

CLINICAL ISLET TRANSPLANTATION PROTOCOL CIT-06
Islet Transplantation versus Intensive Insulin Therapy in Type 1 Diabetic Kidney
Allograft Recipients
Efficacy of Islet after Kidney Transplantation
Version 2.0F (8 Nov 2007), [BB-IND 9336]

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National Institute of Diabetes & Digestive & Kidney Diseases (NIDDK)

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Protocol Approval

Protocol Number CIT-06	Version/Date: 2.0F /8 Nov 2007
IND: BB-IND 9336	CIT-06 Principal Investigator: Bernhard Hering, MD; Ali Naji, MD, PhD ; Camillo Ricordi, MD; James Shapiro, MD, PhD
Short Title: <i>Efficacy of Islet after Kidney Transplantation</i>	
Study Sponsors: The National Institute of Allergy and Infectious Diseases (NIAID) The National Institute of Diabetes & Digestive & Kidney Diseases (NIDDK)	
INSTRUCTIONS: Please have the Principal Investigator print, sign, and date at the indicated location below. A copy should be kept for your records and the original signature page sent to the Data Coordinating Center (DCC). After signature, please return the original of this form by surface mail to: ATTN: Clinical Trials Statistical and Data Management Center 201 S Clinton St Department of Biostatistics College of Public Health University of Iowa Iowa City, IA 52242-4034 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 current Good Clinical Practice (cGCP) 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 accordance with local, legal, and regulatory requirements. As the site Principal Investigator, I agree to conduct protocol CIT-06, “Efficacy of Islet after Kidney Transplantation,” 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 Versus Intensive Insulin Therapy in Type 1 Diabetic Kidney Allograft Recipients
Short Title	Efficacy of Islet After Kidney Transplantation
Clinical Phase	Phase 3
IND Sponsor	DAIT/NIAID/ NIH
IND Number	BB-IND 9336
Activation Date	January 2007
Accrual Objective	65/group (90% power, at 5% level in two-tailed test)
Accrual Period	2 years
Study Duration	5 years (Accrual plus follow-up)
Study Design	Prospective, multi-center, controlled, randomized trial comparing the benefit of islet transplantation versus intensive insulin therapy (IIT) in type 1 diabetic (T1D) kidney transplant recipients.
Treatment Description	Experimental treatment group patients will receive an infusion(s) of in vitro cultured islets with the goal of achieving insulin independence. For the first islet transplant, patients will receive induction therapy with rabbit anti-thymocyte globulin (ATG, 5 doses) plus etanercept and will remain on their calcineurin-based maintenance immunosuppression regimen already in place for their renal allograft. Induction therapy for subsequent transplants will be 5 biweekly doses of daclizumab.
Primary Endpoint	The proportion of subjects with both an HbA1c $\leq 6.5\%$ and an absence of severe hypoglycemic events at 1 year after the first islet transplant or a reduction in HbA1c of at least 1 point and an absence of severe hypoglycemic events at 1 year after the <i>first</i> islet transplant. Study endpoints are listed relative to the islet transplant occurring on Day = 0. For IIT randomized subjects, "1 year after the first islet transplant" is equivalent to "1 year after Day = 0."

Key Secondary Endpoints

Key secondary endpoints at 365 ± 14 days after the first islet transplant:

1. Diabetes related hypoglycemia avoidance behavior score on the Hypoglycemia Fear Survey
2. Diabetes related worry score on the Hypoglycemia Fear Survey
3. HbA1c
4. The number of severe hypoglycemic events
5. The Ryan HYPO score
6. The glycemic lability index (LI)
7. Urinary albumin creatinine ratio
8. Serum creatinine (SCr)
9. Iohexol measured glomerular filtration rate (GFR)

Key secondary endpoint at 365 ± 14 days after the *last* islet transplant:

10. The primary endpoint measured at one year after the *last* islet transplant. That is a comparison of the proportion of subjects with both an $\text{HbA1c} \leq 6.5\%$ and an absence of severe hypoglycemic events at 1 year after the last islet transplant or a reduction in HbA1c of 1 point and an absence of severe hypoglycemia at 1 year after the *last* islet transplant

Other Secondary Endpoints

1. Insulin independence at 75 ± 5 days after the first islet transplant, at 1 year after the first islet transplant, and at 1 year after the last islet transplant
2. Change in renal biopsy from baseline to three years

At 365 ± 14 days following the *first* islet transplant:

3. Cardiovascular events [death, cerebrovascular accident (CVA), myocardial infarction (MI)], changes in carotid intima-medial thickness, atherogenic profile, ratio of Apolipoprotein A1 and B, and subject death
4. Impact on diabetic neuropathy
5. Impact on vision
6. Diabetes related and general quality of life (SF-36, EQ-5D, and DDS)

7. Differences in cost of care for islet transplanted versus IIT
8. Renal impact measures including renal allograft survival
9. Measures of metabolic control including: Basal c-peptide levels; mixed-meal tolerance test (MMTT); β -score; reduction in insulin requirement; Clarke Survey and mean amplitude of glycemic excursions (MAGE); c-peptide to glucose, creatinine ratio (CPGCR), acute insulin response to glucose (AIR_{glu}), insulin sensitivity, and disposition index derived from the insulin modified frequently-sampled intravenous (IV) glucose tolerance (FSIGT) test; and glucose variability and hypoglycemia duration derived from the continuous glucose monitoring system[®] (CGMS)

At 365 ± 14 days following the *last* islet transplant:

10. Renal impact measures including renal allograft survival and renal allograft function measured by SCr and spot urine albumin creatinine ratio
11. Measures of metabolic control including: Basal c-peptide levels; MMTT; β -score; reduction in insulin requirement; Clarke Survey and MAGE; c-peptide to glucose, creatinine ratio (CPGCR), acute insulin response to glucose (AIR_{glu}), insulin sensitivity, and disposition index derived from the insulin modified frequently-sampled IV glucose tolerance (FSIGT) test; and glucose variability and hypoglycemia duration derived from the CGMS[®]
12. The proportion of subjects who abandon study treatment due to whole organ pancreas transplants

In addition, the following time to event analyses (survival analyses) are also other secondary endpoints:

13. Time to death (all cause and also cardiac related) and time to cardiovascular events (death, CVA, MI)
14. Time to global treatment failure composite outcome measure

**Inclusion
Criteria**

Subjects who meet *all* of the following criteria are eligible for enrollment:

1. Male and female subjects age 18 to 68 years.
2. Subjects who are able to provide written informed consent and to comply with the procedures of the study protocol.
3. Clinical history compatible with T1D with disease onset < 40 years of age and insulin-dependence for ≥ 5 years at the time of enrollment, and a sum of subject age and insulin dependent diabetes duration of ≥ 28 .
4. Absent stimulated c-peptide (< 0.3 ng/mL) in response to a MMTT [Boost® 6 mL/kg body weight (BW) 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 start of consumption.
5. Subjects who are ≥ 3 months and < 60 months post-renal transplant who are taking appropriate calcineurin inhibitor (CNI) based maintenance immunosuppression ([tacrolimus alone or in conjunction with sirolimus, mycophenolate mofetil, myfortic, or azathioprine; or cyclosporine in conjunction with sirolimus, mycophenolate mofetil, or myfortic] $\pm$ Prednisone ≤ 10 mg/day).
6. Stable renal function as defined as creatinine of no more than one third greater than the average creatinine determination performed in the 3 previous months prior to islet transplantation, until rejection, obstruction or infection is ruled out.

Subjects who meet the following criterion are eligible for randomization:

7. Prior to randomization, all subjects must receive IIT for at least 4 months under the care of an experienced diabetologist and at the end of this period have either an HbA1c ≥ 7.0 ;

OR

HbA1c < 7.0 and reduced awareness of hypoglycemia manifested by a Clarke score of 4 or more and at least 1 severe hypoglycemic event 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 (BG) level < 54 mg/dL (3.0 mmol/L) or prompt recovery after oral carbohydrate, IV glucose, or glucagon administration while receiving IIT.

**Exclusion
Criteria**

Subjects who meet *any* of these criteria are *not* eligible for enrollment:

1. Weight more than 80 kg or body mass index (BMI) > 30 kg/m².
2. Insulin requirement > 55 Units/day.
3. Other (non-kidney) organ transplants except prior failed pancreatic graft where the graft failed within the first two weeks due to thrombosis, followed by pancreatectomy and the pancreas transplant occurred more than 6 months prior to enrollment.
4. Untreated or unstable proliferative diabetic retinopathy.
5. Blood Pressure: SBP > 160 mmHg or DBP >100 mmHg.
6. Measured GFR using iohexol of ≤ 40 mL/min/1.73 m² (or for subjects with an iodine or seafood allergy, calculated using the subject's measured SCr and the Modification of Diet in Renal Disease [MDRD] study estimation formula). Strict vegetarians (vegans) with a calculated GFR ≤ 35 mL/min/1.73 m² are excluded. The absolute (raw) GFR value is used for subjects with body surface areas >1.73 m².
7. Proteinuria (albumin/creatinine ratio or ACr > 300mg/g) of new onset since kidney transplantation.
8. Either Class I or Class II panel-reactive anti-HLA antibodies > 50%.
Subjects with either Class I or Class II panel reactive anti-HLA antibodies $\leq 50\%$ will be excluded if any of the following are detected:
 - a. Positive cross-match,
 - b. Islet donor-directed anti-HLA antibodies detected by Luminex Single Antigen/specificity bead assay, even if the antibodies are very weak and even if the cross-match is compatible, or
 - c. Antibodies to the renal donor (*i.e.* presumed denovo).
9. For female subjects: Positive pregnancy test, presently breast-feeding, or unwillingness to use effective contraceptive measures for the duration of the study and 4 months after discontinuation. For male subjects: intent to procreate during the duration of the study or within 4 months after discontinuation or unwillingness to use effective measures of contraception. Oral contraceptives, Norplant®, Depo-Provera®, and barrier devices with spermicide are acceptable contraceptive methods; condoms used alone are not acceptable.
10. Active infection including hepatitis B, hepatitis C, HIV, or TB (tuberculosis) as determined by a positive skin test or clinical presentation, or under treatment for suspected TB. Positive tests are acceptable only if associated with a history of previous vaccination in

the absence of any sign of active infection. Positive tests are otherwise not acceptable, even in the absence of any active infection at the time of evaluation.

11. Negative screen for Epstein-Barr virus (EBV) by IgG determination at time of screening or previous kidney transplant.
12. Invasive aspergillus, histoplasmosis, and coccidioidomycosis infection within one year prior to study enrollment.
13. Any history of malignancy except for completely resected squamous or basal cell carcinoma of the skin.
14. Known active alcohol or substance abuse.
15. Evidence of Factor V Leiden mutation.
16. Any coagulopathy or medical condition requiring long-term anticoagulant therapy (*e.g.* warfarin) after transplantation (low-dose aspirin treatment is allowed) or subjects with international normalized ratio (INR) > 1.5.
17. Severe co-existing cardiac disease, characterized by any one of these conditions:
 - a. Recent MI (within past 6 months);
 - b. Evidence of ischemia on functional cardiac exam within the last year;
 - c. Left ventricular ejection fraction < 30%; or
 - d. Valvular disease requiring replacement with prosthetic valve.
18. 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]), alkaline phosphatase or total bilirubin, with values > 1.5 times normal upper limits will exclude a subject.
19. Active infections (except mild skin and nail fungal infections).
20. Acute or chronic pancreatitis.
21. Active peptic ulcer disease, symptomatic gallstones, or portal hypertension.
22. Treatment with any anti-diabetic medication other than insulin within 4 weeks of enrollment.
23. Use of any investigational agents within 4 weeks of enrollment.
24. Administration of live attenuated vaccine(s) within 2 months of enrollment.
25. Any medical condition that, in the opinion of the investigator, will interfere with the safe participation in the trial.

- 26. Male subjects with elevation of prostate specific antigen (PSA) > 4 unless malignancy has been excluded.
- 27. Any condition other than T1D as the primary cause of end stage renal disease (ESRD) in the native kidney.
- 28. Positive screen for BK virus by polymerase chain reaction (PCR) determination at time of screening.
- 29. A previous islet transplant.

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Glossary of Abbreviations

ABO	ABO blood type group
ACE	American College of Endocrinology
ACR _{Arg}	Arginine
ADA	American Diabetes Association
AE	Adverse Event
AIR _{glu}	Acute Insulin Response to Glucose
ATG	Anti-thymocyte Globulin
AZA	Azathioprine
BG	Blood Glucose
BID or bid	Twice daily
BK	BK Virus
BMI	Body Mass Index
BW	Body Weight
CBC	Complete Blood Count
CD3	Total T lymphocyte count
CFR	Code of Federal Regulations
cGCP	Current Good Clinical Practice
cGMP	Current Good Manufacturing Practices
CGMS	Continuous Glucose Monitor System®
CIT	Clinical Islet Transplant Consortium
CITR	Collaborative Islet Transplant Registry
CMV	Cytomegalovirus
CNI	Calcineurin Inhibitor
CrCl	Creatinine Clearance
CRF	Case Report Form
CsA	Cyclosporine
CSII	Continuous Subcutaneous Insulin Infusion
CT	Computed Tomography
CIT-TCAE	CIT Terminology Criteria for Adverse Events
CTCAE	Common Terminology Criteria for Adverse Events
CVA	Cerebrovascular Accident (stroke)
d	Day
DAIT	Division of Allergy, Immunology, and Transplantation
DCC	Data Coordinating Center

DCCT	Diabetes Control and Complications Trial
DDS	Diabetes Distress Scale
DIC	Disseminated Intravascular Coagulation
dL	Deciliter
DSMB	Data Safety Monitoring Board
EBV	Epstein Barr Virus
EC	Ethics Committee (also Institutional Review Board)
ECG or EKG	Electrocardiogram
EQ-5D (EuroQol)	EuroQol group Quality of Life instrument
F	Fahrenheit (temperature scale)
FDA	Food and Drug Administration
FSIGT	Frequently-Sampled Intravenous Glucose Tolerance
G-CSF	Granulocyte-Colony Stimulating Factor
GFR	Glomerular Filtration Rate
GBM	Glomerular Basement Membrane
h or hr	Hour (time)
Hb	Hemoglobin
HbA1c	Glycosylated Hemoglobin
HDL	High Density Lipoprotein
HFS	Hypoglycemia Fear Survey
HIV	Human Immunodeficiency Virus
HLA	Histocompatibility Leucocyte Antigen
hOKT3γ1	Anti-CD3 Monoclonal Antibody ala-ala
HSA	Human Serum Albumin
HTK	Histidine-tryptophan-ketoglutarate
HTLV	Human T-lymphotropic Virus
HTLV1	Human T-lymphotropic Virus 1
HTLV2	Human T-lymphotropic Virus 2
IAK	Islet After Kidney transplant
ICH	International Conference on Harmonization
IEq	Islet Equivalent
IIT	Intensive Insulin Therapy
IL-2	Interleukin 2
IND	Investigational New Drug
INR	International Normalized Ratio
IP	Initial Portal Pressure

