidence of abnormal liver ultrasound and (LFTs) within 1.5 times ULN range.

10. 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).

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

7.6 Visit Windows

Study visits should take place within the time limits specified on the Schedule of Events (Appendices 1, 2, and 4).

8. SAFETY MONITORING

AEs that are classified as serious according to the definition set forth by the health authorities must be reported promptly to NIAID/NIDDK, Clinical Research Organization (CRO)/Data Coordinating Center (DCC), health authorities, PIs, and IRBs. This section defines the types of AEs and outlines the procedures for appropriately collecting, grading, recording, and reporting them. Information in this section complies with *International Conference on Harmonization (ICH) Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting* and *ICH E6: Guideline for Good Clinical Practice,* and applies the standards set forth in the *Terminology Criteria for Adverse Events in Trials of Adult Pancreatic Islet Transplantation (CIT-TCAE).* This document, created by the CIT Consortium, modifies the National Cancer Institute (NCI), *Common Terminology Criteria for Adverse Events (CTCAE)* version 3.0 (June 10, 2003), to ensure applicability in the setting of Islet Transplantation.

8.1 Definitions

8.1.1 Adverse Event

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

8.1.2 Serious Adverse Event

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

1. Death.

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

3. Inpatient hospitalization or prolongation of existing hospitalization. Please note that hospital admissions for the purpose of conducting protocol-mandated procedures do not need to be reported as SAEs, unless the hospitalization is prolonged due to complications.

4. Persistent or significant disability.

5. Congenital anomaly or birth defect.

6. An event that required intervention to prevent permanent impairment or damage. An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based on appropriate medical

judgment, it may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

7. Other conditions specified in the protocol

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

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

8.1.3　Unexpected Adverse Event

An AE is considered "unexpected" when its nature (specificity) or severity is not consistent with available product information, 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 **severe hypoglycemic events** (see study definitions) 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.   For transplants that occur as a standard of care procedure at the University of Alberta, adverse events will be collected and submitted by the site investigator until all CIT07 study visits have been completed. 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:

- Observing the subject.

- Questioning the subject, which should be done in an objective manner.

- Receiving an unsolicited complaint from the subject.

- An abnormal value or result from a clinical or laboratory evaluation (*e.g.*, a radiograph, an ultrasound, or an electrocardiogram) can also indicate an AE. If this is the case, then the evaluation that produced the value or result should be repeated until the value or result returns to normal or can be explained and the subject's safety is not at risk. If an abnormal

value or result is determined by the investigator to be clinically significant, it must be reported as an AE.

8.2.2 Recording Procedure

Throughout the study, the investigator will record all adverse events 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 document provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all AEs.

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

Grade 1 = Mild AE.

Grade 2 = Moderate AE.

Grade 3 = Severe and undesirable AE.

Grade 4 = Life-threatening or disabling AE.

Grade 5 = Death.

Table 4: General severity definition of adverse event

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

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

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, 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) or immunosuppression/infection prophylaxis will be defined by using the descriptors provided below.

Table 5:   Attribution of adverse event

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.

 For transplants that occur as a standard of care procedure at the University of Alberta, serious adverse events will be collected and submitted by the site investigator until all CIT07 study visits have been completed. 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 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 a 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 by study sponsor 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.*, will be included in the investigational new drug [IND] annual report to the health authorities). This requirement applies if the AE is classified as any of the following:

  Serious, expected, and drug related.

  Serious, expected, and *not* drug related.

  Serious, *unexpected,* and not drug related.

- **Expedited reporting.** This requirement applies if the AE is considered serious, unexpected, and drug related as defined in 21 CFR 312.32. This type of SAE must be

reported by the sponsor to the appropriate health authorities within 15 days; fatal or life-threatening events must be reported within 7 days.

8.3.3.2 REPORTING TIMELINE– FROM THE SITE TO THE DCC

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

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

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

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

8.3.3.4 NOTIFYING THE DATA AND SAFETY MONITORING BOARD

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

8.3.3.5 NOTIFYING THE INSTITUTIONAL REVIEW BOARD AND ETHICS COMMITTEE

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

8.3.3.6 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.

8.3.4 Updating Source Documentation

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

9. MECHANISTIC ASSAYS

In addition to the specific metabolic and immunologic procedures listed below and in the Schedule of Events (Appendix 1), subjects enrolled at the University of Miami and the University of Pennsylvania will have additional assays performed as outlined in the University of Miami Sub-study (Appendix 5) and University of Pennsylvania Sub-study (Appendix 6) respectively.

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 HbA1c <7.0% and absence of hypoglycemic episodes addresses the clinically important outcome. Insulin-independence will be used as a clinically relevant measure of islet graft function for the secondary endpoint, and additional stimulatory tests of islet graft function utilizing meal (MMTT) and glucose (FSIGT) challenges will be performed to assess additional 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. (See section 4.1 for endpoint description).

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 until one year after their final islet transplant. The timing of these metabolic assessments is provided in Appendix 1.

Subjects may use any glucometer for the metabolic assessments in Appendix 4, during the second year after final islet transplant.

9.1.2.1 INSULIN REQUIREMENTS

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

9.1.2.2 GLYCEMIC CONTROL

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

9.1.2.3 GLYCEMIC LABILITY

Glycemic lability will be assessed by both the MAGE[1] and the LI[2].

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 $mmol/L^2/h \cdot wk^{-1}$. A LI greater than or equal to the 90th percentile (433 $mmol/L^2/h \cdot wk^{-1}$) of values derived from an unselected group of T1D patients is evidence for severe glycemic lability.

9.1.2.4 HYPOGLYCEMIA

An episode of severe hypoglycemia is defined as an event with one of the following symptoms: memory loss; confusion; uncontrollable behavior; irrational behavior; unusual difficulty in awakening; suspected seizure; seizure; loss of consciousness; or visual symptoms, in which the subject was unable to treat him/herself and which was associated with either a blood glucose level <54 mg/dL (3.0 mmol/L) or prompt recovery after oral carbohydrate, IV glucose, or glucagon administration.[28]

In addition, composite indices of hypoglycemia frequency, severity, and symptom recognition will be assessed by both the Clarke survey[155] and the HYPO score[2].

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

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

9.1.2.5 MIXED-MEAL TOLERANCE TEST (MMTT)

Basal (fasting) and stimulated glucose and c-peptide levels will be determined using the MMTT. Subjects will be instructed not to eat or inject short-acting (or bolus) insulin after 8 PM the night before the test. Evening or bedtime administration of long-acting insulin will be permitted, as

will consumption of water. Subjects receiving CSII (insulin "pump" therapy) may remain on the basal rate of insulin. Subjects will arrive fasting to the transplant or diabetes clinic where the capillary BG will be checked. If the BG is <70 mg/dl (3.89 mmol/L) or >180 mg/dl (10 mmol/L), the test will be rescheduled for the next possible day. If the BG is 70 – 180 mg/dl (3.89 – 10 mmol/L), basal glucose and c-peptide levels will be drawn. Immediately after, the subject will receive 6 mL per kg body weight (to a maximum of 360 mL) of Boost® High Protein Drink (or a nutritionally equivalent substitute) to consume in 5 minutes starting at time = 0. Then, at time = 60 and 90 minutes, stimulated glucose and c-peptide levels will again be drawn.

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

9.1.2.6   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 stimulated c-peptide as developed by Ryan et al[3]. 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[156,157]. The CPGCR is calculated as [c-peptide (ng/mL) * 100]/[glucose (mg/dL) * creatinine (mg/dL)]. An index of islet graft function, this measure correlates well with both the 90-minute glucose levels during a MMTT and the β-score[158].

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 transplantation [4]. These results require confirmation by longitudinal analysis.

The insulin-modified FSIGT test[5] involves blood sampling at baseline (t = -10, -5, and -1 min) and at t = 1, 2, 3, 4, 5, 7, 10, 12, 14, 16, 18, 20, 22, 25, 30, 40, 50, 70, 100, 140, & 180 minutes post-

injection of glucose at t = -30 seconds with an injection of insulin at t = 20 min. Each 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, and -1 min) and t = 1, 2, 3, 4, 5, 7, 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, 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 (DI = AIR_{glu} • 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[124,159].

9.1.2.10 QUALITY OF LIFE (QOL)

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

Generic Measures

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

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

option. This shorter version would derive eight scales and two summary components and would be also be normed to the 1998 data (general population and disease-specific groups).

EQ-5D (EuroQoL)

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

Disease-targeted Measures

Diabetes Distress Scale

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

Hypoglycemic Fear Survey

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

9.2   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 T1Dconsortia (*e.g.*, ELISPOT, tetramer staining, T cell proliferation assays) to determine which tests provide the most reliable data with regards to distinguishing between patients with T1D vs. normal controls (for autoantigen) and to improve techniques for assessing recipient anti-donor reactivity.

9.2.1 Immune Assays

9.2.1.1 HLA TYPING OF DONORS AND RECIPIENTS, CROSSMATCHING

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

9.2.1.2 ALLOANTIBODY

Development of alloantibody is generally associated with longer term graft loss. 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 SAMPLES

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. 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.

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

10.   STATISTICAL CONSIDERATIONS AND ANALYTICAL PLAN

10.1 Statistical Analyses

At each center, subjects are randomized to this study, CIT-07, or one of the phase 2 trials. Subjects and data from the phase 2 trials will not be included in the analysis of the CIT-07 results. 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 has been initiated 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. In addition, a per-protocol analysis will include all subjects who are randomized to CIT-07 and in whom the islet infusion procedure is initiated. The procedure will be considered initiated when the operator has started the process of obtaining access to the portal vein (*i.e.*, entered the body with a needle or scalpel).

Subjects who are randomized to CIT-07 may never receive immunosuppression: either because a compatible pancreas never became available or because a usable islet isolation could not be obtained. The numbers of these patients will be reported, but they will not be included in the intention-to-treat population.   Subjects for whom planned islets are not released for transplant or for whom the planned islets are not compatible, will return to the waiting list. Should an alternative pancreas become available later, they will be transplanted under the study to which they were randomly assigned. That is, once a subject is randomized to CIT-07, she/he will always be assigned to CIT-07. All subjects in the intention-to-treat population will be included in the safety analysis.