IRB	Institutional Review Board (also Ethics Committee)
ITN	Immune Tolerance Network
IV	Intravenous
kg	Kilogram (10^3 gram)
K-P (see also SPK)	Kidney pancreas transplant
LDL	Low Density Lipoproteins
LI	Lability Index
MAGE	Mean Amplitude Glycemic Excursion
MDI	Multiple Daily Injections
MDRD	Modification of Diet in Renal Disease
mg	Milligram (10^{-3} gram)
min	Minute (time)
MI	Myocardial Infarction
mL	Milliliter (10^{-3} gram)
MMF	Mycophenolate Mofetil
MMTT	Mixed-meal Tolerance Test
MRI	Magnetic Resonance Imaging
μ g	Microgram (10^{-6} gram)
μ mole	Micromole (10^{-6} mole)
NCI	National Cancer Institute
ng	Nanogram (10^{-9} gram)
NIAID	National Institute of Allergy and Infectious Diseases
NIDDK	National Institute of Diabetes & Digestive & Kidney Diseases
NIH	National Institutes of Health (United States)
nmole	Nanomole (10^{-9} mole)
OPO	Organ Procurement Organization
PAID	Problem Areas in Diabetes Scale
PAK	Pancreas After Kidney transplant
PBMC	Peripheral Blood Mononuclear Cell
PCP	<i>Pneumocystis carinii</i> pneumonia
PCR	Polymerase Chain Reaction
PI	Principal Investigator
PICC	Peripherally Inserted Central Catheter
pit-hGH	Pituitary Growth Hormone

PLT	Platelet Count
PNF	Primary Non-function
PPD	Purified Protein Derivative
PRA	Panel Reactive Antibodies
Pred	Prednisone
PSA	Prostate Specific Antigen
PT	Prothrombin Time
PTT	Partial Thromboplastin Time
PTA	Pancreas Transplant Alone
PTLD	Post-transplant Lymphoproliferative Disease
QOL	Quality of Life
Rapa	Rapamune
RNA	Ribonucleic Acid
SAE	Serious Adverse Event
sc	Subcutaneous
SCr	Serum Creatinine
sec	Second (time)
SF-36	Short-form 36 (functional health and well-being instrument)
SGOT	Serum Glutamic-oxaloacetic Transaminase
SGPT	Serum Glutamate Pyruvate Transaminase
SIK	Simultaneous Islet Kidney transplant
SOE	Schedule of Events
SOP	Standard Operating Procedure
SPK (see also K-P)	Simultaneous Pancreas Kidney transplant
T1D	Type 1 Diabetes
Tac	Tacrolimus
TAT	Thrombin-antithrombin
TB	Tuberculosis
TCAE	Terminology Criteria for Adverse Events
tid	Three times daily
TNF	Tumor Necrosis Factor
ULN	Upper Limit of Normal
UNOS	United Network of Organ Sharing
WHO	World Health Organization
y.o.	Years old

1. BACKGROUND AND RATIONALE

1.1 Background

Of the nearly 15 million Americans afflicted with diabetes, almost 2 million have the Type 1 form of the disease in which insulin-producing islet β (beta) cells are destroyed by autoimmunity. This β cell selective attack disrupts normal glucose homeostasis and renders the host dependent on exogenous insulin administration for effective glucose utilization. While administration of daily exogenous insulin is a life-saving therapy, patients still have a spectrum of microvascular and macrovascular pathologies due to the imperfect glycemic control provided by even the most modern insulin therapy regimens¹. The resulting morbidity and mortality from complications of the disease represents a significant health care burden to the US and other countries and accounts for a large percentage of US health care related expenditures².

Convincing data exist to document the close association of the degree of glucose control with the risk of developing diabetes-related complications. In the seminal Diabetes Control and Complications Trial (DCCT), an IIT regimen improved HbA1c levels versus conventionally treated patients (mean 9.1 vs. 7.2% at the trials end) and progression of retinopathy, neuropathy, and nephropathy were tightly linked to glycemic control³⁻⁵. In fact, in general a 30-35% reduction in microvascular complications was achieved by an average of 1% reduction in HbA1c. Moreover, there was no threshold HbA1c below which a benefit was not observed, nor was there a minimal reduction in a high HbA1c that was not associated with benefit. Some patients receiving IIT also demonstrated sustained endogenous insulin responses as measured by induced c-peptide⁴. Unfortunately, the attempt at rigorous control was associated with a marked increase in hypoglycemic events and related morbidity⁶.

As a result of these findings, HbA1c has become the “gold standard” for monitoring the degree of glycemic control and thus is used for estimating the risk of complications. For this reason both the American Diabetes Association (ADA) and the American College of Endocrinology (ACE) have issued recommendations detailing targets for glycemic control based on HbA1c level in an attempt to minimize complications without excessive development of hypoglycemia related problems^{7,8}. The ADA has suggested a target HbA1c of 7.0% (about the average HbA1c reached in the DCCT trial with intensive therapy). The ACE recommendations are more stringent and suggest a near normal target of HbA1c of $\leq 6.5\%$. For both ADA and ACE recommendations, the goal of HbA1c reduction is tempered by the potential for development or worsening of hypoglycemic events due to an attempt at tight control. Therefore, consideration of both glycemic control by HbA1c and potentially morbid hypoglycemic events must be gauged in parallel to glean a full assessment of optimal care of the diabetic patient. This guiding principle engendered the selection of the primary endpoint of the proposed trial.

At present, the only therapeutic option to restore entirely normal glucose homeostasis requires β cell replacement by transplantation. To date, whole organ pancreas transplantation has been the primary method of β cell replacement used for Type 1 diabetic patients and has proven highly efficacious in normalizing glucose control⁹⁻¹¹. Considerable evidence suggests that

successful pancreas transplantation can retard the progression, and in some cases reverse some of the long-term complications, of the disease including nephropathy and neuropathy¹²⁻¹⁶. Despite this evidence, the procedure is considered appropriate for only a few diabetics because of its highly invasive nature and the potential for significant morbidity and life-threatening complications. In fact, a recent database analysis questioned the survival benefit of pancreas transplantation in the setting of pancreas alone (PTA) transplants and in pancreas after kidney (PAK) transplants^{17, 18}. However, a marked survival benefit was evident for those patients receiving a combined kidney-pancreas transplant (though the relative benefit of the kidney versus the pancreas has not been differentiated). While these data remain both unconfirmed and controversial, at a minimum they suggest a narrow therapeutic margin for the whole organ pancreas transplant procedures in terms of overall survival benefits. Similar conclusions were supported by a recent analysis by Grussner¹⁹ in response to the report of Venstrom et al¹⁷. Their analysis challenged the assertion that PAK or PTA was accompanied by a survival disadvantage, but they were unable to find evidence for a clear survival advantage conferred by the procedures. Quality of life (QOL) from being rendered insulin independent was not considered in either study and is an important weakness in these investigations. Of note, QOL measures will serve as secondary endpoints in the proposed trial.

Interest in replacement of the β cell mass by a less invasive means using isolated islets has been piqued by the report from investigators from Edmonton in 2000²⁰. Their results suggested for the first time that diabetes could be consistently reversed by transplantation of isolated islets. In the four years since this landmark report, nearly 500 islet transplants have been performed by more than 40 centers worldwide²¹. Although the results have been variable depending on the experience of the transplanting center, at the most experienced sites, reversal of hyperglycemia has been routinely achieved²²⁻²⁷.

Controlled trials to compare the safety and efficacy of islet transplantation with conventional insulin therapy have not been reported and are critically required for definitive conclusions regarding the appropriateness of islet transplantation as a standard therapy for T1D. In this study, we propose to evaluate the net health benefit of islet transplantation by comparing in a randomized multi-center trial the metabolic, QOL, cardiovascular and renal effects of islet transplantation in patients who have had a successful kidney transplant, versus intensive insulin therapy (IIT) for T1D. The study is a response to a Congressional mandate requiring Medicare patient inclusion in an islet transplantation trial. Medicare's role is to provide partial funding for this trial.

1.2 Preclinical and Clinical Experience

1.2.1 Published Preclinical Studies Using Rabbit Anti-thymocyte Globulin with Islets

- 1) Hirschberg et al²⁸ performed allogeneic transplants in cynomolgus monkeys rendered diabetic with streptozotocin. Rabbit ATG (20 mg/kg) was administered IV for 7 days. Sirolimus monotherapy was used as maintenance immunosuppression with target trough levels of 10-15 ng/mL. Rabbit ATG resulted in marked peripheral lymphocyte depletion (> 95%) that gradually recovered beginning after a month. Three of 4 animals died while insulin independent between 3 and 4 weeks from toxicities attributed to rapamycin. The surviving animal maintained insulin independence for 169 days after which the islets succumbed to rejection following a 2-month period of sub-therapeutic rapamycin levels (< 3 ng/mL).

1.2.2 Published Clinical Studies of IAK Transplantation

- 1) Davalli et al²⁹ reported a case of successful IAK in a 43 y.o. following a thrombosed pancreas graft as part of a simultaneous pancreas kidney (SPK) transplant. The patient was immunosuppressed with cyclosporine, azathioprine (AZA), and prednisone (10 mg/d) for the kidney and received an additional steroid bolus and rabbit ATG (125 mg/d x 7 days) at the time of islet transplantation. The patient received an islet prep pooled from 2 donors that consisted of ~613,000 IEq's. Insulin independence was achieved after about 6 months and persisted for more than 4 years though graft function deteriorated slowly but progressively over time. By year 3-4, mild glucose intolerance was evident with HbA1c mildly abnormal at 6.8%. The patient died of a MI while still off insulin therapy and morphometric analysis of the liver at autopsy was conducted. This analysis suggested an islet mass of about 99.9 mg or about 15% of a normal pancreas. There was an absence of both alloreactive and autoreactive antibodies and there was no histological evidence of rejection. The authors' concluded that the gradual graft loss was a result of non-immune mediated graft deterioration.
- 2) Bertuzzi et al³⁰ described 15 successful IAKs (using 23 islet preparations and 24 hours of in vitro culture) defined by increased c-peptide and reduced insulin requirement. Fourteen of fifteen had a greater than 50% reduction in insulin requirement and 10 of 15 (66%) were rendered fully insulin independent. Immunosuppression consisted of steroids and cyclosporine for the prior renal transplant and was modified at the time of islet transplant to include rabbit ATG (125 mg/d x 10 days), mycophenolate mofetil (2 gm/day), cyclosporine (1 patient received tacrolimus instead), and methylprednisolone (500 mg before infusion and tapered to 5 mg/day). Five patients remained insulin independent in the long term (13-43 months). Five other patients initially gaining insulin independence resumed insulin therapy 1-4 months later. In two cases, presumed rejection was reportedly reversed with hOKT3γ1 (Anti-CD3 Monoclonal Antibody) ala-ala therapy.