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                        ┌─────────────────┐
                        │ All subjects    │
                        │ randomized to   │
                        │ CIT-07          │
                        └─────────────────┘
        ┌───────────────────────┼───────────────────────┐
        ▼                       ▼                        ▼
┌─────────────────┐                          ┌─────────────────────┐
│ The islet       │                          │ No protocol-directed │
│ infusion        │                          │ therapy is ever      │
│ procedure is    │                          │ initiated            │
│ initiated       │                          │ = EXCLUDED FROM      │
│ = PER PROTOCOL  │                          │ ANALYSIS             │
│ SAMPLE          │                          └─────────────────────┘
└─────────────────┘
                  ┌──────────────────────┐
                  │ Any protocol-directed │
                  │ therapy is initiated =│
                  │ INTENTION TO TREAT    │
                  │ SAMPLE                │
                  └──────────────────────┘
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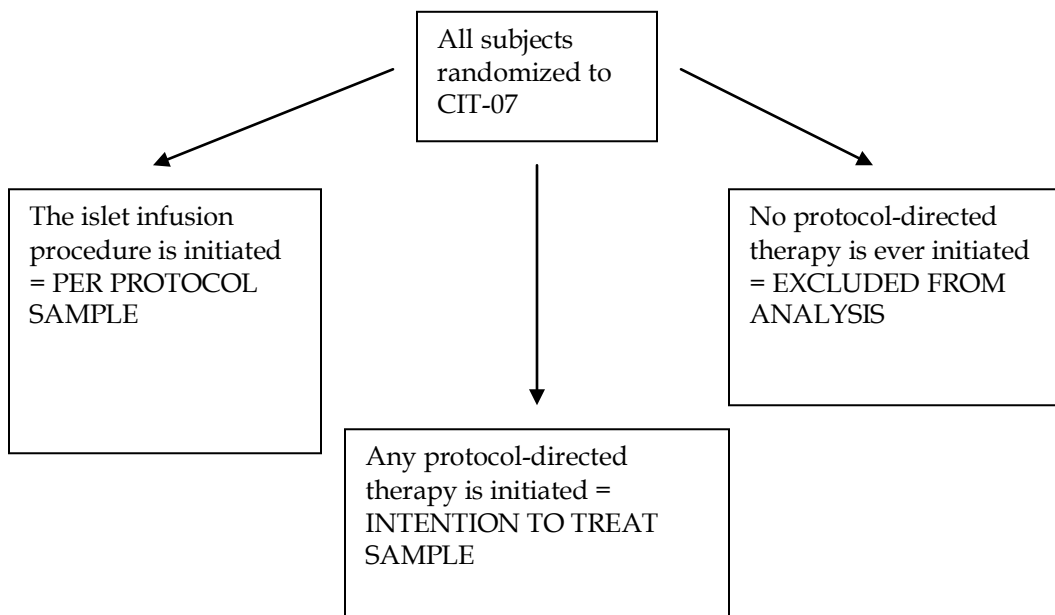
Figure 3: Selection of intention to treat sample

10.2 Study Endpoint Assessment

10.2.1 Primary Endpoint

The primary endpoint is defined in section 4.1.1. The primary endpoint for this study will be the proportion of subjects with an HbA1c <7.0% AND free of severe hypoglycemic events from Day 28 to Day 365, inclusive, following the first islet transplant, with the day of transplant designated Day 0. For brevity in the following discussion, we will denote this outcome as a "favorable outcome" at one year.

The primary aim of the analysis is to estimate the true rate of favorable outcome at one year in eligible subjects who receive protocol-directed therapy. The observed rate will be used as the point estimate. The primary analysis will compute an exact one-sided binomial test of the hypothesis that the true rate is 50% against the alternative hypothesis that the true rate is greater than 50%. The analysis will be considered significant if this test rejects the null hypothesis at the 5% level of significance.

The primary endpoint should be available for all subjects who receive any protocol-directed therapy. Subjects who withdraw consent for participation in the study, those who die in the first year after receiving their first transplant, and those who receive protocol-directed therapy but do not receive an islet transplant, will be classified as having failed to achieve a favorable outcome. 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 one year after transplant, in which case, that later value will be imputed. All imputations will be reported with the primary analysis.

10.2.2 Secondary Endpoints

Bayesian Analysis

The rate of favorable outcome at each of the centers preparing islets for transplant will be estimated as a planned subgroup analysis, as described in the SAP, using a Bayesian random-effects model. Any missing endpoints will be accounted for as described for the primary endpoint in Section 10.2.1. Any imputations will be reported along with the estimates. The results of the Bayesian analysis will serve as the basis for an individual islet manufacturing center to submit a Biological License Application. It will have no bearing upon the efficacy and safety analyses conducted for the study as a whole. Therefore, Bayesian analysis will not be considered in the adjustments for multiplicity that are described below under "Key Secondary Endpoints".

For the planned Bayesian subgroup analysis, a one-sided 90% probability interval will be constructed to estimate a 90% posterior probability lower bound for efficacy at each center. This analysis is required for the proposal for the licensure of islet preparation labs but will not be used to make decisions for efficacy. Details are in the SAP.

Key Secondary Endpoints

The key and other secondary endpoints are defined in section 4.1.2. Because there are a large number of secondary endpoints, it is impractical to account for all multiple comparisons. However, the analyses will account for the multiplicity of the key secondary endpoints. Details of this method will be included in the Statistical Analysis Plan.

Analysis of key secondary endpoints

We will use the Benjamini and Hochberg[160] method to account for the multiplicity of the key secondary tests. This method controls the false discovery rate (FDR) rather than the more familiar family-wise error rate (FWER). It provides a powerful approach for identifying those positive tests that are not likely to be true while controlling a reasonable measure of the number of false positive tests (the FDR). Because this is a single intervention study, the tests will be one sided tests for whether the true rates are greater than the endpoint's' predetermined "minimum rate for efficacy". These minimum rates were determined by the investigators to be large enough to have credibility for the islet transplant community. The minimum rate for efficacy is provided in Table 6 for each of the key secondary outcomes. The procedure orders the p-values from all of the key secondary tests and rejects those hypotheses for which the p-values are less than or equal to an upper bound defined by the total number of tests and the predetermined maximum FDR. We will assure that the FDR does not exceed 10%.

As with the primary endpoint, the key secondary endpoints should be available for all transplanted subjects. If an endpoint is not available for a randomized subject, then it will be imputed using the same rules that were used for the primary. The observed rate for each key secondary outcome will be used as the point estimate. The following table displays the smallest rate that will be considered to support efficacy for each of the key secondary endpoints.

Table 6: Key secondary outcomes

	Key Secondary Outcome	Minimum* Rate for Efficacy
1	The proportion of subjects with an HbA1c <7.0% AND free of severe hypoglycemic events from Day 28 to Day 730, inclusive, after the first islet transplant.	50%
2	The proportion of subjects with HbA1c ≤ 6.5% at one year after the first islet transplant AND free of severe hypoglycemic events from Day 28 to Day 365 after the first transplant.	50%
3	The proportion of subjects with HbA1c ≤ 6.5% at two years after the first islet transplant AND free of severe hypoglycemic events from Day 28 to Day 730 after the first transplant.	40%
4	The proportion of subjects free of severe hypoglycemic events from Day 28 to Day 365 after the first islet transplant.	50%
5	The proportion of subjects free of severe hypoglycemic events from Day 28 to Day 730 after the first islet transplant.	40%
6	The proportion of subjects with HbA1c <7.0% at one year after the first islet transplant.	50%

	Key Secondary Outcome	Minimum* Rate for Efficacy
7	The proportion of subjects with HbA1c <7.0% at two years after the first islet transplant.	40%
8	The proportion of subjects with HbA1c ≤6.5% at one year after the first islet transplant.	50%
9	The proportion of subjects with HbA1c ≤6.5% at two years after the first islet transplant.	40%
10	The proportion of insulin-independent subjects at one year after the first islet transplant.	20%
11	The proportion of insulin-independent subjects at two years after the first islet transplant.	10%

Analysis of other secondary endpoints

Because the questions addressed are primarily explanatory or supportive of the other findings in the trial, analysis of the other secondary endpoints will use confidence intervals. There will be no adjustment for multiple comparisons. Any purported findings should be treated as hypothesis generating only.

When the endpoint is a proportion, the observed rate will be used as the point estimate, and an exact 95% one-sided binomial confidence interval will be reported. Continuous variables will be treated in a similar fashion. If the necessary normality assumption is valid, then the sample mean will be used as the point estimate, and the usual 95% one-sided normal confidence intervals will be computed. When the normality assumption is not valid, we will attempt to identify an appropriate transform that will yield normality. An appropriate confidence interval will be calculated in the transformed scale. Where the normality assumptions are not valid and an appropriate transform will achieve normality, then the inverse of the mean of the transformed data will be used as the point estimate, and the inverse of the endpoint for a standard 95% one-sided confidence interval for the transformed mean will be reported for the confidence interval. If no valid transformation can be found, then we will use the bootstrap method to construct a point estimate and a 95% one-sided confidence interval.

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, 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.

10.3.2 Medical History

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

10.3.3 Use of Medications

All medications used will be coded using the World Health Organization (WHO) drug dictionary. The number and percentage of subjects receiving concomitant medications or therapies will be presented. Statistical presentation of concomitant medications or therapies may be further summarized by withdrawal status, favorable outcome status at one year, and other characteristics to be determined by the study investigators.

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

10.4 Sample Size and Power Calculations

The following table describes the data for favorable outcome at the University of Alberta, the University of Miami, the University of Minnesota, University of Pennsylvania, Emory University, and Northwestern University as of January 2006. In 131 recipients of islet transplantation without prior or concurrent kidney transplantation, 76% have achieved a favorable outcome at one year.

Table 7: Transplants and favorable outcomes at CIT centers

Center	Number of subjects transplanted	Number with favorable outcome	Percent with favorable outcome
University of Alberta	68	49	72
University of Miami	21	17	81
University of Minnesota	20	16	80
University of Pennsylvania	9	6	75
Emory University	8	7	88
Northwestern University	5	4	80
Total	131	99	76

A way to select a sample size is to consider which favorable outcome rates the 95% confidence interval would exclude. The following table displays the lower bounds for exact one-sided lower 95% confidence intervals for selected observed favorable outcome rates. The confidence interval excludes any value smaller than the tabulated value. That is, we are 95% confident that the true favorable outcome rate is not lower than the tabulated value.

Table 8: Lower confidence bounds for selected observed favorable outcome rates

Lower Confidence Bounds for Exact One-sided Binomial Confidence Intervals For Favorable Outcome Rate (%)				
Observed Favorable Outcome Rate	Sample Size			
	24	36	48	60
10%	1.5	3.9	4.2	4.4
20%	8.6	9.5	11.8	12.0
30%	14.6	18.2	18.6	20.4
40%	24.6	25.3	27.7	29.3
50%	31.9	35.3	37.4	38.7
60%	39.7	46.0	47.5	48.6
70%	52.1	54.5	58.2	58.8
80%	61.1	66.6	67.2	69.6
90%	76.0	76.3	79.3	81.2

The best clinical judgment of the investigators is that 50% or larger favorable outcome rate would be clinically meaningful. Based on past experience we would expect to observe a rate larger than 70%. With a sample size of 48 transplanted subjects, 31 subjects (65%) would need to achieve a favorable outcome for the exact 95% lower confidence bound to rule out a 50% or lower true rate. The proposed sample size is 48 subjects. Each participating center is expected to enroll at least 6 subjects.