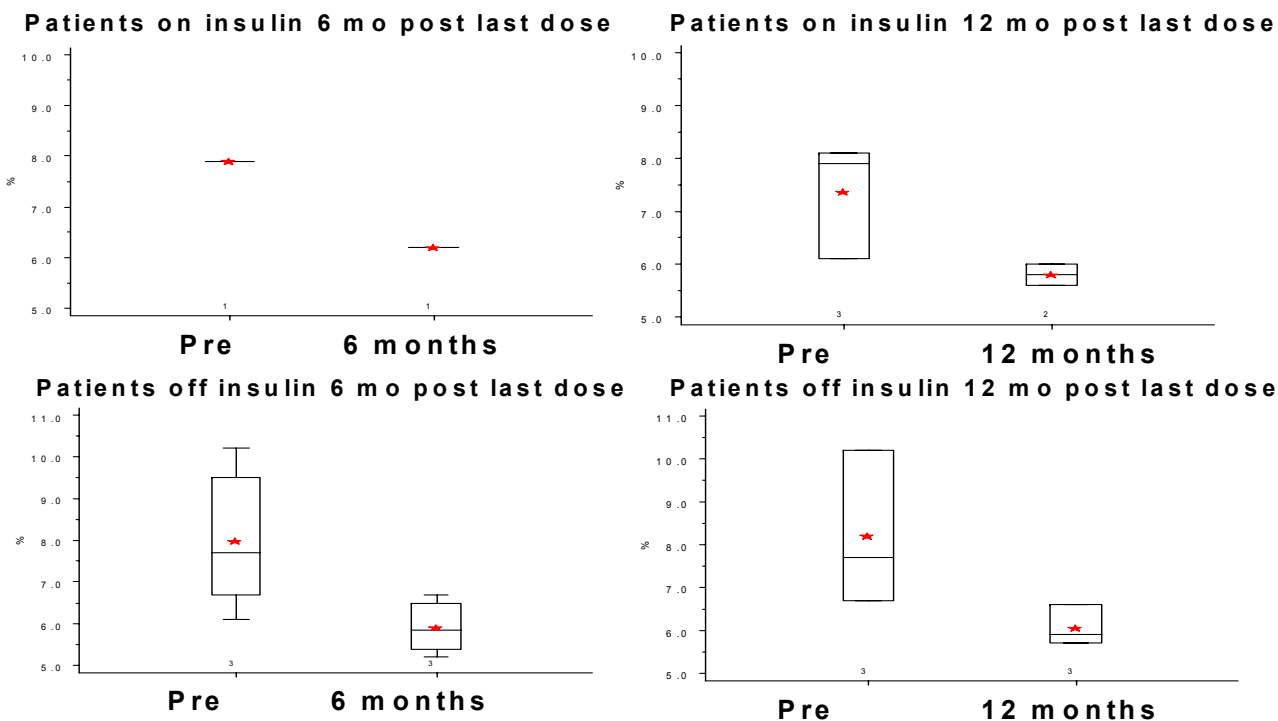
- 3) Kessler et al³¹ compared glucose variability in medically treated patients with HbA1c < 7.5% (with implantable intraperitoneal insulin pump, n=10), pancreas (SPK, n=9), or islet (IAK, n= 7) patients, using continuous glucose monitoring for 72 hours. Islets (~10,000 IEq/kg/recipient) were transplanted immediately or cultured in vitro for 2 to 10 days in 5 cases. Immunosuppression was variable and included ATG, cyclosporine, and steroids (tapered to 5 mg/day) or Simulect, cyclosporine, mycophenolate mofetil, and steroids (5 mg/day). Glucose variability was equivalent in islet and pancreas patients and both were superior to that in patients treated only with medical therapy. Hypoglycemic events were not seen in insulin independent IAK or SPK patients. Patients with partially functioning islet grafts (still requiring some insulin therapy) experienced shorter hypoglycemic events than patients on medical therapy. HbA1c was significantly less in islet and pancreas recipients (5.2% and 5.5% respectively) versus patients on the insulin pump (7.1%).
- 4) Kaufman et al³² reported a successful case of IAK using the Edmonton protocol. Insulin independence was achieved following administration of only 4100 IEq/kg prompting speculation that chronically immunosuppressed IAK recipients may be more receptive to islet transplantation. The islet donor was 20 years of age and the cold ischemia time was < 8 hours. HbA1c levels declined from 6.9% pretransplant to 5.3% post-transplant.
- 5) Fiorina et al³³ compared patient survival, cardiac death rate, atherothrombotic profile and endothelial morphology (via skin biopsy) in IAK patients (n= 37) versus kidney pancreas recipients (K-P, n=162), diabetic kidney alone recipients (n=42) and diabetics still on dialysis (n=196). After an average follow-up of about 5 years, patient survival was superior in IAK and K-P patients than kidney transplanted alone or non-transplanted diabetics on dialysis. Survival was also superior in patients with long-term islet function than in those who lost function. The cardiovascular death rate was less in successful IAK patients (n=24) and similar to K-P patients. Differences were also detected in endothelial morphology and in coagulation parameters (Protein C, ATIII, etc.); however, this comparison was primarily with the diabetics still on dialysis, and thus may be in part attributable to the benefit of the kidney transplant.
- 6) Fiorina et al³⁴ examined the effect of islet transplantation on renal function in 36 IAKs. Again, recipients were stratified by the success of the islet graft as defined by continued c-peptide production (c-peptide > 0.5 x 1 year). There were 24 successful grafts (IAK-s) and 12 unsuccessful (IAK-u). The IAK-s demonstrated superior renal graft survival at 1, 4, and 7 years, though GFRs were similar. IAK-u also developed increased microalbuminuria over time. IAK-s showed decreased natriuresis and increased Na/K ATPase immunoactivity in renal tubules (and RBCs) versus IAK-u. The latter findings were better correlated with c-peptide to Creatinine ratios than to HbA1c levels.
- 7) Fiorina et al³⁵ reported their series of 34 IAK [8 actually simultaneous islet kidney (SIK) transplants], and stratified again by successful (IAK-s) or unsuccessful (IAK-u) based on whether the patient had maintained c-peptide for > 1 year post-transplant. Immunosuppression consisted of rabbit ATG induction (125 mg/day x 10 days) and basal kidney graft immunosuppression (cyclosporine, mycophenolate mofetil 2 gm/d, and pred 1

mg/d). Steroids were withdrawn by 4 to 6 months. Patient survival was better and cardiovascular death rates lower in patients with successful grafts but no statistically significant difference in HbA1c levels was observed. Carotid intimal-media thickness progressed in unsuccessful but not successful transplants.

1.2.3 Unpublished Clinical Studies with IAK Transplantation

Collaborative Islet Transplant Registry (CITR) data on IAK (Provided by CITR with permission of the contributing PIs): As of July 2004 in their year-end report, CITR had recorded 86 islet transplant recipients reported by 16 centers. Of these, 80 were Islet alone and only 6 were IAKs (12 total infusions). The survey was through June 2004, thus it does not include the last half of 2004. Since July, an additional 7 IAKs have been reported. Currently (1/13/05), three centers have submitted IAK data to CITR, for a total of 13 patients. There haven't been any updates to the survey since 08/24/04. Figure 1 shows data from relevant CITR Annual Report sections for the small number of IAK patients entered in the CITR database. Figure 1 shows pre-infusion HbA1c levels at 6 and 12-month post-transplant for those IAK recipients who were on or off insulin at 6 months and 12 months. The limited number of cases entered in the system to date prevents the formulation of definitive conclusions. However, the data suggest that the patients in this series continued to be insulin independent 12 months post last islet transplant³⁶.

Figure 1 HbA1c levels in insulin dependent and independent subjects



1. *Nordic Network experience with IAK transplantations 2001-2004 (Annika Tibell MD, Uppsala, Sweden, personal communication):* When starting the Nordic Network for Clinical Islet Transplantation in 2001, six clinical centers decided on sharing one central isolation lab (The Rudbeck laboratory in Uppsala, Sweden). In the first study, 27 uremic patients were transplanted with functioning kidney grafts and therefore already on immunosuppressant medication.

Suitable kidney transplanted Type 1 diabetics were switched to the Edmonton Protocol (tacrolimus, sirolimus, and daclizumab). In most cases this was performed in a two-step fashion. When admitted to the program, the patients were switched to low dose tacrolimus and 5 mg of prednisone daily. At the time of the transplantation, the patients were started on sirolimus and given 5 doses (every second week) of daclizumab starting on the day of transplantation. Prednisone was discontinued at the transplantation or reduced to 2.5 mg for a couple of weeks and then discontinued. Patients with a stable kidney function transplanted more than 6 months earlier were eligible for IAK. The patients were in general good health and had been screened with the same protocol as patients for planned pancreas transplantation. There was no upper limit for the patient's insulin requirements per kilogram BW. The upper formal BMI limit has been 30 although most patients have had BMI around 25. There has been no formal requirement for glucose instability although several patients have had problems with their metabolic control. Pancreata were harvested at the participating centers and sent to the Rudbeck lab in Uppsala, Sweden, by car or airplane. After isolation the islets were transplanted at a center with a suitable ABO compatible patient. In the first two patients, fresh islets were transplanted; in the following patients, islets were cultured for 24 to 96 hours. The transplantations were performed through a percutaneous transhepatic portal injection and repeated to a maximum of four transplantations.

There have been no rejections of kidney grafts following the switch of immunosuppressive regimen when put on the waiting list. Seventy-three transplantations have been carried out in 27 patients. Patients have been transplanted at 5 centers.

Neither of the two patients transplanted with fresh islets at the start of the program has become insulin independent, but insulin independence was achieved in 11/15 of the subsequent patients after 2-4 transplantations (a total of 7,500 to 28,000 IEq/kg). After 1 to 37 months of follow-up, nine patients are still insulin free. Three of them have been treated at a center more than 500 kilometers from the isolation lab. Four patients at four different centers have not become free of exogenous insulin, even though they have received four transplants each. The mean insulin requirement per kilogram BW before transplantation for these four patients was slightly higher than patients becoming insulin free (0.85 ± 0.34 vs. 0.66 ± 0.18). These four patients came from four different centers and in two cases; the patients were the first patients at that center. Among the patients currently free of exogenous insulin, the mean HbA1c is $5.8\% \pm 0.43$ and among the patients who have received four transplants without becoming free of exogenous insulin, the mean HbA1c is $6.6\% \pm 2.00$. The latter group's mean insulin requirements is currently 6.75 ± 5.67 units per day.

Nine patients have received 1-3 doses of islets and are waiting for other transplants. The vast majority of these “ongoing” patients have partial function (c-peptides above 0.2 nmol/L) with a reduction in insulin requirements and stabilization of blood glucose (BG).

One patient died of cerebral hemorrhage six months after the last islet transplant. Two patients have had pulmonary infiltrates, possibly caused by sirolimus. Both of these patients required treatment in the intensive care unit (ICU). One of the insulin free patients had a minor pneumothorax after his first transplantation. Two patients have received seven units of blood due to hemorrhage following four of seven transplantations. Two out of six patients with a creatinine higher than 175 $\mu\text{mol/L}$ before the first transplantation have had worsened renal function and gone back to dialysis treatment. In one case there has been transfer of cytomegalovirus (CMV) infection. Other, more frequent adverse events (AE) include leukopenia and mouth ulcers.

2. *UPenn experience in IAK (James Markmann, MD, PhD, personal communication):* The islet transplant center at UPenn has performed 14 infusions in 8 IAK recipients to date. Pre-infusion HbA1c averaged 7.95%. To minimize compromising renal graft function, the maintenance immunosuppression used for the renal graft was not modified. Five of eight patients have gained insulin independence following an average of 14,285 IEq/kg. Three patients are awaiting an additional dose. Islet potency was equivalent to islets transplanted to islet alone patients (not shown). Immunosuppression consisted of daclizumab induction with maintenance renal immunosuppression. This consisted of tacrolimus-rapamycin in 1, cyclosporine-mycophenolate mofetil in 1, and tacrolimus-mycophenolate mofetil in 6 patients. Six of eight patients were on 5 mg of prednisone/day. One patient developed a serious adverse event (SAE), a left PV thrombosis detected upon portal venography at the time of PV cannulation for planned (but aborted) second infusion. This patient remains on 4 units/day of insulin with excellent control (HbA1c's of 5.7%).

Table 1 Summary of UPenn Experience with IAK Transplantation

	Immunosuppressive Regimen	# of Infusions	Total # of IEq	Total IEq/kg	Obtained insulin independence?	Insulin independent at 1 year?	Number of days insulin independent	Units of Insulin Required	HbA1C				
									Post-transplant				
									Pre-transplant	3 mos	6 mos	9 mos	12 mos
1	Tac, Siro	2	943,795	20,081	Yes	Yes	684	0	9.8	6.8	6	5.8	5.9
2	Pred, CYA, MMF	2	888,875	12,328	Yes	Yes	524	0	7.6	6.4	6.2	6.2	6.1
3	Pred, TAC, MMF	2	1,090,698	16,450	Yes	No	352	10	7.5	4.4	NA	5.9	6.1
4	Pred, TAC, MMF	2	730,000	15,466	Yes	No	266	7	9.5	5.7	6.7	8.2	
5	Pred, TAC, MMF	2	500,000	7,000	Yes	No	85	10	9.5				
6	Pred, TAC, MMF	2	1,020,000	13,783	No	No	0	17	6.1	5.5			
7	TAC, MMF	1	400,000	8,000	No	No	0	4	6.6	5.7			
8	Pred, TAC, MMF	1	800,000	8,800	No	No	0	14	7	5.6			

3. *Miami experience with IAK (Rodolfo Alejandro, MD, personal communication):* As of 1/13/05, six IAK patients have received 10 total islet transplants comprised of between 8,300 and 20,190

total IEq/kg. Each patient gained insulin independence and in two of five this lasted for more than 1 year (one patient was transplanted recently and is insulin independent about 2 months following a single infusion). Daclizumab was administered at 1 mg/kg x 5 doses then monthly for 1 year. Maintenance immunosuppression included tacrolimus and sirolimus in each patient. Five patients received adjunctive therapy with: mycophenolate mofetil (1), mycophenolate sodium (2), or prednisone 5 mg/day (2).

Table 2 Summary of Miami Experience with IAK Transplantation

	Immunosuppressive Regimen	# of Infusions	Total # of IEq	Total IEq/kg	Obtained insulin independence?	Insulin independent at 1 year?	Number of days insulin independent	Units of Insulin Required	HbA1c	
									Pre-transplant	Post-transplant (last)
1	Siro(2), Tac(0.5), Myfortic(360)	1	576,000	8,471	Yes	No	281	21	6.1	5.2
2	Siro(5), Tac (2.5), Pred(5)	2	897,333	16,400	Yes	Yes	539	8	7.7	6.8
3	Siro(6), Tac(4), Pred(5)	2	914,483	15,356	Yes	Yes	579	6	6.7	6.2
4	Siro(2), Tac(1.5)	2	1,118,687	20,190	Yes	No	325	2	8.2	5.4
5	Siro(6), Tac(2.5), MMF(500)	2	1,083,617	17,141	Yes	No	190	2	7.3	4.9
6	Siro(8), Tac(2.5), Myfortic(360)	1	495,934	8,308	Yes		61+	0	11	7

4. *Northwestern experience with IAK (Dixon Kaufman, MD, PhD, FACS):* As of 6/27/06, four Northwestern patients have received 9 total islet transplants. Two of the four patients achieved insulin independence within one year of transplanatation. One recipient has remained insulin independent at three years following the first infusion. Immunosuppression of all patients prior to islet transplant was tacrolimus and sirolimus without prednisone. All islet transplant recipients received Daclizumab induction therapy and were maintained on the tacrolimus / sirolimus / no prednisone maintenance immunotherapy. No change in renal function or rejection episodes has occurred.

Table 3 Summary of Northwestern Experience with IAK Transplantation

	Immunosuppressive Regimen	# of Infusions	Total # of IEq	Total IEq/kg	Obtained insulin independence?	Insulin independent at 1 year?	Number of days insulin independent	Units of Insulin Required 12 months	HbA1c			
									Pre-transplant	Post-transplant		
										3 mos	6 mos	12 mos
1	Siro(7), Tac(2)	2	557,888	8,492	Yes	No	30	13	6.5	5.4	6.9	7.5
2	Siro, Tac	3	701,203	11,358	No	No		10	8.4	7.5	7.5	
3	Siro, Tac	2	595,184	10,515	No	No		15	9.2	8.4		
4	Siro(6), Tac(2)	2	1,017,430	19,414	Yes	Yes	365+	0	7.6	7	7.1	6.9

6. *European non-Nordic Network (Annika Tibell MD, personal communication):*

a. Dr. Secchi, Milan, personal communication:

The islet center at Milan has performed 38 IAK and 8 SIK to date. Most patients received rabbit ATG induction with maintenance therapy by cyclosporine plus AZA with steroid tapering (similar to the general kidney population at their center). A SCr cut-off at 2 mg/dL was suggested as exclusion criteria. At 1 year post-transplant, 58% gained insulin independence, 30% demonstrated partial function, and 13% had rejection. No major complications from the procedure have been observed.

b. Roger Lehman, personal communication, Abteilung Endokrinologie und Diabetes, Universitätsspital Zürich:

To date, this group has not performed any islet after kidney (IAK) transplants. They have performed SIK transplants in patients who have later received additional islet transplants. They now have a few patients on the waiting list for IAK transplantation.