The following table displays the probability that the study would conclude that the true favorable outcome rate is at least 50% for several selected values of the true favorable outcome rate. The tabulated probabilities are the power that a one-sided binomial test of the null hypothesis H_0: $p<0.5$ versus the alternative hypothesis H_a: $p \geq 0.5$ would conclude that the true favorable outcome rate is at least 50%, given that the selected value is the true underlying favorable outcome rate.

Table 9: Power to rule out a 50% favorable outcome rate for selected true favorable outcome rates

True Favorable Outcome Rate	Power to Rule Out Favorable Outcome Rate
10%	0.0000
20%	0.0000
30%	0.0000
40%	0.0005
50%	0.0297
60%	0.3111
70%	0.8359
80%	0.9962
90%	1.0000

This table shows that if the true favorable outcome rate is 70%, then the power of concluding that the rate is over 50% is 0.8359 for a 5% level test.

10.5 Interim Analyses to Ensure Patient Safety

The DSMB will be convened to review safety and efficacy data following NIH policy. When requested, formal interim analyses to assess safety and efficacy will be performed. Formal interim analyses will include distributions of endpoints, biomarkers and AEs. Additional analyses may be requested by the DSMB.

The monitoring plan will be reviewed by the DSMB.   Details of this plan will be included in the SAP. Because this is a small study and it is important to collect as much safety data as possible, it is not likely that the DSMB or the investigators will recommend stopping early for evidence of efficacy. Therefore, the monitoring plan recommends early stopping only if there is sufficient evidence to conclude that the rate of favorable outcome is unacceptably low.

Should the monitoring boundaries be crossed, the DSMB will be provided with an analysis for the primary endpoint, all secondary endpoints, and AEs. The DSMB will make recommendations on stopping to the NIH using all available interim information.

The following table provides information on our planned strategy for stopping when the favorable outcome rate is too low.   We will use the Lan and Demets[161] error spending approach with the O'Brien-Fleming[162] spending function. These calculations are based on using the O'Brien-Fleming method to calculate boundary values for a one-sided test of the hypothesis that the proportion achieving favorable outcome is no lower than a selected minimum value. The calculations assumed a 2.5% level for the overall type I error. The procedure recommends terminating enrollment when there is overwhelming evidence that the favorable outcome rate is unacceptably low.   The procedure requires that the investigators and/or the DSMB specify the value that should be considered unacceptably low. The table provides stopping boundaries for 20%, 30%, and 40%.   Entries in the table are the numbers of favorable outcomes that would result in recommending stopping because the favorable outcome rate is unacceptably low. Numbers are provided for 3 (2 interim and a final) and 4 (3 interim and a final) equally spaced analyses.

For example, for three analyses and if the lowest acceptable favorable outcome rate were 20% then the rule could not recommend stopping at the first interim analysis. It would recommend stopping at the second interim analysis (after 32 patients had completed their one-year follow-up) if none of the 32 patients experienced a favorable outcome.   The study would conclude that the favorable outcome rate was less than 20% at the end of the trial if 4 or fewer patients experienced a favorable outcome. If the lowest acceptable favorable outcome rate were 30% then the rule would recommend stopping at the second interim analysis if 3 or fewer patients experienced a favorable outcome. It would conclude that the true favorable outcome rate was less than 30% after 48 patients had completed the study if 8 or fewer patients experienced a favorable outcome.

Table 10: Stopping boundaries for selected unacceptable low favorable outcome rates

Number of Interim Analyses	Interim Analysis	Number of Patients	Minimally Acceptable Favorable Outcome Rate		
			20%	30%	40%
			Number Experiencing Favorable Outcome To Recommend Stopping		
3	1	16	--	--	--
	2	32	0	≤3	≤6
	3	48	≤4	≤8	≤12
4	1	12	--	--	--
	2	24	--	0	≤2
	3	36	≤1	≤4	≤7
	4	48	≤3	≤7	≤12

It is anticipated that the DSMB will meet twice a year. We plan for at least 4 interim analyses and that the minimally acceptable favorable outcome rate will be 30%.

10.6 Reporting Deviations from Original Statistical Plan

The principal features of the study design and of the plan for statistical analysis of the data are outlined in this protocol and in the subsequent SAP. Any changes in these principal features will require a protocol or an SAP amendment, which would be subject to review by the 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

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*[163], 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.

15. REFERENCES

1. Service F, Molnar G, Rosevear J, Ackerman E, Gatewood L, Taylor W. Mean amplitude of glycemic excursions, a measure of diabetic instability. Diabetes. 1970 Sep;19(9):644-55.
2. Ryan E, Shandro T, Green K, et al. Assessment of the severity of hypoglycemia and glycemic lability in type 1 diabetic subjects undergoing islet transplantation. Diabetes. 2004 Apr;53(4):955-62.
3. Ryan E, Paty B, Senior P, Lakey J, Bigam D, Shapiro A. Beta-score: an assessment of beta-cell function after islet transplantation. Diabetes Care. 2005 Feb;28(2):343-7.
4. Rickels M, Naji A, Teff K. Insulin Sensitivity, Glucose Effectiveness, and Free Fatty Acid Dynamics after Human Islet Transplantation for Type 1 Diabetes. J Clin Endocrinol Metab. 2006;91(6):2138-44.
5. Saad M, Anderson R, Laws A, et al. A comparison between the minimal model and the glucose clamp in the assessment of insulin sensitivity across the spectrum of glucose tolerance. Insulin Resistance Atherosclerosis Study. Diabetes. 1994 Sep;43(9):1114-21.
6. Kessler L, Passemard R, Oberholzer J, et al. Reduction of blood glucose variability in type 1 diabetic patients treated by pancreatic islet transplantation: interest of continuous glucose monitoring. Diabetes Care. 2002 Dec;25(12):2256-62.
7. Streja D. Can continuous glucose monitoring provide objective documentation of hypoglycemia unawareness? Endocr Pract. 2005 Mar-Apr;11(2):83-90.
8. Stevens L, J C, CH S, et al. Estimating GFR using serum cystatin C alone and in combination with serum creatinine: a pooled analysis of 3,418 individuals with CKD. Am J Kidney Dis. 2008 3/2008;51(3):395-406.
9. DCCT Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med. 1993 Sep;329(14):977-86.
10. Hirsch I. Drug therapy: Insulin and analogues. N Engl J Med. 2005 Jan;352(2):174-83.
11. Cryer P, Davis S, Shamoon H. Hypoglycemia in diabetes. Diabetes Care. 2003 Jun;26(6):1902-12.
12. Larsen J. Pancreas transplantation: Indications and consequences. Endocrine Rev. 2004 Dec;25(6):919-46.
13. Shapiro A, Lakey J, Ryan E, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N Engl J Med. 2000 Jul 27;343(4):230-8.
14. Ryan E, Paty B, Senior P, et al. Five-year follow-up after clinical islet transplantation. Diabetes. 2005 Jul;54(7):2060-9.
15. Reckard C, Barker C. Transplantation of isolated pancreatic islets across strong and weak histocompatibility barriers. Transplant Proc. 1973 Mar;5(1):761-3.
16. Posselt A, Barker C, Tomaszewski J, Markmann J, Choti M, Naji A. Induction of donor-specific unresponsiveness by intrathymic islet transplantation. Comment in: Science. 1990 Sep 14;249(4974):1248. Science. 1990 Sep;249(4974):1293-5.
17. Lacy P, Davie J, Finke E. Prolongation of islet allograft survival following in vitro culture (24 degrees C) and a single injection of ALS. Science 1979. 1979 Apr;204(4390):312-3.
18. Hirshberg B, Preston EH, Xu H, et al. Rabbit antithymocyte globulin induction and sirolimus monotherapy supports prolonged islet allograft function in a nonhuman primate islet transplantation model. Transplantation. 2003 Jul 15;76(1):55-60.
19. Hering B, Kandaswamy R, Ansite J, et al. Single-donor, marginal-dose islet transplantation in patients with type 1 diabetes. JAMA. 2005 Feb;293(7):830-5.
20. Cryer P. Hypoglycemia: the limiting factor in the management of IDDM. Diabetes 1994 Nov;43(11):1378-89.

21.   Cryer P. Iatrogenic Hypoglycemia as a Cause of Hypoglycemia-Associated Autonomic Failure in IDDM: A Vicious Cycle. Diabetes. 1992 Mar;41(3):255-60.
22.   Cryer P, Fisher J, Shamoon H. Hypoglycemia. Diabetes Care. 1994 Jul;17(7):734-55.
23.   Cryer P. Diverse causes of hypoglycemia-associated autonomic failure in diabetes. N Engl J Med. 2004 May;350(22):2272-9.
24.   Gerich J, Langlois M, Noacco C, Karam J, Forsham P. Lack of glucagon response to hypoglycemia in diabetes: Evidence for an intrinsic pancreatic alpha cell defect. Science. 1973 Oct;182(108):171-3.
25.   Amiel S, Sherwin R, Simonson D, Tamborlane W. Effect of intensive insulin therapy on gylcemic thresholds for counter-regulatory hormone release. Diabetes. 1988 Jul;37(7):901-7.
26.   White N, Skor D, Cryer P, Levandoski L, Bier D, Santiago J. Identification of type 1 diabetic patients at increased risk for hypoglycemia during intensive therapy. N Engl J Med. 1983;308(9):485-91.
27.   Bolli G, De Feo P, De Cosmo S, et al. A reliable and reproducible test for adequate glucose counterregulation in type 1 diabetes. Diabetes. 1984 Aug;33(8):732-7.
28.   DCCT Research Group. Epidemiology of Severe Hypoglycemia in the Diabetes Control and Complications Trial. *Am J Med.* 1991;90(4):450-9.
29.   Muhlhauser I, Berger M, Sonnenberg G, et al. Incidence and management of severe hypoglycemia in 434 adults with insulin dependent diabetes mellitus. Diabetes Care. 1985 May-Jun;8(3):268-73.
30.   Muhlhauser I, Bruckner I, Berger M, et al. Evaluation of an intensified insulin treatment and teaching programme as routine management of type 1 (insulin-dependent) diabetes. The Bucharest-Dusseldorf Study. Diabetologia. 1987 Sep;30(9):681-90.
31.   Mecklenburg R, Benson E, Benson JJ, et al. Acute complications associated with insulin infusion pump therapy. Report of experience with 161 patients. JAMA. 1984 Dec 21;252(23):3265-9.
32.   Hepburn D, Patrick A, Eadington D, Ewing D, Frier B. Unawareness of hypoglycemia in insulin-treated diabetic patients: prevalence and relationship to autononomic neuropathy. Diab Med. 1990 Sep-Oct;7(8):711-7.
33.   Ryder R, Owens D, Hayes T, Ghatei M, Bloom S. Unawareness of hypoglycemia and inadequate glucose counterregulation: no casual relationship with diabetic autonomic neuropathy. Br Med J. 1990;301(6755):783-7.
34.   Dagogo-Jack S, Craft S, Cryer P. Hypoglycemia-associated autonomic failure in insulin-dependent diabetes mellitus. Recent antecedent hypoglycemia reduces autonomic responses to, symptoms of, and defense against subsequent hypoglycemia. J Clin Invest. 1993 Mar;91(3):819-28.
35.   Gold A, Macleod K, Frier B. Frequency of Severe Hypoglycemia in Patients with Type-1 Diabetes with Impaired Awareness of Hypoglycemia. Diabetes Care. 1994 Jul;17(7):697-703.
36.   DCCT Research Group. Hypoglycemia in the Diabetes Control and Complications Trial. Diabetes. 1997 Feb;46(2):271-86.
37.   Gill G. Socioeconomic problems of hypoglycemia. In: Frier BM, Fisher BM, eds. Hypoglycemia and Diabetes. 1993:362-70.
38.   Irvine A, Cox D, Gonder-Frederick L. Fear of hypoglycemia: relationship to physical and psychological symptoms in patients with insulin dependent diabetes mellitus. Health Psychol. 1992;11(2):135-8.
39.   Irvine A, Cox D, Gonder-Frederick L. Methodological issues in examination of fear of hypoglycemia (Letter). Diabetes Care. 1991 Jan;14(1):76.
40.   Pramming S, Thorsteinsson B, Bendtson I, Binder C. Symptomatic hypoglycemia in 411 type 1 diabetic patients. Diabet Med. 1991 Apr;8(3):217-22.
41.   Dagogo-Jack S, Rattarasarn C, Cryer P. Reversal of hypoglycemia unawareness, but not defective glucose counterregulation, in IDDM. Diabetes. 1994 Dec;43(12):1426-34.
42.   Fanelli C, Epifano L, Rambotti A, et al. Meticulous prevention of hypoglycemia normalizes the glycemic thresholds and magnitude of most neuroendocrine responses to, symptoms of and cognitive function during hypoglycemia in intensively treated patients with short-term IDDM. Diabetes. 1993 Nov;42(11):1683-9.