This group uses the Edmonton protocol for islet transplants. Of note, they exclude patients with coronary heart disease and poor kidney graft function, and suggest a creatinine clearance (CrCl) of 60 or better prior to islet transplantation.

Besides HbA1c and QOL, Roger Lehmann emphasizes the need to monitor both hypoglycemia and metabolic stability. Before deciding on islet transplantation, all patients are required to trial an insulin pump. They also keep their pumps during the initial period after islet transplantation. By choosing this patient group, Lehmann selects patients who are highly motivated to do something to improve their metabolic control. Dr. Lehmann believes that this also facilitates keeping a good blood sugar level during the engraftment period. He also argues that this means that islet transplantation is compared to the best available insulin therapy, namely pumps. He recommends the use of sensors to get good metabolic data, and in his experience quite a few patients have undetected hypoglycemia during night time. The use of sensor data could then be one way to really find a difference between the islet-transplanted patients and the controls.

In 2 of 14 SIK-patients, sirolimus-induced interstitial pneumonitis has occurred.

c. Mathias Brendel, Giessen, personal communication.

Dr. Brendel's team has performed 24 IAK transplants. In the initial 20, either antilymphocyte globulin (ALG) or ATG was used as induction therapy. The basic renal immunosuppression protocol was not changed, but doses of cyclosporine were increased and prednisolone was also increased to 20 mg in conjunction with the islet transplant and gradually reduced to 5 mg at 2 weeks. Most patients were already on AZA or mycophenolate mofetil; otherwise one of those drugs was instituted at the time of islet transplantation.

Since 2000, they have only used interleukin 2 (IL-2) receptor antibody induction. In the last 2 patients, they have also switched from cyclosporine to tacrolimus and a low dose of rapamycin, 2-4 mg/day, (providing a trough level of 3 to 4). In the recent cases, no

steroids were used. This group is the least favorable in the Giessen experience. Fifty percent had partial function and 6 out of 20 of the early patients had temporary insulin independence, the longest for approximately 3 years. At 5 years, 20% of the patients had partial function.

d. Bart Keymeulen, Brussels, personal communication.

The Brussels groups have performed 18 IAK transplants between 1 and 5 years after kidney transplantation. Maintenance immunosuppression consisted of cyclosporine, AZA, and steroids. In 12 patients, the protocol was not changed at the time of transplantation. Ten of those were comparable while in one the intraperitoneal site was used and in one only cryopreserved tissue was given. At the time of transplantation 0.5 mg of methylprednisolone was given, otherwise the immunosuppression was not modified.

The 10 patients received different induction therapy prior to kidney transplantation. Of the 5 patients that received, ATG as induction therapy, four were insulin independent at one year. After 2-4 years these four patients returned to low doses of insulin, but still had significant c-peptide production and stable metabolic control for up to 7 years after the transplantation. The fifth patient, the first in the series, received a low number of islets and had no long-term c-peptide production.

The other five patients had no history of ATG treatment. They all became c-peptide negative within a month.

In a later series, 6 patients were switched from cyclosporine, AZA, and steroids to cyclosporine, mycophenolate mofetil, and steroids at the time of transplantation. None of those 6 patients had received ATG in conjunction with the kidney transplant. None became insulin independent and all were c-peptide negative within 3 months.

The Brussels groups recommend transplant recipients be < 55 y.o. and have BW below 80 kg. Kidney function was not defined in this series only that it should be stable, but good kidney function was recommended. Patients were not entered if they had experienced kidney rejections within 6 months before evaluation for islet transplantation.

The Brussels groups also reported no serious complications. Transplants were performed intraportally by laparoscopy. HbA1c, QOL, and variability of glucose control were recommended as follow-up parameters. In one patient, there were signs of improved microangiopathy and improved healing of wounds on the feet after islet transplantation.

e. Information provided by the Swiss-French GRAGIL (Groupe Rhin-Rhone-Alpes-Geneve pour la Transplantation d'Ilots de Langerhans) collaborative network:

Six out of nine IAK patients receiving 10,000 IEq/kg had temporary insulin independence, but only one was insulin independent at one year. The GRAGIL network used the Edmonton protocol for immunosuppression, except that Everolimus was used instead of rapamycin. The window for Everolimus trough concentration was very wide, 3-15 ng/mL, and the trough concentrations were infrequently measured. After the study was

completed, it was determined that the patients were all in the lower trough concentration range (between 3 and 8 ng/mL) and it was suggested the low trough concentration may have contributed to rejection and the poor results.

A single center study was then performed in Geneva by Thierry Berney. Seven patients received IAK transplants, the majority had two islet infusions, and the islet dose was approximately 10,000 IEq/kg. An immunosuppressive regimen consisting of daclizumab-sirolimus-tacrolimus was used. Insulin-independence was achieved in all patients for at least 3 months, with an actual rate of 71% at 1 year after transplantation.

1.2.4 Summary of International Experience with IAK Transplantation

Important conclusions that are relevant to the proposed Protocol CIT-06 trial can be drawn from the extensive experience by the European transplant centers and the growing US experience with IAK transplantation:

1. IAK transplantation was found to be safe for the recipient. A specific critical safety concern in IAK transplantation is the function of the renal allograft that is already in place. The IAK transplant experience to date suggests that islet transplantation can be performed without unduly jeopardizing the kidney graft. In fact, no instance of renal graft loss due to the islet transplant has been documented. In addition, the results reported by Fiorina et al suggest that a successful islet transplant may provide significant benefit to the renal graft manifest by improved survival and function.³⁴
2. With IAK, insulin independence can be achieved at a rate comparable to that seen with islet alone transplants in the Edmonton protocol. In each of the sites participating in the Clinical Islet Transplant Consortium (see section 1.2.3) that have performed IAK transplantation, insulin independence has been achieved by the majority of the patients completing the planned islet transplant regimen.
3. It was theorized that an IAK recipient might be more receptive to islet transplantation because of the presence of a chronically immunosuppressed state. Unfortunately, also similar to the islet alone experience using an Edmonton-like regimen of immunosuppression. This observation in part provides a strong rationale for more intensive immunosuppression in the proposed trial, such as that reported successful by Hering et al in islet alone patients ³⁷.
4. As summarized in section 1.2.3, a variety of data from unpublished trials suggest that islet transplantation may afford the IAK recipient considerable net health benefit. This includes a reduction in risk of long-term microvascular complications, improved QOL, and a marked reduction or elimination of morbid hypoglycemia. Moreover, this was evident even in patients with partial graft function.

The current trial will attempt to substantiate these findings for the first time in a prospective, randomized, controlled fashion, enrolling a sufficient number of patients to draw statistically meaningful conclusions.

1.3 Rationale for Selection of Study Population

Individuals with T1D and a successful kidney allograft are particularly appropriate candidates for an islet transplant procedure because they are already relegated to life-long chronic immunosuppression. Thus, the nominal risk of the islet transplant procedure itself is minimal compared to the risk exposure of non-uremic patients with diabetes receiving new immunosuppression in addition to the transplant procedure^{29, 30, 32}. Also relevant is the preliminary evidence from uncontrolled studies suggesting that transplanted islets in patients who have already received a kidney transplant may provide beneficial renal and cardiovascular effects^{31, 33-35}.

Critical in IAK transplantation is avoiding disruption of the function of a successful life-saving kidney transplant. For this reason, only kidney recipients > 3 months post-transplant who demonstrate stable graft function and the absence of rejection episodes in the previous three months will be considered for enrollment. This will avoid the early post-transplant period in which complications and allograft rejection are most common. Similarly, the most appropriate immunosuppression must be selected to avoid islet and kidney graft rejection as well as deleterious side effects to either the islets or the kidney from the medications.

Patients will also be selected based on inadequate glucose control following a period of diabetes management by an experienced diabetologist using IIT. IIT is defined as intensive insulin therapy with target HbA1c levels of $\leq 6.5\%$ as suggested by the ACE Consensus Statement on Guidelines for Glycemic Control⁷. Following a period of ≥ 4 months on this therapy, only patients who evidence continued inadequate glucose control characterized by either HbA1c $\geq 7.0\%$ or HbA1c $< 7.0\%$ but with severe hypoglycemic episodes (≥ 1 severe episode while receiving IIT) as defined by the DCCT trial (and Clarke hypoglycemia score > 4) will be considered for randomization. This approach will target the group of post renal transplant diabetics most likely to benefit from isolated islet transplantation.

1.4 Rationale for Selection of Study Treatment Regimen

1.4.1 Investigational Product: Allogeneic Islets

The Purified Pancreatic Islets isolated for transplantation in Type 1 diabetics have been evaluated in phase 1 and 2 clinical trials and found to be capable of normalizing BG levels and to have an acceptable safety profile²²⁻²⁷. Isolated pancreatic islets are recovered from cadaveric pancreata that have been declined for use in whole organ transplantation. Donors of pancreata for manufacture of allogeneic islets are subjected to rigorous and stringent evaluations. Donor suitability must conform to the standards established in the U.S. guidances (cGTP and HCT/P) and regulations (69 FR 29786, May 25, 2004 and 21 CFR 314). Donor eligibility determination is based on results of donor screening and testing, including transmissible infectious disease by serological assessment, and screening for high-risk behavior. This information is provided by the relevant Organ Procurement Organization (OPO). The recovered organs are preserved by standard methods used for whole organ grafts [UW or histidine-tryptophan-ketoglutarate

(HTK) solution] or by the two-layer method using an oxygenated perfluorocarbon³⁸. The pancreata are processed using aseptic techniques within 12 hours of cold ischemia time.

Pancreas processing consists of enzymatic digestion, mechanical dissociation of the pancreas and density gradient purification of the islet endocrine component³⁹. The processing procedure will be standardized among CIT participating centers. Islets will be cultured for < 72 hours prior to transplantation. The final product formulation is presented in Table 5.

Islet preparations are tested prior to transplantation to ensure that the final product meets the pre specified biological and biochemical criteria for safety, purity, potency and identity. Only product meeting the pre-specified lot release criteria will be considered for clinical use. Standardized lot release assays will be used to document comparability of the product between centers (for detailed information regarding islet preparation and manufacturing, consult the Investigator's Brochure).

1.4.2 Immunosuppressive Medications for Initial Islet Transplant

1.4.2.1 RABBIT ANTI-THYMOCYTE GLOBULIN (THYMOGLOBULIN[®])

Rabbit ATG is a polyclonal antibody preparation that depletes T and B cells following IV administration^{27, 40-43}. It is approved by the Food and Drug Administration (FDA) for treatment of rejection in kidney transplant recipients receiving adjunctive immunosuppression. It will be administered IV on day -2 (0.5 mg/kg), -1 (1 mg/kg), 0, 1, and +2 (1.5 mg/kg/dose). The dose will be limited to 3 mg/kg total (0.5mg/kg on day -2, 1.0 mg/kg on day -1 and 1.5mg/kg on day 0), in patients treated with depleting antibody induction therapy (hOKT3γ1 ala-ala, rabbit ATG, or Campath) for their renal transplant within the last year. Patients previously treated with rabbit ATG will be tested for efficacy of rabbit ATG therapy at day -2 by assessment of T cell depletion by CD3 (total T lymphocyte) counts. If lack of efficacy is detected, Zenapax[®] can be substituted for rabbit ATG using the regimen detailed below for subsequent transplants. Methylprednisolone [see Section 1.4.2.2 Methylprednisolone (Solumedrol[®])], acetaminophen, pentoxifylline, and diphenhydramine will be administered as premedications 1 hour prior to the first dose of rabbit ATG.

1.4.2.2 METHYLPREDNISOLONE (SOLUMEDROL[®])

Methylprednisolone is a synthetic glucocorticoid with potent antiinflammatory and immunosuppressive properties that has been approved by the FDA for prevention and treatment of rejection in organ transplant recipients. This drug will be administered at a dose of 1mg/kg IV 1 hour prior to and repeated half way through the day -2 dose of the rabbit ATG. Patients will not receive any additional doses of this drug.

1.4.2.3 DACLIZUMAB (ZENAPAX[®])

Daclizumab is a humanized monoclonal antibody specific for the human IL-2 receptor that targets activated T cells. It is approved for use in solid organ transplant recipients to prevent

rejection^{20, 43, 44}. With second or third islet transplants, as a substitute for rabbit ATG, daclizumab will be administered (2 mg/kg pre-transplant, then 1 mg/kg biweekly x 4 doses).

1.4.2.4 ETANERCEPT (ENBREL®)

Etanercept is an engineered soluble tumor necrosis factor (TNF) receptor-Fc that blocks TNF binding and reduces inflammation⁴⁵⁻⁵⁰. It is approved for use in severe rheumatoid arthritis, juvenile arthritis, ankylosing spondylitis, and psoriatic arthritis. It will be administered 50 mg IV prior to transplant and 25 mg (subcutaneous) sc on days +3, +7, and +10 post-transplant for each islet transplant.

1.5 Rationale for Selection of Study Drug Regimen

Pancreatic islet transplantation using the Edmonton approach has demonstrated that at experienced centers, almost all patients gain insulin independence and experience a rate of insulin independence at 1 year that is equivalent to that achieved by whole organ pancreas transplantation⁵¹. Long-term insulin independence, however, is inferior, and a high proportion of islet-alone patients have required reinstitution of small doses of insulin over time²². Recently reported and unpublished preliminary data (see section 1.2.3) from US centers performing IAK transplants suggest that under Edmonton-like immunosuppression, islets transplanted even to chronically immunosuppressed individuals may experience a similar fate³².

It is a tenable hypothesis that the encountered barrier to long-term success derives from an inadequate control of the deleterious allo- and auto-immune responses that threaten the islet mass immediately post-transplant and in the long-term. For this reason, we suggest that more potent non-islet toxic immunosuppression will lead to an increase in engrafted islet mass and prolongation of its survival.

To date, Hering et al have reported what are arguably the most successful series of islet-alone transplants using potent induction immunosuppression and high quality islets. In the first study, 4 of 6 consecutive patients were rendered insulin independent with single donor islet transplants using hOKT3γ1 ala-ala²⁵. A comparable if not even more impressive experience has been gained with rabbit ATG in islet alone recipients transplanted for severe hypoglycemia. In a series of 10 consecutive patients treated with rabbit ATG induction therapy (6mg/kg total dose), 9 became insulin independent with a single infusion of islets.³⁷ The patient who did not become insulin independent had the benefit of a reduced insulin requirement to 4 U/day. No SAEs have been observed. The available information from these studies suggest that restoration of insulin independence with a lower islet mass prepared from a single-donor pancreas may be largely attributable to the administration of etanercept in the peritransplant period and more potent induction immunosuppressive therapy.