43.   Fanelli C, Pampanelli S, Epifano L, et al. Long-term recovery from unawareness, deficient counterregulation and lack of cognitive dysfunction during hypoglycaemia, following institution of rational, intensive insulin therapy in IDDM [published erratum appears in Diabetologia 1995 Feb; 38(2): 254]. Diabetologia. 1994 Dec;37(12):1265-76.
44.   Cranston I, Lomas J, Maran A, Macdonald I, Amiel S. Restoration of hypoglycaemia awareness in patients with long duration insulin-dependent diabetes. Lancet. 1994 Jul 30;344(8918):283-7.
45.   Bottini P, Boschetti E, Pampanelli S, et al. Contribution of autonomic neuropathy to reduced plasma adrenaline responses to hypoglycemia in IDDM: evidence for a nonselective defect. Diabetes. 1997 May;46(5):814-23.
46.   Fanelli C, Pampanelli S, Lalli C, et al. Long-term intensive therapy of IDDM patients with clinically overt autonomic neuropathy: effects on hypoglycemia awareness and counterregulation. Diabetes. 1997 Jul;46(7):1172-81.
47.   Cryer P. Hypoglycemia begets hypoglycemia in IDDM. Diabetes. 1993 Dec;42(12):1691-3.
48.   Kendall D, Rooney D, Smets Y, Salazar B, Robertson R. Pancreas transplantation restores epinephrine response and symptom recognition during hypoglycemia in patients with long-standing type 1 diabetes and autonomic neuropathy. Diabetes. 1997 Feb;46(2):249-57.
49.   Meyer C, Hering B, Grossmann R, et al. Improved glucose counterregulation and autonomic symptoms after intraportal islet transplants alone in patients with long-standing type 1 diabetes mellitus. Transplantation. 1998 Jul 27;66(2):233-40.
50.   Paty B, Ryan E, Shapiro A, Lakey J, Robertson R. Intrahepatic islet transplantation in type 1 diabetic patients does not restore hypoglycemic hormonal counterregulation or symptom recognition after insulin independence. Diabetes. 2002 Dec;51(12):3428-34.
51.   Ryan E, Lakey J, Rajotte R, et al. Clinical outcomes and insulin secretion after islet transplantation with the Edmonton Protocol. Diabetes. 2001 April;50(4):710-9.
52.   Tattersall R. Brittle Diabetes. Clin Endocrinol Metab. 1977 Jul;6(2):403-19.
53.   Gill G. Does brittle diabetes exist? Gill GV, Pickup JC, Williams G, Eds. Difficult Diabetes. 2001:151-67.
54.   Tattersall R, Gregory R, Selby C, Kerr D, Heller S. Course of brittle diabetes: a twelve year follow-up. BMJ. 1991 May;302(6787):1240-3.
55.   Gill G, Lucas S, Kent L. Prevalence and characteristics of brittle diabetes in Britain. Q J Med. 1996 Nov;89(11):839-43.
56.   Dandona P, Foster M, Healey F, Greenbury E, Beckett A. Low-dose insulin infusions in diabetic patients with high insulin requirements. Lancet. 1978 Aug;2(8084):283-5.
57.   Schade D, Duckworth W. In search of the subcutaneous-insulin-resistance syndrome. N Engl J Med. 1986 Jul;315(3):147-53.
58.   Schlichtkrull J, Munck O, Jersild M. The M-value, an index of blood-sugar controls in diabetics. ACTA MED SCAND. 1965;177:95-102.
59.   Diabetes Research in Children Network Study Group. Accuracy of the GlucoWatch G2 Biographer and the Continuous Glucose Monitoring System during hypoglycemia: experience of the Diabetes Research in Children Network. Diabetes Care. 2004 Mar;27(3):722-6.
60.   Markmann J, Deng S, Huang X, et al. Insulin independence following isolated islet transplantation and single islet infusions. Ann Surg. 2003 Jun;237(6):741-9; discussion 9-50.
61.   Froud T, Ricordi C, Baidal D, et al. Islet transplantation in type 1 diabetes mellitus using cultured islets and steroid-free immunosuppression: Miami experience. Am J Transplant. 2005 Aug;5(8):2037-46.
62.   Rickels M, Schutta M, Markmann J, Barker C, Naji A, Teff K. Beta-cell function following human islet transplantation for type 1 diabetes. Diabetes. 2005 Jan;54(1):100-6.
63.   Brennan D, Flavin K, Lowell J, et al. A randomized, double-blinded comparison of Thymoglobulin versus Atgam for induction immunosuppressive therapy in adult renal transplant recipients. Transplantation. 1999 Apr;67(7):1011-8.

64.   Gaber A, First M, Tesi R, et al. Results of the double-blind, randomized, multicenter, phase III clinical trial of Thymoglobulin versus Atgam in the treatment of acute graft rejection episodes after renal transplantation. Transplantation. 1998 Jul 66(1):29-37.

65.   Bourdage J, Hamlin D. Comparative polyclonal antithymocyte globulin and antilymphocyte/antilymphoblast globulin anti-CD antigen analysis by flow cytometry. Transplantation. 1995 Apr;59(8):1194-200.

66.   Michallet M, Preville X, Flacher M, Fournel S, Genestier L, Revillard J. Functional antibodies to leukocyte adhesion molecules in antithymocyte globulins. Transplantation. 2003 Mar;75(5):657-62.

67.   Bonnefoy-Berard N, Vincent C, Revillard J. Antibodies against functional leukocyte surface molecules in polyclonal antilymphocyte and antithymocyte globulins. Transplantation. 1991 Mar;51(3):669-73.

68.   Rebellato L, Gross U, Verbanac K, Thomas J. A comprehensive definition of the major antibody specificities in polyclonal rabbit antithymocyte globulin. Transplantation. 1994 Mar;57(5):685-94.

69.   Shoskes D, Halloran P. Delayed graft function in renal transplantation: etiology, management and long-term significance. J Urol. 1996 Jun;155(6):1831-40.

70.   Bartlett S, Chin T, Dirden B, Quereshi A, Hadley G. Inclusion of peripancreatic lymph node cells prevents recurrent autoimmune destruction of islet transplants: evidence of donor chimerism. Surgery. 1995 Aug;118(2):392-7.

71.   Stegall M, Lafferty K, Kam I, Gill R. Evidence of recurrent autoimmunity in human allogeneic islet transplantation. Transplantation. 1996 Apr 61(8):1272-4.

72.   Terada M, Salzler M, Lennartz K, Y M. The effect of H-2 compatibility on pancreatic beta cell survival in the nonobese diabetic mouse. Transplantation. 1988 Mar;45(3):622-7.

73.   Wang Y, McDuffie M, Nomikos I, Hao L, Lafferty K. Effect of cyclosporine on immunologically mediated diabetes in nonobese diabetic mice. Transplantation. 1988;46:101S-6S.

74.   Stegall M, Loberman Z, Ostrowska A, Coulombe M, Gill R. Autoimmune destruction of islet grafts in the NOD mouse is resistant to 15-deoxyspergualin but sensitive to anti-CD4 antibody. J Surg Res. 1996 Aug;64(2):156-60.

75.   Genestier L FS, Flacher M, Assossou O, Revillard JP, Bonnefoy-Berard N.   . Induction of Fas (Apo-1, CD95)-mediated apoptosis of activated lymphocytes by polyclonal antithymocyte globulins. Blood. 1998;91:2360-8.

76.   Bartlett S, Schweitzer E, Kuo P, Johnson L, Delatorre A, Hadley G. Prevention of autoimmune islet allograft destruction by engraftment of donor T cells. Transplantation. 1997;63(2):299-303.

77.   Uchikoshi F, Ito T, Kamiike W, et al. Pancreas transplantation, but not islet transplantation, protects recurrence of IDDM in diabetic BB rats. Transplant Proc. 1997;29(1-2):753-5.

78.   Sibley R, Sutherland D, Goetz F, Michael A. Recurrent diabetes mellitus in the pancreas iso- and allograft. A light and electron microscopic and immunohistochemical analysis of four cases. Lab Invest. 1985;53(2):132.

79.   Lampeter E, Homberg M, Quabeck K, et al. Transfer of insulin-dependent diabetes between HLA-identical siblings by bone marrow transplantation. Lancet. 1993;341(8855):1243-44.

80.   Maki T, Ichikawa T, Blanco R, Porter J. Long-term abrogation of autoimmune diabetes in nonobese diabetic mice by immunotherapy with anti-lymphocyte serum. Proc Nat Acad Sci U S A. 1992;89(8):3434-8.