Thus, in order to mimic the approach used by the Minnesota team, the proposed protocol will include peritransplant administration of the soluble form of the p75 receptor for TNF (etanercept). Etanercept is a dimeric fusion protein consisting of the extracellular ligand-binding portion of the human 75 kilodalton (p75) TNF receptor linked to the constant fraction

(Fc) portion of human IgG1. Etanercept inhibits binding of both TNF- α and TNF- β to cell surface TNF receptors, rendering TNF biologically inactive.

The scientific rationale for inclusion of a peritransplant course of etanercept is that it 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 unresponsiveness. 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 autoimmunity⁵². Blockade of TNF in the neonatal period results in a dramatic increase in the levels of CD4⁺CD25⁺ regulatory T cells in NOD mice^{52,53}. Such an effect of etanercept on CD4⁺CD25⁺ regulatory T cells in islet transplant recipients could prove critical for the long-term survival of transplanted islets.

In addition, increasing evidence suggests that blocking TNF- α in the early post-transplant period will diminish nonspecific islet β 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. TNF- α is known to be cytotoxic to human islet β cells⁵⁴. In murine models, selective inhibition of TNF- α in the peritransplant period has promoted reversal of diabetes after marginal-mass islet isografts³².

Peritransplant administration of etanercept has subsequently been studied in a mouse islet

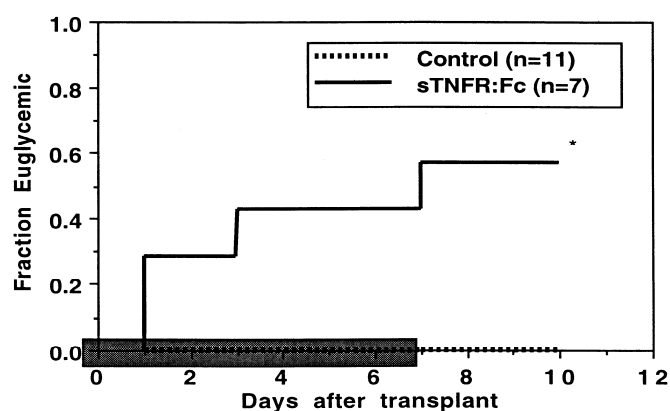


Figure 2 TNF- α Blockade Improves Islet Mass Survival

allograft model by Farney A et al (unpublished data, provided by Dr. Hering). Figure 2 (above) shows actuarial islet graft function in streptozotocin diabetic C57BL/6 recipients of 150 allogeneic B10.BR islets. The hatched box represents the duration of intraperitoneal administration of either etanercept (100 μ g at -24hrs, 50 μ g at +24, +72, +120, and +168 hrs post-transplant) or saline. The proportion of euglycemic recipient animals was significantly higher in the etanercept group (4/7 vs. 0/11, $p=0.01^*$). 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 peritransplant period.

Temporary etanercept administration has previously been studied in globally immunosuppressed kidney^{45, 48-50} and bone marrow transplant recipients^{46, 47}. In renal transplant recipients, etanercept was combined with depleting T cell antibodies (hOKT3 γ 1 ala-ala or ATG). These studies demonstrated that etanercept is well tolerated and may limit the severity of the acute cytokine release syndrome associated with hOKT3 γ 1 ala-ala and ATG administration. The most significant observation by Wee S et al⁵⁰ 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 hOKT3 γ 1 ala-ala induction therapy. Recent studies in bone marrow transplant recipients^{46, 47} 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.

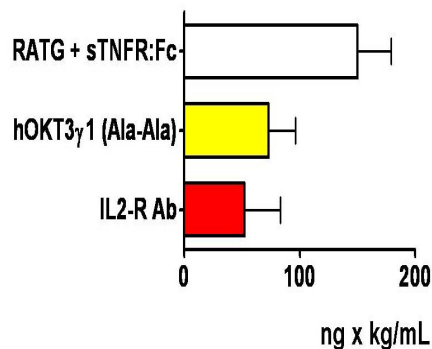


Figure 3 TNF- α Blockade Improves Engraftment Index

Compared with the hOKT3 γ 1 ala-ala trial,²⁵ in which 4 of 6 single-donor islet recipients achieved and maintained insulin independence, the 8 recipients with sustained insulin independence in the ATG plus etanercept trial³⁷ had a significantly higher acute c-peptide response to arginine (ACR_{Arg}) on days ≥ 180 post-transplant: 1.07 ± 0.15 ng/mL (vs. 0.74 ± 0.21 ng/mL in the hOKT3 γ 1 ala-ala trial²⁶; $p=0.028$). This improvement occurred despite transplantation of fewer islets: $7,271 \pm 1,035$ IEq/kg (vs. $10,302 \pm 2,594$ IEq/kg in the hOKT3 γ 1 ala-ala trial²⁶; $p=0.01$). To facilitate comparison of the proportion of engrafted islets between studies, the ACR_{Arg} was corrected for implanted IEq/kg, and expressed as the engraftment index. As shown in Figure 3, the engraftment index in our ATG plus etanercept islet transplant trial would be thus $150 \pm 29 \times 10^{-6}$ ng · kg/mL, as compared with $73 \pm 23 \times 10^{-6}$ ng · kg/mL in our hOKT3 γ 1 ala-ala trial²⁶ and $52 \pm 31 \times 10^{-6}$ ng · kg/mL in a study (estimated from figures) using the Edmonton protocol²⁰.

The success with a rabbit ATG based regimen is perhaps not unexpected in light of data confirming it to be highly efficacious in prevention and treatment of allogeneic rejection^{27, 40-44, 55, 56}. In many kidney series, 1-year rejection rates as low as 5% have been achieved using a brief course of rabbit ATG induction. For this reason, it has become the preferred induction agent for kidney and kidney-pancreas transplant at many centers. It has also been applied with success for non-renal organs⁴¹.

Rabbit ATG has been found to be more effective at preventing rejection compared to an anti-IL-2 receptor agent (basiliximab) in a randomized multi-center kidney transplant trial including sites in the US and Europe⁴³. The incidence of rejection was 1.8 times higher in patients receiving a standard 2-dose course of basiliximab compared with a 5-day induction with rabbit

ATG (1.5 mg/kg/day). The rate of AEs, SAEs, and infections was similar between groups. In addition, in large registry studies, the incidence of post-transplant lymphoproliferative disease (PTLD) in rabbit ATG treated patients was quite low (0.3 to 0.5%)^{40, 57}.

The current protocol plans to evaluate the efficacy of isolated islets transplanted to T1D patients who have already been transplanted with a renal allograft. In order to promote optimal islet engraftment and long-term survival, induction immunosuppression will rely on rabbit ATG and etanercept at the time of the first islet transplant. If additional islet transplants are required, daclizumab will be substituted for rabbit ATG to avoid potentially over immunosuppressing the recipient.

1.6 Known and Potential Risks and Benefits to Human Participants

1.6.1 Risks of Use of Investigational Agent: Transplantation of Allogeneic Islets

Transplantation of islets is associated with several potential risks. These risks may be categorized in terms of: a) transmission of disease from donor to recipient, b) risk of microbial contamination of islet preparations, c) sensitization of the recipient to donor antigens, d) acceleration of retinopathy with acute correction in glycemic control, e) risk of triggering renal graft failure in subjects who have already undergone renal transplant, and f) psychological impact of successful or failed islet transplantation. Other risks including portal thrombosis, portal hypertension, bleeding or hepatic steatosis have been discussed separately in Section 1.6.4.3 entitled "The Procedural Risks of Islet transplantation."

1.6.1.1 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 diseases or malignancies.

A potential donor must have a favorable medical and social history, and clear all standard laboratory tests for low-risk of transmission of donor disease. Donor families are therefore questioned about high risk lifestyle and detailed medical history. Donor blood samples are screened for conditions including (but not limited to) Human Immunodeficiency Virus (HIV)1, HIV2, Human T-cell Lymphotropic Virus Type 1 (HTLV1) or HTLV2, hepatitis B, hepatitis C, CMV, EBV disease, and syphilis.

Donors are excluded if: a) there is known pre-existing metabolic disease including Type 1 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 resected basal or squamous cell carcinoma or intracranial tumor, 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, 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 United Network for Organ Sharing (UNOS) or similar procedures as required by competent OPOs 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 subjects 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 who 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 anti-EBV antibody positive are acceptable for the current trial. It is recognized that this criterion might exclude pediatric patients from IAK transplantation; therefore, there is the possibility of changing this criterion at a later time in the study. EBV by PCR monitoring will be carried out routinely after transplantation at defined intervals throughout the trial. EBV disease and the risk of PTLT have not been reported in the recent era of clinical islet transplantation, suggesting that the risk of this complication may be less than 2%.

1.6.1.2 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 (less than 5 EU/kg of 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, and there have been no reported cases of transmission of bacterial or fungal disease through islet transplantation, when islets are prepared using cGMP. 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 using cGMP.

There have been previous reports of two cases of islet transplantation-related septicemia (*Enterobacter cloacae*) due to transplantation of contaminated cryopreserved pancreatic islets⁵⁸. Additionally, the University of Minnesota investigators have previously reported on the incidence and significance of contaminated islet preparations in clinical islet auto- and allotransplantation⁵⁹. Positive cultures from islet tissue preparations were identified in 11 of 29 subjects (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 subjects (21%) received tissue that retrospectively was determined to be contaminated. None of these subjects 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 low or of low virulence, which allowed 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 subjects (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 subjects 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 Good Manufacturing Practices (cGMP) regulations. Overall, the risk of islet transplantation-related septicemia is considered very low due to the manufacturing and clinical precautions described in the islet manufacturing protocol.

1.6.1.3 SENSITIZATION OF THE RECIPIENT TO DONOR ANTIGENS

As with any allogeneic transplant, the recipient of an organ transplant may become sensitized against donor antigens. Islet transplant recipients who lose their islet graft due to allorejection may develop alloantibodies directed at donor alloantigens. Data on the development of cytotoxic antibodies against donor histocompatibility leucocyte antigens (HLA) in islet allotransplant recipients with failing grafts have been communicated from several islet transplant centers⁶⁰⁻⁶⁴. Once an islet graft fails in islet alone patients, immunosuppressive medicines are usually stopped which can lead to the development of antibodies against the

transplanted tissues. IAK patients are also at risk for developing antibodies against the transplanted islet tissue; however, the exact chance of developing these antibodies in islet-kidney transplant patients is not known, but it is thought to be less than in patients who receive islet alone transplants, since patients with kidney transplants will continue taking maintenance immunosuppressive medications even if the islet graft fails. This is also supported by data from whole organ pancreas transplantation. It is estimated that overall risk of significant levels of additional sensitization from the islet graft is approximately 5-10% in IAK patients who continue on maintenance immunosuppressive medications (unpublished data).

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 panel reactive antibody (PRA) testing. Sensitization of T1D individuals may limit the number of compatible donor kidneys or other organs should a transplant be needed in the future⁶⁵.

1.6.1.4 ACCELERATION OF RETINOPATHY WITH ACUTE CORRECTION IN GLYCEMIC CONTROL

In the DCCT study⁶, about 10% of subjects 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 subjects 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 T1D 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 baseline⁶⁶. 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.

1.6.1.5 RISK OF TRIGGERING RENAL GRAFT REJECTION

A risk unique to IAK subjects is the possibility that the immune response to the islet transplant could trigger renal graft rejection. While the magnitude of this risk is unknown, data from IAKs performed to date, simultaneous kidney-pancreas transplants, and PAK transplants suggests that the risk should be small (see summary above). Naturally, an important component of follow-up in these subjects will include monitoring the function of the renal allograft and prompt treatment of rejection if it ensues.

1.6.1.6 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. Subjects who appear to be incapable of understanding and/or coping with the possibility of failure will not be transplanted.

1.6.2 **Risks of Induction and Maintenance Immunosuppressive Therapies**

Administration of immunosuppressive and immunomodulatory therapies used presently to prevent rejection of transplanted tissues carries 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. All subjects in this trial will already be on an immunosuppression regimen for their kidney transplant. Subjects who receive islet transplantation as part of this study will have the additional risks from administration of a) daclizumab, b) rabbit ATG, and c) anti-inflammatory therapy (*i.e.*, etanercept).

1.6.2.1 DACLIZUMAB (ZENAPAX®)

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

Daclizumab is known to impair the immune system's response to antigenic challenge while the drug is in circulation. Quoting from the package insert, "whether the ability to respond to repeated or ongoing challenge with those antigen returns to normal after daclizumab is cleared is unknown." Because the regimen is to be repeated, there is a possibility of sensitization to the monoclonal antibody, especially after conclusion of the first series of five doses and a period of washout. As with any protein product, anaphylaxis can occur but has been reported only rarely.

As with the other immunosuppressive agents, the risk of opportunistic infections and certain malignancies may be increased. Daclizumab is listed as pregnancy category C. The agent is not recommended for nursing mothers, and it is recommended that women of childbearing potential use effective contraception before, during, and for at least 4 months following daclizumab administration.

1.6.2.2 RABBIT ANTI-THYMOCYTE GLOBULIN (THYMOGLOBULIN®)

Rabbit ATG (Thymoglobulin®) was approved by the FDA in 1999 for the treatment for acute renal graft rejection in conjunction with concomitant immunosuppression (see Thymoglobulin® package insert 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 occurred in 13% and PTLN in 2% of subjects. 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 relatively 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®²⁸. Hering et al described a beneficial role of Thymoglobulin® induction (6mg/kg) in 8 subjects with T1D receiving single donor islet grafts, all of whom achieved insulin independence and were protected against recurrence of hypoglycemia³⁷. Acute islet rejection was described in subjects receiving calcineurin-free immunosuppression when sirolimus levels fell below 9 ng/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®) is utilized³⁷.

1.6.3 Risks of Immunosuppressive / Anti-inflammatory Therapy

1.6.3.1 ETANERCEPT (ENBREL®)

Etanercept is a dimeric soluble form of the p75 TNFR receptor that blocks TNF binding and reduces inflammation^{45, 47-50}. In the United States, it is FDA-approved for use in severe rheumatoid arthritis, juvenile arthritis, ankylosing spondylitis, and psoriatic arthritis. In controlled trials, approximately 37% of subjects treated with etanercept developed injection site

reactions (see Enbrel[®] 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 patients⁶⁷⁻⁶⁹, and based on post-marketing studies a warning has been issued about serious infections and sepsis, including fatalities, which have been reported with the use of Enbrel. Many of the serious infections occurred in patients on concomitant immunosuppressive therapy.

The incidence of tuberculosis has been shown to be statistically higher in anti-TNF-alpha-treated subjects⁶⁷⁻⁶⁹. 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 mice³². Hering et al used etanercept in a recent trial of 8 T1D subjects receiving single donor islet transplants, and all 8 achieved insulin independence suggesting a beneficial role for anti-TNF therapy in clinical islet transplantation³⁷.

1.6.3.2 METHYLPREDNISOLONE (SOLUMEDROL[®])

Methylprednisolone is a synthetic glucocorticoid with potent immunosuppressive properties that has been approved by the FDA for prevention and treatment of rejection in organ transplant recipients. It has also been approved for treatment of several autoimmune and rheumatologic disorders. This drug will be administered at a dose of 1mg/kg I.V. 1 hour prior to and repeated half way through the day -2 dose of the rabbit ATG. Patients will not receive any additional doses of this drug. Side effects are usually minor and self-limited during a short treatment course but may include hyperglycemia, hypertension, euphoria, visual disturbances, anaphylactic reactions, nausea and vomiting.

1.6.4 Risks of Study Procedures

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

1.6.4.1 BLOOD DRAW TESTING

Peripheral blood draws performed during these research studies will not exceed 450 mL per eight-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.

1.6.4.2 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, and displacement, interstitial infusion of fluids, local vein thrombosis, infection, or thrombophlebitis.

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

1.6.4.3 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.

1.6.4.3.1 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 venous thrombosis, hepatic abscess, hepatic infarction, mesenteric ischemia, and mesenteric venous 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.

1.6.4.3.2 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 shoulder 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, this agent has some nephrotoxicity and can cause local or systemic allergic reactions in some subjects.

1.6.4.4 RISK OF BLEEDING AFTER PERCUTANEOUS ISLET TRANSPLANTATION

In the 158 islet transplant procedures submitted to the CITR, the reported SAEs associated with bleeding include hemoperitoneum (n=1), intraabdominal bleed (n=2), low hemoglobin (Hb) (n=1), right hemothorax (n=1), and subcapsular hematoma (n=1) of the liver⁷⁰. 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.⁷¹ One instance of injury to the hepatic artery leading to death during percutaneous transhepatic catheterization of the portal vein has been reported previously to the International Islet Transplant Registry². Reports on intra-abdominal (n=1)²⁴ and intrathoracic bleeding (n=1)³⁰ have been published. The risk of significant hemorrhage after percutaneous islet transplantation as defined by a drop in Hb of more than 25 g/L or the need for transfusion or surgery was 9% in the Edmonton series⁵¹. Subsequently, a further increase in risk of bleeding has been observed by the Edmonton program and has been attributed in part to concomitant aspirin therapy⁷². The risk has since been ameliorated by avoidance of pre-transplant aspirin and more effective measures to seal the catheter tract in the liver⁷². 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 bleeding⁷³. At the University of Minnesota, no bleed-related complications occurred in 20 consecutive subjects when the catheter tract was sealed with coils and Gelfoam³⁷.

1.6.4.5 HYPOGLYCEMIA

Severe hypoglycemia is a risk associated with the transplantation of islets. Iatrogenic hypoglycemia in the immediate post-transplant period is a rare event. Frequent BG 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 CITR⁷⁰. For these six occurrences, the events occurred at the following time intervals; 59 days post the third transplant, 230 days post the second transplant, 296 days post the second transplant, 360 days post the third transplant, 673 days post the third transplant, and 318 days post the second transplant. The local CITR investigators did not attribute any of the six events to the transplant procedure or to the immunosuppression medication.

1.6.4.6 HYPOTENSION

Hypotension not associated with bleeding but induced by transplantation 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 hours 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 posttransplant period is part of the protocol-required 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 surgery⁷⁴.

1.6.4.7 DISSEMINATED INTRAVASCULAR COAGULATION (DIC)

DIC has been documented after autologous islet transplantation of dispersed pancreatic islet tissue in 3 out of about 400 subjects expected to have undergone this procedure⁷⁵⁻⁷⁷.

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 posttransplant period will be part of the protocol-required safety assessments.

1.6.4.8 HEPATIC DYSFUNCTION AND STEATOSIS

Transient abnormalities in liver enzyme tests have been observed immediately following intraportal islet transplantation^{27, 78}. Three of the 86 islet transplant recipients reported to the CITR have experienced transient elevations of liver enzymes requiring prolongation of posttransplant hospitalization or admission⁷⁰. 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 weeks²⁷. 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^{79, 80} 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,⁷⁹ 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.

1.6.4.9 PORTAL HYPERTENSION

Portal hypertension following intraportal infusion of unpurified allogeneic islet tissue resulted in a tear of the splenic capsule requiring splenectomy in one case⁷¹. The elevation in portal pressure (P) following intraportal islet transplantation is temporary in most instances. In 1981, Cameron et al reported on 4 subjects who developed portal hypertension during intraportal infusion of only partially-purified auto-islet transplants, and in whom direct or indirect measurements of portal pressure were performed 3 to 12 months later⁸¹. In all subjects, 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)⁹. The baseline pressures were normal in all cases, suggesting absence of chronic portal hypertension.⁹

1.6.4.10 PORTAL VEIN THROMBOSIS

Transplanted islets release tissue factor and exhibit prothrombotic properties when infused to an intravascular site such as the portal vein⁸². A partial portal vein thrombosis has been reported in one of six subjects transplanted at the intramural National Institutes of Health

(NIH) program²⁴. In the Edmonton single-center experience, the risk of partial portal vein thrombosis was 3% in more than 100 intraportal islet transplants⁷². The management of partial vein thrombosis includes anticoagulation therapy which may lead to intra-abdominal hemorrhage requiring transfusion and/or surgical intervention⁸³. There is one published report of complete thrombosis of the portal vein after transplantation of partially purified pancreatic islets in a combined islet/liver allograft, which necessitated emergency re-transplantation of the liver⁸⁴. 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 was performed on islet transplant recipients on days 1 and 7 posttransplant. 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 subjects who have experienced prior portal thrombosis.

1.6.4.11 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 International Islet Transplant Registry⁷¹. 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.⁷⁰ Gall bladder hematoma (n=1) and gall bladder opacification (n=2) have been observed as well.

1.6.5 **Risks associated with Follow-up Procedures**

1.6.5.1 GFR

Risks associated with the GFR are minimal and are related to the blood draw process. Rarely, the following will occur: excessive bleeding at the 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 occur⁸⁵.

1.6.5.2 RENAL BIOPSY

Subjects randomized to each treatment arm will have a renal biopsy performed. The risks associated with a needle biopsy of the kidney include the possibility of significant bleeding around the kidney which could result in some pain. This occurs in less than 1 out of 200 biopsies. Such bleeding usually lasts for a very short period and involves a small amount of blood. Bleeding into the urine collecting system of the kidney that causes discomfort or temporary blockage of the kidney or bladder may also occur in less than 1 out of 200 biopsies. This could require placing a catheter in the kidney or bladder to drain the urine until the problem has been resolved.

Biopsies can have a risk of creating arteriovenous fistula formations or repeated episodes of bleeding due to damage to a blood vessel (artery) within the kidney. This occurs in less than 1 in 1,000 biopsies. In that case, the damaged vessel may need to be obstructed with material delivered through a catheter (a small tube) guided into the damaged artery from an artery in

the leg. This would result in the permanent loss of blood flow to a small portion of the transplanted kidney, but is unlikely to measurably affect overall kidney function.

Blood-tinged or bloody urine occurs in one out of 10 to 15 biopsies, but this almost always (about 90% of the time) clears without treatment within 24 hours. Perforation of the intestine or of a large blood vessel is extremely unlikely as is injury to other organs such as the liver or bowel.

In general, the chance is less than 1 in 3,000 that a subject may need to have an operation because of bleeding or other complications following biopsy. The chance is less than 1 in 7,500 that the kidney would have to be removed.

1.6.6 Procedures for Minimizing Risks:

A number of features of the proposal have been designed to maximize the safety of subjects in the trial. Important selection criteria include avoidance of any medical condition that might significantly increase the risk related to islet transplantation. Importantly this includes selection of kidney recipients who have stable graft function and who are at a low risk for rejection of their graft. In addition, renal graft function in potential trial subjects has to be sufficient (CrCl of > 40 mL/min) so that it is unlikely that they will experience imminent graft failure and face the need for ongoing immunosuppression for an islet graft in the absence of a functioning kidney. To ensure further that there is no detrimental impact on renal graft function, the maintenance immunosuppression regimen already in place for the renal graft will be continued without marked changes to benefit the islet transplant. This approach will avoid immunosuppressant drug changes that could potentially precipitate renal allograft rejection. In addition, the induction immunosuppression regimen based on rabbit ATG is well studied and has been demonstrated to have a favorable safety profile in solid organ transplantation.

The islet preparation to be infused will be isolated from cadaveric donors thoroughly tested for transmissible infectious agents and will be free of recent high-risk behaviors. In addition, the islet product will be tested preinfusion to verify a suitable level of purity, viability, mass, and endotoxin.

Since gaining insulin independence is dependent on the recovery and infusion of a sufficient islet mass per recipient BW, the trial has been limited subjects < 80 kg in an attempt to maximize the likelihood that insulin independence will be achieved with one (or two) islet transplants. It is recognized that subjects ≥ 80 kg should benefit equally for this therapy should a sufficient islet mass be obtainable.

The informed consent process is carefully organized to minimize unrealistic expectations. We will also reject volunteer subjects who are so desperate as to be incapable of understanding and/or coping with the possibility of failure. We believe that our process leading to informed consent is purposefully organized so as to minimize psychological risk to the recipient.

1.6.7 Benefits of Allogeneic Islet Transplantation

The benefits of islet transplantation include improved glycemic control in subjects who have been unable to achieve an HbA1c of less than 7.0% after a dedicated trial (> 4 months) of IIT by

an experienced diabetologist. Transplantation should also benefit subjects who are well-controlled but have multiple episodes of hypoglycemia. The benefits of islet transplantation on long-term complications of diabetes as compared to IIT are not known but will be studied as part of this clinical trial²⁴.

Although an important goal of this trial is to gain insulin independence in islet transplanted subjects, there is a growing recognition that a partially functioning islet graft in conjunction with low doses of insulin may also reduce or eliminate hypoglycemic events and improve glucose control^{10, 86, 87}. In addition, experimental evidence suggests that even with partial graft function, providing endogenously produced c-peptide may contribute to restoration of physiological function³³⁻³⁵.

From a scientific standpoint, demonstrating the efficacy of islet transplantation in a controlled, randomized trial will provide an important foundation for eventual extension of islet transplantation to other subjects.

2. OBJECTIVES

2.1 Primary Objective

The primary objective is to test the hypothesis that islet transplantation in patients with established kidney transplants leads to a reduced risk of diabetes-related complications as assessed by improved metabolic control measured by serial HbA1c levels and/or reduced occurrence of hypoglycemic events compared with IIT. Comparisons will be made between IIT control subjects and islet transplanted subjects at 1 year after the first islet transplant.

2.2 Secondary Objectives

Secondary objectives of this study will assess whether successful islet transplantation compared to IIT leads to improved QOL, improved metabolic control and reduced cardiovascular, renal, and neurologic complications from diabetes. The cost of care for islet-transplanted subjects versus subjects treated by IIT will also be assessed.

3. SELECTION AND WITHDRAWAL OF SUBJECTS

3.1 Inclusion Criteria

Subjects who meet *all* of the following criteria are eligible for enrollment:

1. Male and female subjects age 18 to 68 years.
2. Subjects who are able to provide written informed consent and to comply with the procedures of the study protocol.
3. Clinical history compatible with T1D with disease onset < 40 years of age and insulin-dependence for ≥ 5 years at the time of enrollment, and a sum of subject age and insulin dependent diabetes duration of ≥28.
4. Absent stimulated c-peptide (< 0.3 ng/mL) in response to a MMTT (Boost® 6 mL/kg BW 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 start of consumption.
5. Subjects who are ≥ 3 months and < 60 months post-renal transplant who are taking appropriate calcineurin inhibitor based maintenance immunosuppression ([tacrolimus alone or in conjunction with sirolimus, mycophenolate mofetil, myfortic, or azathioprine; or cyclosporine in conjunction with sirolimus, mycophenolate mofetil, or myfortic] ± Prednisone ≤ 10 mg/day).
6. Stable renal function as defined as creatinine of no more than one third greater than the average creatinine determination performed in the 3 previous months prior to islet transplantation, until rejection, obstruction or infection is ruled out.

Subjects who meet the following criterion are eligible for randomization:

7. Prior to randomization, all subjects must receive IIT for at least 4 months under the care of an experienced diabetologist and at the end of this period have either an HbA1c ≥ 7.0;

OR

HbA1c < 7.0 and reduced awareness of hypoglycemia manifested by a Clarke score of 4 or more and at least 1 severe hypoglycemic event 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 BG level < 54 mg/dl (3.0 mmol/L) or prompt recovery after oral carbohydrate, IV glucose, or glucagon administration while receiving IIT.