81.   Keymeulen B, Ling Z, Gorus F, et al. Implantation of standardized beta-cell grafts in a liver segment of IDDM patients: graft and recipient characteristics in two cases of insulin-independence under maintenance immunosuppression for prior kidney graft. Diabetologia. 1998;41(4):452-9.

82.   Brendel M, Hering B, Schultz A, Bretzel R. International Islet Transplant Registry. Newsletter. 2001 June 8(9):1.

83.   Birkeland S. Steroid-free immunosuppression after kidney transplantation with antithymocyte globulin induction and cyclosporine and mycophenolate mofetil maintenance therapy. Transplantation 1998;66(9):1207-10.

84.   Cantarovich D, Giral-Classe M, Hourmant M, et al. Low incidence of kidney rejection after simultaneous kidney-pancreas transplantation after antithymocyte globulin induction and in the absence of corticosteroids: results of a prospective pilot study in 28 consecutive cases. Transplantation. 2000;69(7):1505-9.

85.   Brennan D, Group TIS. Thymoglobulin versus Simulect for induction immunosuppression in cadaveric renal transplant recipients: expanded results from a prospective, randomized, multicenter trial. Oral presentation at the American Society of Transplantation 2003 American Transplant Congress, May 30-Jun 4, 2003 Washington, DC. 2003 April 26- May 1;Abstract 1121.

86.   Vincenti F, Kirkman R, Light S, et al. Interleukin-2-receptor blockade with daclizumab to prevent acute rejection in renal transplantation. N Engl J Med. 1998 Jan;338(3):161-5.

87.   Jindal R, Sidner R, Milgrom M. Post-transplant diabetes mellitus. The role of immunosuppression. Drug Saf. 1997 Apr;16(4):242-57.

88.   Christiansen E, Andersen H, Rassmussen K, et al. Pancreatic beta-cell function and glucose metabolism in human segmental pancreas and kidney transplantation. Am J Physiol Endocrinol Metab. 1993;264(3):E441-E9.

89.   Shapiro A, Gallant H, Hao E, et al. Portal vein immunosuppressant levels and islet graft toxicity. Transplant Proc. 1998 Mar;30(2):641.

90.   Gores P, Sutherland D. Immunosuppression for islet transplantation. In: Lanza RP, Chick WL, eds. Pancreatic Islet Transplantation. 1994;II(Immunomodulation of Pancreatic Islets):63-74.

91.   Sutherland D, Gores P, Hering B, Wahoff D, McKeehen D, Gruessner R. Islet transplantation: an update. Diab Met Rev. 1996;12(2):137-50.

92.   Hering B, Wijkstrom M. Sirolimus and islet transplants. Transplant Proc. 2003 May;35(3 Suppl):187S-90S.

93.   Groth C, Backman L, Morales J, et al. Sirolimus (rapamycin)-based therapy in human renal transplantation: similar efficacy and different toxicity compared with cyclosporine. Sirolimus European Renal Transplant Study Group. Transplantation. 1999 Apr 15;67(7):1036-42.

94.   Kahan B, Podbielksi J, Napoli K, Katz S, Meier-Kriesche H, VanBuren C. Immunosuppressive effects and safety of a sirolimus/cyclosporine combination regimen for renal transplantation. Transplantation. 1998;66(8):1040-6.

95.   McAlister V, Gao Z, Peltekian K, Dominguas J, Mahalati K, MacDonald A. Sirolimus-tacrolimus combination immunosuppression. Lancet. 2000;355(9201):376-7.

96.   McDevitt H, Munson S, Ettinger R, Wu A. Multiple roles for tumor necrosis factor-alpha and lymphotoxin alpha/beta in immunity and autoimmunity. *Arthritis Res*. 2002;4(Suppl 3):S141-S52.

97.   Wu A, Hua H, Munson S, McDevitt H. Tumor necrosis factor-alpha regulation of CD4+CD25+ T cell levels in NOD mice. *Proc Natl Acad Sci USA*. 2002;99(19):12287-92.

98.   Rabinovitch A, Sumoski W, Rajotte R, Warnock G. Cytotoxic effects of cytokines on human pancreatic islet cells in monolayer culture. *J Clin Endocrinol Metab*. 1990;71(1):152-6.

99.   Farney A, Xenos E, Sutherland D, et al. Inhibition of pancreatic islet beta cell function by tumor necrosis factor is blocked by a soluble tumor necrosis factor receptor. *Transplant Proc*. 1993 Feb;25;25(1 Pt 2):865-6.

100.   Eason J, Pascual M, Wee S, et al. Evaluation of recombinant human soluble dimeric tumor necrosis factor receptor for prevention of OKT3-associated acute clinical syndrome. *Transplantation*. 1996 Jan 27;61(2):224-8.

101.   Eason J, Wee S, Kawai T, et al. Inhibition of the effects of TNF in renal allograft recipients using recombinant human dimeric tumor necrosis factor receptors. *Transplantation*. 1995 Jan 27;59(2):300-5.

102.   Wee S, Pascual M, Eason J, et al. Biological effects and fate of a soluble, dimeric, 80-kDa tumor necrosis factor receptor in renal transplant recipients who receive OKT3 therapy. *Transplantation*. 1997;63(4):570-7.

103.   Novak E, Blosch C, Perkins J, et al. Recombinant human tumor necrosis factor receptor Fc fusion protein therapy in kidney transplant recipients undergoing OKT3 induction therapy. *Transplantation*. 1998 Dec;66(12):1732-5.

104. Chiang K, Abhyankar S, Bridges K, Godder K, Henslee-Downey J. Recombinant human tumor necrosis factor receptor fusion protein as complementary treatment for chronic graft-versus-host disease. *Transplantation*. 2002 Feb 27;73(4):665-7.

105. Andolina M, Rabusin M, Maximova N, Di Leo G. Etanercept in graft-versus-host disease. *Bone Marrow Transplant*. 2000 October;26(8):929.

106. Nam M, Reda D, Boujoukos A, et al. Recombinant human dimeric tumor necrosis factor (TNF) receptor (TNFR:Fc): safety and pharacokinetics in human volunteers. Clin Res. 1993;41:249A (Abstract).

107. Lebsack M, Hanna R, Lange M, et al. Absolute bioavailabitilty of TNF receptor fusion protein following subcutaneous injection in healthy volunteers. Pharmacotherapy. 1997;17:1118.

108. Moreland L, Margolies G, Heck Jr L, et al. Recombinant soluble tumor necrosis factor receptor(p80) fusion protein: toxicity and dose finding trial in refractory rheumatiod arthritis. J Rheumatol. 1996;23:1849-55.

109. Hering B, Kandaswamy R, Harmon J, et al. Transplantation of cultured islets from two-layer preserved pancreases in type 1 diabetes with anti-CD3 antibody. Am J Transplant. 2004 Mar;4(3):390-401.

110. Taylor G, Kirkland T, Lakey J, Rajotte R, Warnock G. Bacteremia due to transplantation of contaminated cryopreserved pancreatic islets. Cell Transplant. 1994 Jan-Feb;3(1):103-6.

111. Lloveras J, Farney A, Sutherland D, Wahoff D, Field J, Gores P. Significance of contaminated islet preparations in clinical islet transplantation. Transplant Proc. 1994;26(2):579-80.

112. Rickels M, Kamoun M, Kearns J, Markmann J, Naji A. Evidence for allograft rejection in an islet transplant recipient and effect on {beta}-cell secretory capacity. J Clin Endocrinol Metab. 2007 May;92(7):2410-4.

113. Rickels M, Kearns H, Markmann E, et al. HLA sensitization in islet transplantation. Clinical Transplants. 2006;34:413-20.

114. Alejandro R, Angelico M, Esquenazi V, Kenyon N, Garcia-Morales R, Ciancio G, et al. Development of cytotoxic antibodies against donor HLA antigens in islet autograft recipients. 23rd Scientific Meeting of the American Society of Transplant Surgeons, 1997.

115. Olack B, Swanson C, Flavin K, et al. Sensitization to HLA antigens in islet recipients with failing transplants. Transplant Proc. 1997 Jun;29(4):2268-9.

116. Mao Q, Terasaki P, Cai J, et al. Extremely high association between appearance of HLA antibodies and failure of kidney grafts in a five-year longitudinal study. Am J Transplant. 2007 Apr;7(4):864-71.

117. Terasaki P, Ozawa M. Predicting kidney graft failure by HLA antibodies: a prospective trial. Am J Transplant. 2004 Mar;4(3):438-43.

118. Terasaki P, Ozawa M, Castro R. Four-year follow-up of a prospective trial of HLA and MICA antibodies on kidney graft survival. Am J Transplant. 2007;7(2):408-15.

119. United Network for Organ Sharing, (UNOS). http://www.unos.org/data/about/viewDataReports.asp.

120. Ramsay R, Goetz F, Sutherland D, et al. Progression of diabetic retinopathy after pancreas transplantation for insulin-dependent diabetes mellitus. N Engl J Med. 1988 Jan;318(4):208-14.

121. Crew R, Radhakrishnan J, Cohen D, et al. De novo thrombotic microangiopathy following treatment with sirolimus: report of two cases. Nephrol Dial Transplant. 2005 Jan;20(1):203-9.

122. Marti H, Frey F. Nephrotoxicity of rapamycin: an emerging problem in clinical medicine. Nephrol Dial Transplant. 2005 Jan;20(1):13-5.

123. Fervenza F, Fitzpatrick P, Mertz J, et al. Acute rapamycin nephrotoxicity in native kidneys of patients with chronic glomerulopathies. Nephrol Dial Transplant. 2004 May;19(5):1288-92.

124. Ryan E, Lakey J, Paty B, et al. Successful islet transplantation: continued insulin reserve provides long-term glycemic control. Diabetes. 2002 Jul;51(7):2148-57.

125. Ryan E, Paty B, Senior P, Shapiro A. Risks and side effects of islet transplantation. Curr Diab Rep. 2004 Aug;4(4):304-9.

126.   Senior PA, Shapiro AM, Ackerman TE, Ryan EA, Paty BW, Bhargava R. Magnetic resonance-defined perinephric edema after clinical islet transplantation: a benign finding associated with mild renal impairment. Transplantation. 2004 Sep 27;78(6):945-8.

127.   Digon Br, Rother K, Hirshberg B, Harlan D. Sirolimus-induced interstitial pneumonitis in an islet transplant recipient. Diabetes Care. 2003 Nov;26(11):3191.

128.   Hirshberg B, Rother K, Digon Br, et al. Benefits and risks of solitary islet transplantation for type 1 diabetes using steroid-sparing immunosuppression: the National Institutes of Health experience. Diabetes Care. 2003 Dec;26(12):3288-95.

129.   Ehlers S. Why does tumor necrosis factor targeted therapy reactivate tuberculosis? J Rheumatol Suppl. 2005 Mar;32(Suppl 74):35-9.

130.   Keane J. TNF-blocking agents and tuberculosis: new drugs illuminate an old topic. Rheumatology. 2005 Jun;44(6):714-20.

131.   Ormerod L. Tuberculosis and anti-TNF-alpha treatment. Thorax. 2004;59(11):921.

132.   Close N, Hering B, Eggerman T. Collaborative Islet Transplant Registry - Annual Report. 2006;1:1-278.

133.   Hering B, Ricordi C. Islet Transplantation for patients with type 1 diabetes: Results, research priorities, and reasons for optimism. Graft. 1999;2(1):12-27.

134.   Bertuzzi F, Grohovaz F, Maffi P, et al. Successful [correction of Succesful] transplantation of human islets in recipients bearing a kidney graft. Diabetologia. 2002 Jan;45(1):77-84.

135.   Froud T, Yrizarry J, Alejandro R, Ricordi C. Use of D-STAT to prevent bleeding following percutaneous transhepatic intraportal islet transplantation. Cell Transplant. 2004;13(1):55-9.

136.   Manciu N, Beebe D, Tran P, Gruessner R, Sutherland D, Belani K. Total pancreatectomy with islet cell autotransplantation: anesthetic implications. J Clin Anesth. 1999;11(7):576-82.

137.   Froberg M, Leone J, Jessurun J, Sutherland D. Fatal disseminated intravascular coagulation after autologous islet transplantation. Hum Pathol. 1997 Nov;28(11):1295-8.

138.   Mehigan D, Bell W, Zuidema G, Eggleston J, Cameron J. Disseminated intravascular coagulation and portal hypertension following pancreatic islet autotransplantation. Ann Surg. 1980;191(3):287-93.

139.   Mittal V, Toledo-Pereyra L, Sharma M, et al. Acute portal hypertension and disseminated intravascular coagulation following pancreatic islet autotransplantation after subtotal pancreatectomy. Transplantation. 1981;31(4):302-4.

140.   Rafael E, Ryan E, Paty B, et al. Changes in liver enzymes after clinical islet transplantation. Transplantation. 2003 Nov;76(9):1280-4.

141.   Wahoff D, Papalois B, Najarian J, et al. Autologous islet transplantation to prevent diabetes after pancreatic resection. Ann Surg. 1995 Oct;222(4):562-75; discussion 75-9.

142.   Bhargava R, Senior P, Ackerman T, et al. Prevalence of hepatic steatosis after islet transplantation and its relation to graft function. Diabetes Care. 2004 May;53(5):1311-7.

143.   Markmann J, Rosen M, Siegelman E, et al. Magnetic resonance-defined periportal steatosis following intraportal islet transplantation - A functional footprint of islet graft survival? Diabetes. 2003 Jul;52(7):1591-4.

144.   Cameron J, Mehigan D, Broe P, Zuidema G. Distal pancreatectomy and islet transplantation for chronic pancreatitis. Ann Surg. 1981 Mar;193(3):312-7.

145.   Casey J, Lakey J, Ryan E, et al. Portal venous pressure changes after sequential clinical islet transplantation. Transplantation. 2002 Oct;74(7):913-5.

146.   Moberg L, Johansson H, Lukinius A, et al. Production of tissue factor by pancreatic islet cells as a trigger of detrimental thrombotic reactions in clinical islet transplantation. Lancet. 2002;360(9350):2039-45.

147.   Ryan EA, Paty BW, Senior PA, Shapiro AM. Risks and side effects of islet transplantation. Curr Diab Rep. 2004 Aug;4(4):304-9.

148.   Rother K, Harlan D. Challenges facing islet transplantation for the treatment of type 1 diabetes mellitus. J Clin Invest. 2004 Oct;114(7):877-83.

149.   Shapiro A, Lakey J, Rajotte R, et al. Portal vein thrombosis after transplantation of partially purified pancreatic islets in a combined human liver/islet allograft. Transplantation. 1995;59(7):1060-3.
150.   Brown S, O'Reilly P. Iohexol clearance for the determination of glomerular filtration rate in clinical practice: evidence for a new gold standard. J Urol. 1991 Sep;146(3):675-9.
151.   Johnson J, Kotovych M, Ryan E, Shapiro A. Reduced fear of hypoglycemia in successful islet transplantation. Diabetes Care. 2004 Feb;27(2):624-5.
152.   Steffes M, Sibley S, Jackson M, Thomas W. beta-cell function and the development of diabetes-related complications in the diabetes control and complications trial. Diabetes Care. 2003 Mar;26(3):832-6.
153.   Lesley A Stevens JC, Christopher H Schmid, Harold I. Feldman, Marc Froissart, John Kusek, Jerome Rossert, Frederick Van Lente, Robert D. Bruce, III, Yaping (Lucy) Zhang, Tom Greene, and Andrew S Levey. Estimating GFR using Serum Cystatin C Alone and in Combination with Serum Creatinine: A Pooled Analysis of 3418 Individuals with CKD. Am J Kidney Dis. 2008 March 2008;51(3):395-406.
154.   Wyeth Pharmaceuticals. Sirolimus package insert. 2005.
155.   Clarke W, Cox D, Gonder-Frederick L, Julian D, Schlundt D, Polonsky W. Reduced awareness of hypoglycemia in adults with IDDM. A prospective study of hypoglycemic frequency and associated symptoms. Diabetes Care. 1995 Apr;18(4):517-22.
156.   Zavaroni I, Deferrar iG, Lugari R, et al. Renal metabolism of C-peptide in man. J Clin Endocrinol Metab. 1987 Sep;65(3):494-8.
157.   Zerbini G, Mangili R, Luzi L. Higher post-absorptive C-peptide levels in Type 1 diabetic patients without renal complications. Diabet Med. 1999 Dec;16(12):1048.
158.   Faradji R, Monroy K, Messinger S, et al. Simple Measures to Monitor B-Cell Mass and Assess Islet Dysfunction: Lessons from Islet Transplantation. Unpublished Data (in review process). 2006.
159.   Teuscher A, Kendall D, Smets Y, Leone J, Sutherland D, Robertson R. Successful islet autotransplantation in humans: functional insulin secretory reserve as an estimate of surviving islet cell mass. Diabetes. 1998 Mar;47(3):324-30.
160.   Benjamini YaYH. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J Roy Statist Soc. 1995;B 57(1):289-300.
161.   Lan K, DeMets D. Changing frequency of interim analysis in sequential monitoring. Biometrics. 1989;45(3):1017-20.
162.   O'Brien P, Fleming T. A multiple testing procedure for clinical trials. Biometrics. 1979;35:549-56.
163.   ICH Expert Working Group. Guidance for Industry: E6 Good Clinical Practice Consolidated Guidance. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. 1996 April.
164.   Rickels M, Schutta M, Mueller R, et al. Islet Cell hormonal Responses to Hypoglycemia After Human Islet Transplantation for Type 1 Diabetes. Diabetes. 2005 Nov;54(11):3205-11.

Appendix 1. Schedule of Events

Time points (specified in Days relative to transplant)	SCR	WL / BL[1]	0 [2]	3[3]	7	14	21	28	56	75	120	150	180	210	240	270	300	330	365	365 post initial tx
Visit Number	01	02	03	04	05	06	07	08	09	10	11	12	13	13a	13b	14	14a	14b	15	Y1
Visit Windows (specified in days)	N/A	N/A	N/A	N/A	±3	±3	±3	±3	±7	±5	±7	±7	±7	±7	±7	±14	±7	±7	±14	± 14
Equivalent Week/Month	N/A	N/A	N/A	N/A	W1	W2	W3	W4	M2	M2.5	M4	M5	M6	M7	M8	M9	M10	M11	M12	Varies
GENERAL ASSESSMENTS																				
Informed Consent	X[4]	X[5]																		
Med/Diabetes Hx & Demographics	X																			
Evaluation of Inclusion / Exclusion	X	X																		
Retinopathy Evaluation[6]	X	X-yrly[7]																		X
Physical Exam	X	X-yrly	X		X	X		X	X	X	X	X	X			X			X	
Telephone Consult							X													
QOL		X-q3mo								X			X					X	X	X
Chest X-Ray	X	X-yrly																	X	
Abdominal US (including Pelvis/Liver)	X	X-yrly			X														X	
ECG	X	X-yrly																	X	
Cardiac Stress Test or Angiogram	X																			
PPD	X	X-yrly																	X	
AE /Hypoglycemic Events/Toxicity Assess		X	X	X	X	X	X	X	X	X	X	X	X			X			X	
LOCAL LABORATORY ASSESSMENTS																				
CBC (WBC + Diff & Plat)	X	X-q6mo	X		X	X	X[8]	X	X	X	X	X	X			X			X	
Chemistry[9]	X	X-q6mo	X		X	X	X[8]	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[10]																		

Time points (specified in Days relative to transplant)	SCR	WL / BL[1]	0[2]	3[3]	7	14	21	28	56	75	120	150	180	210	240	270	300	330	365	365 post initial tx
Visit Number	01	02	03	04	05	06	07	08	09	10	11	12	13	13a	13b	14	14a	14b	15	Y1
Visit Windows (specified in days)	N/A	N/A	N/A	N/A	±3	±3	±3	±3	±7	±5	±7	±7	±7	±7	±7	±14	±7	±7	±14	± 14
Equivalent Week/Month	N/A	N/A	N/A	N/A	W1	W2	W3	W4	M2	M2.5	M4	M5	M6	M7	M8	M9	M10	M11	M12	Varies
LOCAL LABORATORY ASSESSMENTS (cont'd)																				
Serology[11] (Hep B, Hep C, HIV)	X	X-yrly																		X
EBV IgG	X																			
CMV IgG, CMV IgM		X-yrly[12]																		X[12]
Coagulation (PT, PTT, INR)	X	X-yrly	X																	
Blood Type		X[13]																		
HLA		X																		
Crossmatch		X[14]																		
Fasting & post-prandial c-peptide[15]				X	X															
Glucose (immediately post-transplant)			X[16]																	
PRA by flow cytometry		X[17]																		
CMV by PCR		X								X			X							
EBV by PCR[18]		X																		
CENTRAL LABORATORY / METABOLIC ASSESSMENTS																				
First morning spot urine[19]	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[20]	X	X						X	X	X	X	X	X	X[21]	X[21]	X	X[21]	X[21]	X	X
90 min[22] c-pep/glucose (MMTT) [20]	X									X		X				X			X	X
Insulin modified FSIGT[20]		X-yrly7								X									X	X