3.2 Exclusion Criteria

Subjects who meet *any* of these criteria are *not* eligible for enrollment:

1. Weight more than 80 kg or BMI > 30 kg/m².
2. Insulin requirement > 55 Units/day.
3. Other (non-kidney) organ transplants except prior failed pancreatic graft where the graft failed within the first two weeks due to thrombosis, followed by pancreatectomy and the pancreas transplant occurred more than 6 months prior to enrollment.
4. Untreated or unstable proliferative diabetic retinopathy.
5. Blood Pressure: SBP > 160 mmHg or DBP > 100 mmHg.
6. Measured GFR using iohexol of ≤ 40 mL/min/1.73 m² (or for subjects with an iodine or seafood allergy, calculated using the subject's measured SCr and the Modification of Diet in Renal Disease [MDRD] study estimation formula. Strict vegetarians (vegans) with a calculated GFR ≤ 35 mL/min/1.73 m² are excluded. The absolute (raw) GFR value is used for subjects with body surface areas > 1.73 m².
7. Proteinuria (albumin/creatinine ratio or ACr > 300mg/g) of new onset since kidney transplantation.
8. Either Class I or Class II panel-reactive anti-HLA antibodies > 50%. Subjects with either Class I or Class II panel reactive anti-HLA antibodies $\leq 50\%$ will be excluded if any of the following are detected:
 - a. Positive cross-match,
 - b. Islet donor-directed anti-HLA antibodies detected by Luminex Single Antigen/specificity bead assay, even if the antibodies are very weak and even if the cross-match is compatible, or
 - c. Antibodies to the renal donor (*i.e.* presumed denovo).
9. For female subjects: Positive pregnancy test, presently breast-feeding, or unwillingness to use effective contraceptive measures for the duration of the study and 4 months after discontinuation. For male subjects: intent to procreate during the duration of the study or within 4 months after discontinuation or unwillingness to use effective measures of contraception. Oral contraceptives, Norplant®, Depo-Provera®, and barrier devices with spermicide are acceptable contraceptive methods; condoms used alone are not acceptable.
10. Active infection including hepatitis B, hepatitis C, HIV, or TB as determined by a positive skin test or clinical presentation, or under treatment for suspected TB. Positive tests are acceptable only if associated with a history of previous vaccination in the absence of any sign of active infection. Positive tests are otherwise not acceptable, even in the absence of any active infection at the time of evaluation.

11. Negative screen for EBV by IgG determination at time of screening or previous kidney transplant.
12. Invasive aspergillus, histoplasmosis, and coccidioidomycosis infection within one year prior to study enrollment.
13. Any history of malignancy except for completely resected squamous or basal cell carcinoma of the skin.
14. Known active alcohol or substance abuse.
15. Evidence of Factor V Leiden mutation.
16. Any coagulopathy or medical condition requiring long-term anticoagulant therapy (*e.g.* warfarin) after transplantation (low-dose aspirin treatment is allowed) or subjects with INR > 1.5.
17. Severe co-existing cardiac disease, characterized by any one of these conditions:
 - a. Recent MI (within past 6 months);
 - b. Evidence of ischemia on functional cardiac exam within the last year;
 - c. Left ventricular ejection fraction < 30%; or
 - d. Valvular disease requiring replacement with prosthetic valve.
18. Persistent elevation of liver function tests at the time of study entry. Persistent SGOT (AST), SGPT (ALT), alkaline phosphatase or total bilirubin, with values > 1.5 times normal upper limits will exclude a subject.
19. Active infections (except mild skin and nail fungal infections).
20. Acute or chronic pancreatitis.
21. Active peptic ulcer disease, symptomatic gallstones, or portal hypertension.
22. Treatment with any anti-diabetic medication other than insulin within 4 weeks of enrollment.
23. Use of any investigational agents within 4 weeks of enrollment.
24. Administration of live attenuated vaccine(s) within 2 months of enrollment.
25. Any medical condition that, in the opinion of the investigator, will interfere with the safe participation of the trial.
26. Male subjects with elevation of prostate specific antigen (PSA) > 4 unless malignancy has been excluded.
27. Any condition other than T1D as the primary cause of end stage renal disease (ESRD) in the native kidney.
28. Positive screen for BK virus by PCR determination at time of screening.
29. A previous islet transplant.

4. STUDY DESIGN

The study will be a multi-center, randomized, prospective, controlled clinical trial in kidney transplant recipients with T1D, comparing the effect of islet transplantation versus IIT. Subjects considered for enrollment will be: 1) at least 3 months but not more than 60 months post-renal transplant, 2) have stable renal graft function and a measured GFR using iohexol plasma clearance of ≥ 40 mL/min/1.73 m² (or calculated by measured SCr and the MDRD study estimation formula for subjects with iodine or seafood allergy), and 3) be free of renal rejection episodes for ≥ 3 months.

Subjects will undergo a period of standardized diabetes care by an experienced diabetologist at the transplant center using the current ADA's standards of medical care in diabetes detailed in Appendix 3 (including general diabetes education and nutrition instruction and standardized diabetes management)⁸. After at least 4 months of structured IIT by an experienced diabetologist, subjects not achieving acceptable glycemic control (HbA1c ≥ 7.0) and those with HbA1c $\leq 7.0\%$ but afflicted with one or more severe hypoglycemic events will be consented for randomization and listed for islet transplantation with UNOS.

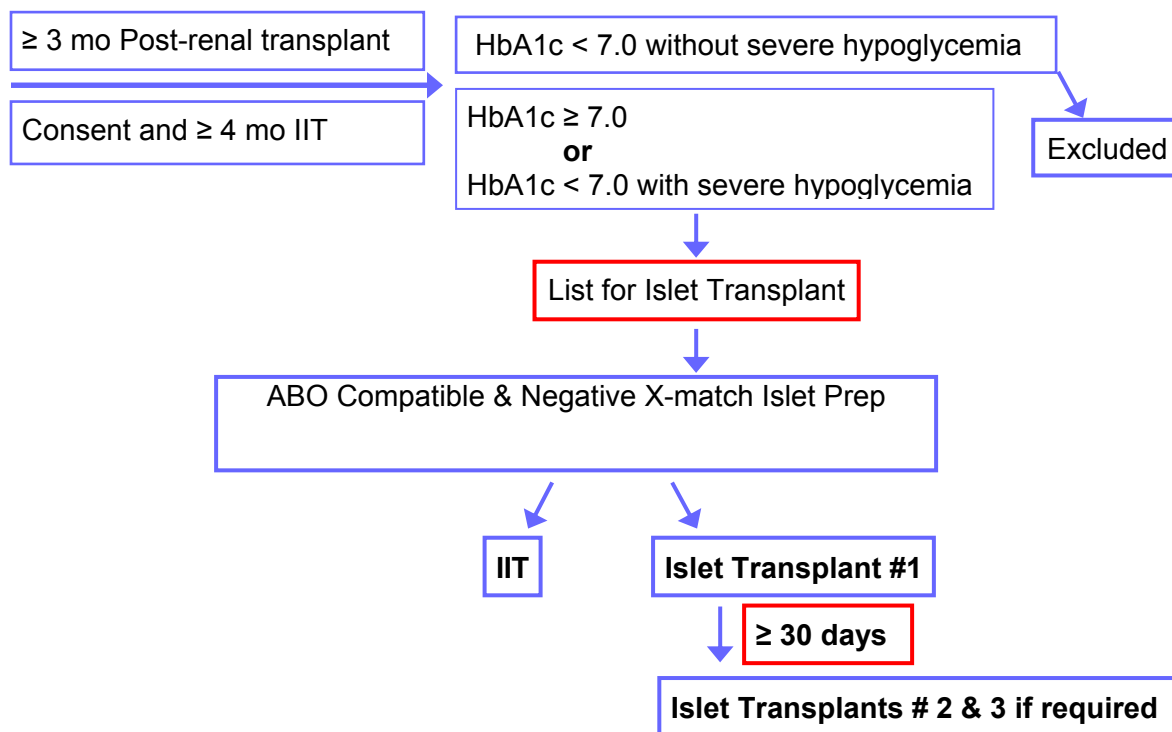


Figure 4 Time Line for Enrollment, Randomization, and Transplantation

In order to maximize enrollment in the trial, participating CIT centers will agree not to conduct competing trials simultaneously that have overlapping criteria for participation. Parallel IAK trials are permitted but must draw from a distinct cohort of IAK subjects.

All subjects fulfilling protocol eligibility criteria will be treated with IIT for at least 4 months prior to potential randomization to islet transplantation or continued IIT. All subjects (islet transplanted and control IIT) will undergo IIT by experienced diabetologists at the transplant center. All subjects will be treated by IIT for diabetes management following current ADA guidelines for glycemic control⁸. Similar guidelines for insulin therapy will also be applied to subjects with partial function following islet transplantation. This will include a target HbA1c of $\leq 6.5\%$, fasting glucose level of < 140 mg/dL and 2-hour postprandial glucose levels of < 180 mg/dL. Target levels can be adjusted as needed based on the development of hypoglycemic episodes. Subjects will be expected to perform self BG monitoring at least 4 times/day and to utilize an insulin regimen consisting of at least 3 insulin injections/day (the type of insulin used should be tailored to the individual subject and include currently available insulin analogs). Insulin pump therapy is not prohibited nor is it required, but may be recommended by the treating diabetologist. BG levels will be “downloaded” from the subject’s glucometer during periodic clinic visits and the glucometer accuracy periodically validated.

Clinical evaluations should occur quarterly, with additional diabetes education or nutrition visits (including education in carbohydrate counting) as necessary. Initially, more frequent appointments may be required to convert subjects to a more intensive insulin regimen. The insulin dose needed will be adjusted to obtain and sustain target glucose and HbA1c.

For the purpose of this trial, a severe hypoglycemic event will be 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 (BG) level < 54 mg/dL [3.0 mmol/L] or prompt recovery after oral carbohydrate, IV glucose, or glucagon administration⁶. Hypoglycemic events that occur within the first week after an islet transplant will not be considered as severe hypoglycemic events if the subject is receiving insulin therapy at that time.

When a suitable islet preparation becomes available, a subject having a blood type compatible with the islet donor and crossmatch negative will be selected from the wait list and randomized to either islet transplantation or continued IIT. If a subject randomized to receive the islet transplant begins induction immunosuppression and then the islet preparation ultimately proves unsuitable for transplantation and an adequate islet preparation replacement is not available within a 90 day period, the subject will continue the immunosuppression regimen indicated for the kidney transplant, but the case will be considered an IAK treatment failure (See Section 5.8.2).

A subject randomized to islet transplantation will be invited to the hospital upon determination of preliminary islet suitability based on count, quality, ABO and negative serum crossmatch. After preliminary testing, detailed in Appendix 1, Schedule of Events (SOE), the subject will

begin the planned induction therapy with rabbit ATG and solumedrol premedication. These drugs can be administered via central vein catheter, peripherally inserted central catheter (PICC) line, or peripheral IV⁵⁶. A period of in vitro culture is considered essential to the protocol because the use of rabbit ATG induction immunosuppression may cause a transient cytokine release associated with the initial doses. The period of in vitro culture after treatment with rabbit ATG will allow any cytokine release to dissipate. The culture period will also permit microbiological and potency assessment of the islet preparation prior to transplant.

Following induction therapy, subjects will remain on a calcineurin-based maintenance immunosuppression regimen. This can consist of tacrolimus alone or in conjunction with sirolimus, mycophenolate mofetil, myfortic, or azathioprine; or cyclosporine in conjunction with sirolimus, mycophenolate mofetil, or myfortic. Subjects on CsA alone will be excluded. Up to 10 mg/day of steroids are also acceptable in conjunction with either regimen. Mycophenolate sodium is an acceptable alternative to mycophenolate mofetil. Drug target levels can be adjusted at the discretion of the treating transplant physician based on subject care needs including islet graft rejection, drug-related islet graft injury, drug-related side effects, or infection.

The islet transplant procedure will be performed either by the percutaneous transhepatic or open mini-laparotomy approaches based on center preference. Either approach is capable of gaining access to the portal system and there is no reason to suspect and no evidence to suggest that the choice of approach will impact the function of the transplanted islets. Islets will be infused (< 7 mL packed cell volume) together with heparin (70 U/kg BW up to 5,000 units total) with periodic portal pressure monitoring. Heparin will be continued for 48 hours after the procedure as an IV drip with target partial thromboplastin time (PTT) levels of 50-60 seconds. Following completion of heparin, subjects will be treated with enoxaparin 30 mg SC BID through day 7. Subjects will also receive pentoxifylline 400 mg tid beginning as a premed for the first dose of rabbit ATG and continuing through day 7.

Following transplant, subjects will receive prophylactic antibiotics including ampicillin/sulbactam (Unasyn®) (x 24 hours), valganciclovir (x 3 months), and trimethoprim/sulfamethoxazole (Bactrim®) (qd x 1 year post last transplant). Enteric coated aspirin (1 po qd) will be instituted 48 hours post-transplant. Optimal glycemic control will be achieved by IV insulin administration beginning day -2 (following rabbit ATG infusion) and continuing through day +2. Thereafter subjects will be maintained on SC insulin therapy for at least 3 weeks post-transplant in an attempt to minimize work related stress to the islet transplant until engraftment is established.

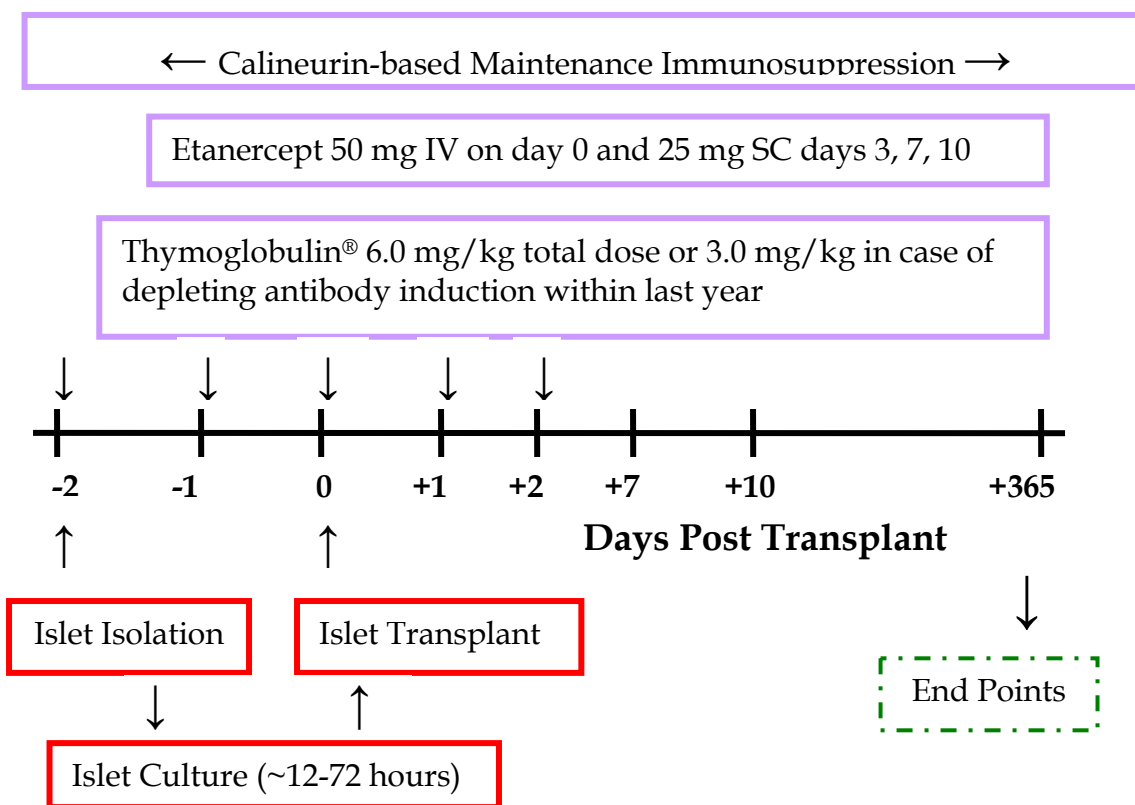


Figure 5 Immunosuppression for first IAK Transplants and Timing of Transplantation

Islets will be administered with the goal of achieving insulin independence as defined below and administering a total of >5,000 IEq/kg recipient BW. It is expected that achieving insulin independence will in all likelihood require more than one islet transplant. Islet transplants will be administered at least 30 days apart and it is recommended that subsequent transplants occur within six months of the preceding transplant. Subjects who have completed 8 months follow-up post-initial transplant will no longer be eligible for additional islet transplants funded under this protocol. It is recognized that availability of suitable donor pancreata may affect the interval between transplants.