Time points (specified in Days relative to transplant)	SCR	WL / BL[1]	0[2]	3[3]	7	14	21	28	56	75	120	150	180	210	240	270	300	330	365	365 post initial tx
Visit Number	01	02	03	04	05	06	07	08	09	10	11	12	13	13a	13b	14	14a	14b	15	Y1
Visit Windows (specified in days)	N/A	N/A	N/A	N/A	±3	±3	±3	±3	±7	±5	±7	±7	±7	±7	±7	±14	±7	±7	±14	± 14
Equivalent Week/Month	N/A	N/A	N/A	N/A	W1	W2	W3	W4	M2	M2.5	M4	M5	M6	M7	M8	M9	M10	M11	M12	Varies
CENTRAL LABORATORY / METABOLIC ASSESSMENTS (cont'd)																				
Atherogenic Profile[23]		X																		X
LOCAL METABOLIC ASSESSMENTS																				
Glycemic Stability (CGMS)[20]		X-yrly[7]								X									X	X
BSR eCRFs[20, 24]	X	X-q6mo								X			X			X			X	X
CALCULATED METABOLIC ASSESSMENTS																				
MAGE		X-q6mo								X			X			X			X	X
LI	X	X-q6mo								X			X			X			X	X
Clarke Score	X	X-q6mo											X						X	X
HYPO	X	X-q6mo								X			X			X			X	X
Beta Score		X								X			X			X			X	X
C-peptide: (glucose X creatinine) ratio	X	X						X	X	X	X	X	X[25]			XError! Bookmark not defined.			X	X
CARDIOVASCULAR ASSESSMENTS																				
Carotid IMT		X[7]																		X
IMMUNOSUPPRESSION LEVELS																				
Sirolimus 24-hour trough levels			X	X	X	X	X[8]	X	X	X	X	X	X			X			X	X
Tacrolimus 12-hour trough levels				X	X	X	X[8]	X	X	X	X	X	X			X			X	X
MECHANISTIC ASSAYS																				

	SCR	WL / BL[1]	0[2]	3[3]	7	14	21	28	56	75	120	150	180	210	240	270	300	330	365	365 post initial tx
Alloantibody	X	X-q6mo[26]								X		X				X			X	X
Autoantibody (GAD, IA-2, IAA)		X								X		X				X			X	X
Time points (specified in Days relative to transplant)	SCR	WL / BL[1]	0[2]	3[3]	7	14	21	28	56	75	120	150	180	210	240	270	300	330	365	365 post initial tx
Visit Number	01	02	03	04	05	06	07	08	09	10	11	12	13	13a	13b	14	14a	14b	15	Y1
Visit Windows (specified in days)	N/A	N/A	N/A	N/A	±3	±3	±3	±3	±7	±5	±7	±7	±7	±7	±7	±14	±7	±7	±14	± 14
Equivalent Week/Month	N/A	N/A	N/A	N/A	W1	W2	W3	W4	M2	M2.5	M4	M5	M6	M7	M8	M9	M10	M11	M12	Varies
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] If participant receives basiliximab with a subsequent transplant, administration of the day 4 dose will be an unscheduled visit.

[4] *Informed Consent #1* includes information on CIT07 or CIT07 and Site Specific Phase 2 protocols.

[5] *Informed Consent #2* includes information specific to CIT07. IC #2 must be signed immediately after randomization.

[6] 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.

[7] 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. Baseline carotid IMT can be collected up to 30 days post-transplant. Even if baseline IMT is missing, follow-up carotid IMTs should be collected.

[8] Can be performed at a local laboratory.

[9] Chemistry includes: Sodium, albumin, magnesium, chloride, potassium, alk phosphatase, total bilirubin, CO_2, creatinine, ALT (SGPT), BUN, gamma GT, glucose, AST (SGOT), calcium, phosphorus.

[10] Complete pregnancy test within 72 hours prior to the initiation of study medication.

[11] Serology includes: HBc Ab, HBs Ab, HBs Ag, HCV Ab, and HIV. Do not repeat Hepatitis B tests if HBs Ab was previously positive.

[12] Repeat only if previous test was negative.

[13] Repeat for subsequent transplant(s).

[14] Sample used for crossmatch may be obtained up to 60 days prior to the start of immunosuppression, as long as there is no evidence of infections or transfusions since the time the sample was drawn. Repeat crossmatch for subsequent transplants.

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

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

[17] Subsequent transplants only. Local result used to determine eligibility for subsequent transplants only.

[18] EBV by PCR should only be done post-randomization if reactivation is suspected.

[19] First morning spot urine includes: albumin, protein, and creatinine

[20] Do 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.

[21] If blood drawn locally, sample should be sent from local lab to study site and then shipped to the central laboratory (Univ of Washington).

[22] MMTT should include 60 and 90 minute c-peptide and glucose measurements for the screening visit and as necessary when determining graft failure.

[23] 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).

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

[25] C-peptide glucose creatinine ratio calculated monthly.

[26] For each transplant, complete alloantibody assessment every 6 months and again on Day -2, regardless of the most recent draw. Central PRA result, current within 6 months, is used to determine subject eligibility for first transplant.

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)[1]
- 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)

---

[1] Only collect if subject has graft function and has not received a non-CIT transplant.

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Appendix 4. 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	20
Visit Window (specified in days)	± 14	± 14	± 14	± 14	± 90
GENERAL ASSESSMENTS					
Physical Exam		X		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
90 min c-pep/glucose (MMTT)[4]		X		X	X
Atherogenic Profile[5]				X	
LOCAL METABOLIC ASSESSMENTS					
Glycemic Stability (CGMS)				X	X
7-Day BSR[6] eCRFs				X	X
CALCULATED METABOLIC ASSESSMENTS					
Clarke Score		X		X	
CARDIOVASCULAR ASSESSMENTS					
Carotid IMT				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	

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[1] Can be performed at a local laboratory.

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

[3] Can be drawn locally.

[4] Also collect as necessary to confirm graft failure.

[5] 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).

[6] Blood Sugar Record (BSR) eCRF is completed using information gathered from subject diary logs, glucometer download data, and insulin requirements for 7 consecutive days. See MOP for guidance.

Appendix 5. University of Miami Sub-study

Rationale for Additional Metabolic and Immunologic Testing:

Conducting additional metabolic and immunologic studies at the University of Miami will allow for generation of control data with which to compare results of the same assays conducted in the site-specific Phase 2 study. These assessments will be performed at the specific time points indicated in the tables below. The goal of these assessments is to identify tests that will lead to informative new assays that can be used in subsequent trials. The specific aims of this sub-study are:

Aim 1: To assess islet engraftment, metabolic function and long-term survival in CIT07 subjects by undertaking additional metabolic tests that we have previously observed to be early indicators of graft dysfunction and comparing the results obtained to data from the site –specific Phase 2 subjects.

Aim 2: To undertake additional immune monitoring strategies in CIT07 subjects that allow verification of the efficacy of immunosuppression and/or identify perturbations of the immune system which precede early loss of function, rejection, or recurrent autoimmunity and comparing the results obtained to data from the site-specific Phase 2 subjects.

Aim 3: To utilize the information obtained from these studies to clearly identify new metabolic and immunologic testing strategies that provide information that is superior to that attainable with more established assays; *i.e.*, the data enables alteration of therapies to achieve enhanced graft survival.

Additional Exploratory Endpoints

The sub-study will allow for analysis of additional exploratory endpoints in relation to graft function and insulin independence. The additional exploratory endpoints include:

1. For the 5 hour MMTT:
   a) 90 Minute C-peptide
   b) Peak C-peptide
   c) Time for C-peptide to peak
   d) Mixed Meal Stimulation Index (MSI = AUC C-peptide/AUC Glucose)
   e) 90 Minute Glucose
   f) Peak Glucose
   g) Time for glucose to peak
   h) AUC for glucagon, amylin, and proinsulin
2. For the (CGMS):
   a) Glucose variability

      b) Percentage Hyperglycemia Time -%HGT: Defined as the percentage of time BG levels are above 140 mg/dL over the total time measured by the CGMS (72-84 hr period)

3. For the additional C-peptide, glucose and creatinine assessments:

      a) C-peptide:glucose ratio (CP/G)

      b) C-peptide: (glucose X creatinine) ratio (CPGCR)

4. Additional Immunologic Testing

      a) Soluble mediators

      b) Granzyme B expression

      c) Phenotype

      d) RNA for microarray

      e) T and B cell assay

A table, containing the time-points for each of the additional metabolic and immunologic tests, is provided on the next two pages.

Table of Additional Testing for the University of Miami Sub-study:

Time points (specified in Days relative to transplant)	SCR	WL / BL[1]	0[2]	3	7	14	21	28	56	75	120	150	180	270	365	365 post initial tx	455	545	635	730
Visit Number	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	Y1	16	17	18	19
Visit Windows (specified in days)	N/A	N/A	N/A	N/A	±3	±3	±3	±3	±7	±5	±7	±7	±7	±14	±14	±14	±14	±14	±14	±14
Equivalent Week/Month	N/A	N/A	N/A	N/A	W1	W2	W3	W4	M2	M2.5	M4	M5	M6	M9	M12	Varies	M15	M18	M21	M24
Nutritional Assessment	X	X-yrly								X			X		X	X				X
MMTT (extra time points = 15, 30, 60, 120, 150, 180, 210, 240, 270, and 300 minutes)										X			X	X	X	X		X		X
Glycemic Stability (CGMS)													X	X	X					
Fasting plasma gluc/c-pep & serum creat (for CP/G and CPGCR)				X	X	X	X	X[3]		X[4]								X		X
Soluble mediators		X[5]	X[6]	X[7]	X	X	X	X												
Granzyme B expression		X[5]	X	X	X	X	X	X[8]	X	X[8]	X[8]	X[8]	X[9]	X[9]	X[9]	X	X	X	X	X
Phenotype		X[5]			X			X		X			X	X	X			X		X
RNA for microarray		X[5]								X			X	X	X			X		X
T and B cell assay		X[5]								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 prior to start of immunosuppression. For repeat WL/BL assessments, record results from test done closest to the start of immunosuppression on the BL CRFs.

[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] Complete once a week (Week 4 is on the CIT07 SOE; therefore, extra time points = Week 5, 6, and 7)

[4] Complete every 2 weeks (M2.5 is on the CIT07 SOE; therefore, extra time points = M3 and M3.5)

[5] Complete one time while subject is on waiting list and again on Day -2 relative to transplant.

[6] Complete pre-infusion, 1 hr, and 6 hrs post-infusion

[7] Complete on Days 1, 2, and 4

[8] Complete every 2 weeks

[9] Complete monthly

Appendix 6. University of Pennsylvania Sub-study

Rationale for Additional Immunology Mechanistic Assays:

Recent evidence supports a pivotal role for B lymphocytes in the pathogenesis of T1D. B lymphocytes not only produce islet-reactive antibodies, but also serve as potent antigen presenting cells, aiding and amplifying autoreactive T lymphocyte responses. In the University of Pennsylvania's site-specific Phase 2 CIT study (Protocol CIT05), B and T lymphocytes will be monitored in two populations of patients: 1. those awaiting islet transplantation and 2. those who received islets and B cell depletion and induction therapy with Thymoglobulin®. The primary goal of this study is to determine if B cell depletion improves islet survival and function in patients with T1D. However, from an immunologic standpoint, the study is somewhat suboptimal because the two groups of patients differ from each other with respect to one or more immunosuppressive agents and these agents (for example, rituximab and ATG) have effects that are synergistic. The CIT07 study therefore provides a very important immunologic and contemporary control group for the CIT05 study. By analyzing the lymphocyte repertoire and phenotype in patients in the CIT07 study, we will be able to obtain a much clearer idea of which immunologic changes are attributable specifically to rituximab depletion.

The analysis of B and T lymphocyte subsets and B cell repertoire at later time points (see below) is important because it is known from other studies that these lymphocyte populations are not fully restored to their baseline state after one year. For example, we have shown that most lupus patients who receive rituximab treatment have mostly transitional B cells at day 365 and sometimes the absolute lymphocyte count overshoots the baseline at the 1-year time point (Sutter, J. et al. Clin Immunol. Vol. 126(3): 282, 2008). Patients receiving combined T and B cell depletion may experience a more prolonged and/or profound period of lymphocyte reconstitution. As mentioned above, the CIT07 group, is a critical control which will provide information on the effects of T cell depletion and suppression on the B lymphocyte compartment. Conversely, the comparison of T cells in the CIT05 and CIT07 groups will allow us to determine to what extent combined B and T cell immunosuppression influences the T cell compartment. Finally, it is possible that the later time points (beyond day 365) will facilitate the correlation of immune parameters and metabolic and clinical assessments of islet function. The longer follow-up period may allow us to determine if particular changes in the lymphocyte subset composition or repertoire precede or accompany islet rejection or tolerance.

The analysis of B and T lymphocyte subsets and B cell repertoire at later time points (see below) is important because it is known from other studies that these lymphocyte populations are not fully restored to their baseline state after one year. For example, we have shown that most lupus patients who receive rituximab treatment have mostly transitional B cells at day 365 and sometimes the absolute lymphocyte count overshoots the baseline at the 1-year time point (Sutter, J. et al. Clin Immunol. Vol. 126(3): 282, 2008). Patients receiving combined T and B cell depletion may experience a more prolonged and/or profound period of lymphocyte reconstitution. As mentioned above, the CIT07 group, is a critical control which will provide information on the effects of T cell

depletion and suppression on the B lymphocyte compartment. Conversely, the comparison of T cells in the CIT05 and CIT07 groups will allow us to determine to what extent combined B and T cell immunosuppression influences the T cell compartment. Finally, it is possible that the later time points (beyond day 365) will facilitate the correlation of immune parameters and metabolic and clinical assessments of islet function. The longer follow-up period may allow us to determine if particular changes in the lymphocyte subset composition or repertoire precede or accompany islet rejection or tolerance.

Rationale for Additional Metabolic Assays:

Islet transplantation can restore endogenous insulin secretion and eliminate the development of severe hypoglycemic episodes in patients with T1D, but usually requires islets isolated from more than one donor pancreas to achieve insulin independence. The majority of islet recipients return to requiring some insulin therapy by two years following transplantation [14]. It has been shown that in insulin-independent islet transplant recipients, the β-cell secretory capacity, a measure of functional β-cell mass, is only ~ 25% of normal, demonstrating that a low engrafted β-cell mass exists even in initially successful cases[62]. Furthermore, the β-cell secretory capacity correlates with measures of glucose-dependent β-cell function, suggesting that a low engrafted β-cell mass can account for the functional defects in glucose-mediated insulin secretion observed after islet transplantation[62]. A low engrafted β-cell mass may be a consequence of inadequate immunosuppression leading to immunologic loss of transplanted islets. Nevertheless, the CNI tacrolimus, part of the immunosuppressive regimen for islet transplantation, has been reported to impair glucose-mediated insulin secretion and has been implicated in the pathogenesis of post-transplant (type 2) diabetes. Thus, generalized β-cell dysfunction caused by tacrolimus might mimic a reduced β-cell mass and contribute to the reduced β-cell secretory capacity in islet transplant recipients. While β-cell function is often impaired after islet transplantation, it is clear that functioning islet grafts lead to stabilization of glycemic lability and elimination of severe hypoglycemic episodes[2], an effect of both endogenous insulin secretion in response to hyperglycemia [62,124] and appropriate inhibition of endogenous insulin secretion in response to hypoglycemia[164]. Importantly, glucagon secretion in response to hypoglycemia, albeit less than normal, is also restored in recipients of islet transplants [164] and epinephrine secretion, while also less than normal, occurs at higher glycemic thresholds in islet recipients than in T1D; both of these effects may contribute to protection from severe hypoglycemia by glucagon and epinephrine mediated increases of endogenous glucose production. To evaluate the effect of a CNI-free immunosuppression regimen on β-cell secretory capacity in islet transplant recipients, and to determine the effect of islet transplantation on endogenous glucose production during hypoglycemia, we propose to 1) determine the β-cell secretory capacity in islet transplant recipients receiving tacrolimus-based immunosuppression (CIT07) versus CNI-free immunosuppression with rituximab (CIT05) and 2) determine the effect of islet transplantation on endogenous glucose production during insulin-induced hypoglycemia.

Additional Exploratory Endpoints

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

- A Glucose-potentiated arginine (GPA) test will be conducted at 75 ± 15 days after each infusion, at 365 ± 30 days and at 730 ± 30 days after the last infusion. The endpoints of acute c-peptide and insulin responses to arginine, the glucose potentiation slope and β-cell secretory capacity will be computed and analyzed.

- Glucose counter-regulation (paired eu- and hypoglycemic clamp studies) will be conducted pre-transplant and at 180 ± 30 days and 545 ± 30 days after the last infusion[164]. The endpoints will include endogenous glucose production in response to hypoglycemia, counter-regulatory hormonal and symptom responses to hypoglycemia, and glycemic thresholds for these responses.

- Immunophenotyping of lymphocyte subsets will be performed at baseline and every six months while subjects are on the waitlist, and on days 7 and 28, and months 2.5, 5, 6, 9, 12, 15, 18, 21, and 24 after the first islet transplant. Assessments occurring after month 12 will occur only if the subject is still in study follow-up. Proposed time points beyond d365 would parallel the metabolic assessments and would occur at approximately 3-month intervals, subject to patient and sample availability.

- HiD (high-resolution multiparameter flow cytometry) of B lymphocyte subsets will be performed at baseline and every six months while subjects are on the waitlist. Additional samples may be studied at or within a few weeks of clinically significant changes in patient status (such as transplant rejection).

- B lymphocyte repertoire analysis will include CDR3 spectratyping and clone tracking. Spectratyping will be performed on CD19+ cells obtained at baseline, and months 6, 9, 12, 15 and 21. If clonal expansions are detected, clone tracking will be performed on whole blood using special primer sets while the patient is on the waitlist and at weeks 1, 2, 3, 4 and months 2, 2.5, 4, 5, 6, 9, 12, and at least one time point after 12 months following the first islet transplant, if the subject is still in study follow-up. In addition, if available, samples drawn at the time of transplant rejection before or after month 12 will be analyzed for clonal expansion by CDR3 spectratyping.

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

Table of Additional Metabolic and Immunologic Testing for the University of Pennsylvania Sub-study:

Time points (specified in Days relative to transplant)	SCR	WL / BL[1]	0 [2]	3	7	14	21	28	56	75	120	150	180	270	365	455	545	635	730
Visit Number	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19
Visit Windows (specified in days)	N/A	N/A	N/A	N/A	±3	±3	±3	±3	±7	±15	±7	±7	±7	±14	±30	±30	±30	±30	±30
Equivalent Week/Month	N/A	N/A	N/A	N/A	W1	W2	W3	W4	M2	M2.5	M4	M5	M6	M9	M12	M15	M18	M21	M24
General B, T & NK cell immunophenotyping[3]		X-q6mo			X			X		X		X	X	X	X	X	X	X	X
HiD B cell tube[4]		X-q6mo																	
CD19+ separation (will include kappa for light chain editing study) [5]		X											X	X	X	X		X	
Clone tracking[6]		X-q3mo			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ELISpot screen[7]		X-q6mo	X		X			X		X			X	X	X				
T cytokine profile[8]		X-q6mo	X		X			X		X			X	X	X				
T and B cell ELISpot		X						X					X						
Functional B cell assay(s)[9]		X						X					X	X	X	X			
Glucose-potentiated arginine (GPA)										X					X				X
Glucose counter-regulation (paired eu- and hypoglycemic clamp studies)		X-yrly											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 prior to start of immunosuppression. For repeat WL/BL assessments, record results from test done closest to the start of immunosuppression the BL CRFs.

[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] General flow cytometry on T and B lymphocyte subsets will be performed using a four-color panel. In order to obtain absolute cell counts and for quality control purposes, a CBC with differential will be performed in parallel with every flow cytometry experiment. General flow will be performed approximately every 3-6 months while subjects are on the waitlist, up to three times. In addition to the time points listed in the table above, late time points (months 15, 18, 21, and 24 after the first islets transplant) will be added, if the subject is still in study follow-up.

[4] The HiD B cell tube refers to using a large number of different fluorochrome conjugated markers in the same tube. High definition flow will be performed approximately every 6 months (up to two times) while subjects are on the waitlist. Additional samples may be studied at or within a few weeks of clinically significant changes in patient status (such as transplant rejection), if deemed necessary by the investigators.

[5] CD19 separation will be performed at baseline while subject is on the waiting list prior to transplantation and at months 6, 9, 12, 15 and 21. The timing of CD19 separation may vary from the proposed schedule if the subject is anemic..

[6] DNA will be extracted from peripheral blood leukocytes every three months while patients are on the waitlist to be transplanted and will be banked at the indicated time points following transplant.

[7] The T cell ELISpot screen will be performed at baseline and repeated every six months while the subject is on the wait list (scheduling can be done at the discretion of other studies and the subject; also, testing will not be performed if the subject has a viral infection.) The screen will be performed using dominant T cell epitopes and pools of peptides for islet antigens, if indicated and if funding is available. The read-outs will be IFN-γ and TGF-β secretion.

[8] If the T cell ELISpot is positive, the specificity of reacting T cells (checked against a panel of peptides) will be profiled and the secretion of different cytokines will be monitored if funding is available.

[9] Functional B and T lymphocyte assays will also be performed at month 15 after the first islets transplant, if funding is available. Mononuclear cells will be purified from samples for functional assays and frozen until all samples from a given patient can be performed in the same assay on the same day.