Centers are encouraged to re-infuse subjects with partial graft function (as defined below) with the goal of gaining a state fully independent of exogenous insulin administration. The pancreatic islet product is obtained by purification of islets of Langerhans from suitable deceased donor pancreata. One batch comprises purified pancreatic islets obtained from one donor pancreas, and processed during a single purification run.

Subjects will be exposed to a maximum of 3 allogeneic donors for the duration of the study.

The first transplant must contain $\geq 5,000$ IEq/kg of recipient BW. Subjects gaining only temporary insulin independence from a first transplant may receive a second transplant if they meet the conditions stated below for partial function and if it can be performed within the first year after the first dose.

Subjects will be considered to be partially insulin independent with partial islet graft function and good metabolic control following the first or subsequent islet transplant if all of the below criteria are met:

- daily insulin requirements reduced by one-half compared with pre-transplant or < 20 U/day;
- basal or stimulated c-peptide levels ≥ 0.3 ng/mL (≥ 0.1 nmol/L);
- HbA1c $\leq 6.5\%$; and
- absence of severe hypoglycemia

For second (or third) transplants, a similar protocol will be followed except that a 5-dose course of daclizumab plus etanercept will be substituted for rabbit ATG and etanercept. Islets will again be maintained in vitro for 12-72 hours and daclizumab will be administered immediately prior to islet transplant (Figure 6).

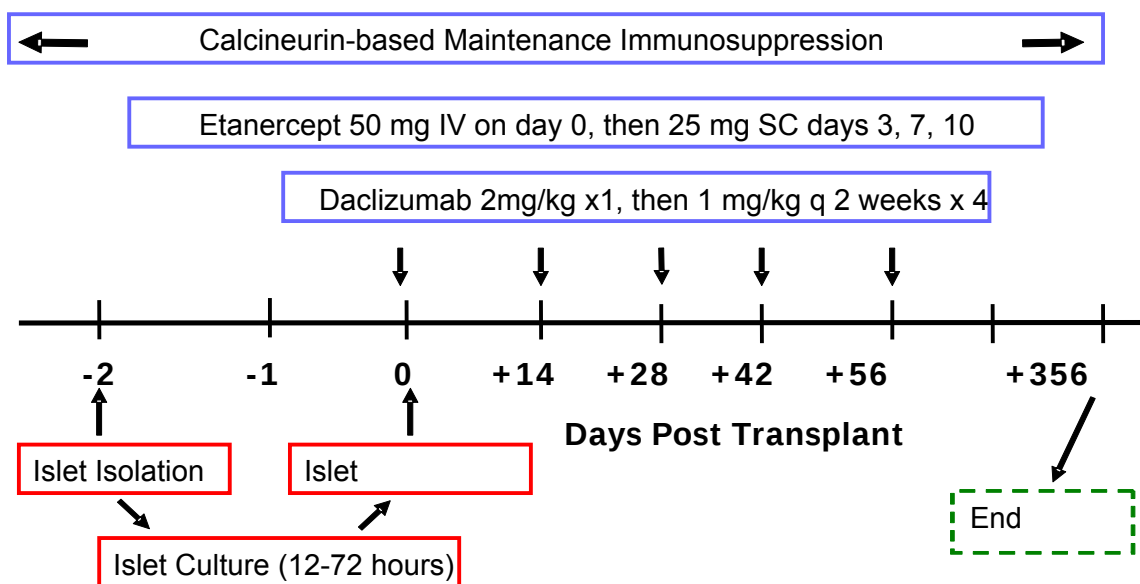


Figure 6 Immunosuppression for Subsequent IAK Transplants and Timing of Transplantation

4.1 Study Endpoints

All subjects will be randomized on Day = -2. Study endpoints are listed relative to the islet transplant occurring on Day = 0. For IIT randomized subjects, "1 year after the first islet transplant" is equivalent to "1 year after Day = 0."

4.1.1 Primary Endpoint

The proportion of subjects with both an HbA1c $\leq 6.5\%$ and an absence of severe hypoglycemic events at 1 year after the first islet transplant or a reduction in HbA1c of at least 1 point and an absence of severe hypoglycemia at 1 year after the first islet transplant will be compared between islet transplanted subjects and control subjects treated by IIT.

4.1.2 Key Secondary Endpoints

These key endpoints are identified because they represent the key beneficial outcomes which the investigators predict the islet transplantation will affect. These domains represent the components of the primary endpoint and important measures of the QOL, metabolic control and glycemic lability, and renal function.

The timing of all assessments is provided in the Schedule of Events (Appendix 1). For logistical purposes, the day 75 timepoint is equivalent to the 3 month evaluation.

4.1.2.1 QUALITY OF LIFE

Whether there is a difference in QOL in the IAK versus IIT group will be assessed by generic and disease-targeted measures.

4.1.2.1.1 *DISEASE-TARGETED MEASURE*

Hypoglycemia Fear Survey

The Hypoglycemia Fear Survey (HFS) is a 23-item self-administered survey for measuring the fear experienced with respect to hypoglycemia^{88, 89}. The HFS measures hypoglycemia avoidance behavior and worry about hypoglycemia. Coefficient alpha for the behavioral and the worry scales were found to exceed 0.90. This instrument has been used in islet transplant candidates and recipients at the University of Minnesota and the University of Alberta.

4.1.2.2 METABOLIC CONTROL

Whether there is an improvement in metabolic control in the IAK versus the IIT group will be evaluated based on improvement in: a) HbA1c levels, b) the number of severe hypoglycemic events, c) the Ryan HYPO score, and d) glycemic lability index.

4.1.2.2.1 *GLYCEMIC CONTROL*

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

4.1.2.2.2 *HYPOGLYCEMIA*

Episodes of severe hypoglycemia are as defined by 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 BG level < 54 mg/dL (3.0 mmol/L) or prompt recovery after oral carbohydrate, IV glucose, or glucagon administration.⁶

4.1.2.2.3 *RYAN HYPO SCORE*

Composite indices of hypoglycemia frequency, severity, and symptom recognition will be assessed by the HYPO score⁸⁷. 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 subjects indicates severe problems with hypoglycemia.

4.1.2.2.4 *GLYCEMIC LABILITY INDEX*

The LI⁸⁷ 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 $(\text{mmol/L}^2) \cdot \text{hr}^{-1} \cdot \text{wk}^{-1}$. A LI greater than or equal to the 90th percentile ($433 \text{ mm}^2 \cdot \text{hr}^{-1} \cdot \text{wk}^{-1}$) of values derived from an unselected group of T1D subjects is evidential for severe glycemic lability.

4.1.2.3 RENAL IMPACT

Whether islet transplantation has beneficial effects on the renal allograft in IAK subjects versus IIT will be assessed by measuring renal allograft function by:

- a. Albumin/creatinine ratio on first morning voided urine;
- b. Serum creatinine; and
- c. GFR using the plasma clearance of iohexol technique (or calculated using a subject's measured SCr and the MDRD study estimation formula for subjects with iodine or seafood allergy).

4.1.2.4 THE PRIMARY ENDPOINT DETERMINED AT 1 YEAR AFTER THE LAST TRANSPLANT

The proportion of subjects with both an HbA1c $\leq 6.5\%$ and an absence of severe hypoglycemic events at 1 year after the last islet transplant or a reduction in HbA1c of at least 1 point and an absence of severe hypoglycemia at 1 year after the last islet transplant will be compared between islet transplanted subjects and control subjects treated by IIT.

4.1.3 **Other Secondary Endpoints**

The timing of all metabolic assessments is provided in the Schedule of Events (Appendix 1). For logistical purposes, the day 75 timepoint is equivalent to the 3 month evaluation.

4.1.3.1 INSULIN INDEPENDENCE

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

- HbA1c $\leq 6.5\%$ or a $\geq 2.5\%$ decrease from baseline (within 30 days prior to transplant);
- fasting capillary glucose level should not exceed 140 mg/dL (7.8 mmol/L) more than three times in the past week (based on measuring capillary glucose levels a minimum of 7 times in a seven day period);
- 2-hour post-prandial capillary glucose should not exceed 180 mg/dL (10.0 mmol/L) more than three times in the last week (based on measuring capillary glucose levels a minimum of 21 times in a seven day period);
- fasting glucose level ≤ 126 mg/dL (7.0 mmol/L); if the fasting glucose level is > 126 mg/dL (7.0 mmol/L), it must be confirmed in an additional one out of two measurements;
- evidence of endogenous insulin production defined as fasting or stimulated c-peptide levels ≥ 0.5 ng/mL (0.16nmol/L).

Secondary insulin independence endpoints will be defined at 75 ± 5 days after the first islet transplant and 365 ± 14 days after the first and final islet transplant.

4.1.3.2 ADDITIONAL RENAL IMPACT MEASURES

Whether islet transplantation has beneficial effects on the renal allograft in IAK subjects versus IIT will be further assessed by monitoring:

- a. Loss of renal allograft survival defined as a permanent return to dialysis, retransplant, or death;
- b. Renal allograft function measured by SCr, spot urine albumin creatinine ratios, and protein excretion; and
- c. Progression of diabetic pathologic post-transplant injury by renal graft biopsy. The investigators at each site have agreed that protocol driven renal biopsies should be done for clinical purposes. This will be done following the first islet transplant in patients randomized to each treatment arm from each pair of randomized patients. Work by Nankivell and others^{90, 91} have demonstrated a high frequency ($>50\%$) of chronic allograft nephropathy already present three years following a transplant, often due to CNI nephrotoxicity. These possibilities may require changes in immunosuppressive therapy, and therefore warrants a repeat biopsy 3 years following the initial islet transplant.

4.1.3.3 CARDIOVASCULAR IMPACT

Whether islet transplantation affords cardiovascular benefit versus IIT will be assessed by monitoring:

- a. Subject mortality (all cause and cardiac related). Standardized follow-up history and physical examinations for cardiovascular disease will be conducted.
- b. Cardiovascular events (MI, CVA) will be recorded at this time. See Appendix 5 for definitions of cardiovascular events.
- c. Carotid intima-medial thickness will be measured by standardized carotid duplex examination. Changes in carotid intimal thickness will be assessed.
- d. Atherogenic profile consisting of a fasting lipid panel [triglycerides, total cholesterol, high density lipoprotein (HDL), and low density lipoprotein (LDL, calculated)], c-reactive protein, serum amyloid A, apolipoprotein A1 and apolipoprotein B will be assessed. Each blood sample collected for the atherogenic profile will be analyzed centrally at the University of Washington.
- e. The ratio of apolipoprotein A1 and apolipoprotein B.

4.1.3.4 IMPACT ON DIABETIC NEUROPATHY

In each treatment group, the following will be conducted:

- a. Standardized neurological history and exam,
- b. Nerve conduction studies, and
- c. Neurological function using the neurologic score, motor index, sensory index and autonomic index described by Kennedy et al.⁹²

4.1.3.5 IMPACT ON VISION

Clinical ophthalmologic examination will be performed in each treatment group. Impact on vision will be determined from the frequency of interventions and from changes in reported visual acuity with optical refraction and severity of diabetic retinopathy at these evaluations.

4.1.3.6 METABOLIC ENDPOINTS

Whether there is an improvement in metabolic control in IAK versus the IIT group will be evaluated based on improvement in a) basal c-peptide levels, b) MMTT, c) β -score, d) insulin requirements, e) clarke survey, f) MAGE, and g) continuous glucose monitoring, h) c-peptide to glucose, creatinine ratio (CPGCR), and i) glucose tolerance.

4.1.3.6.1 *BASAL C-PEPTIDE*

Basal c-peptide levels will be measured in both treatment groups. Induced c-peptide levels will be measured in the islet transplant randomized group only as a part of the mixed-meal test below (Section 4.1.3.6.2).

4.1.3.6.2 *MIXED-MEAL TOLERANCE TEST*

Basal (fasting) and stimulated glucose and c-peptide levels will be determined using the MMTT. The MMTT will be performed following transplantation only in the group receiving islets. The IIT arm will have pre-transplant values carried forward, so not be subjected to repeated marked hyperglycemia that develops when this test is performed in subjects without functioning β -cells.

Subjects will be instructed not to eat or inject short-acting (or bolus) insulin after 8 p.m. the night before the test. Evening or bedtime administration of long-acting insulin will be permitted, as will consumption of water. Subjects receiving CSII (continuous subcutaneous insulin infusion) 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, basal glucose and c-peptide levels will be drawn. Immediately after, the subject will receive 6 mL per kg BW (to a maximum of 360 mL) of Boost® (or a nutritionally equivalent substitute) to consume in 5 minutes starting at time = 0. Then, at time = 90 minutes, stimulated glucose and c-peptide levels will again be drawn.

4.1.3.6.3 B-SCORE: A COMPOSITE INDEX OF POST-TRANSPLANT GRAFT FUNCTION

The β -score will be determined in both treatment groups, using the HbA1c, insulin requirements, fasting (basal) glucose, and basal or stimulated c-peptide as developed by Ryan et al⁸⁶. 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.

4.1.3.6.4 REDUCTION IN INSULIN REQUIREMENTS

The total daily insulin dose required to achieve the fasting capillary glucose level ≤ 140 mg/dL (7.8 mmol/L) 4 or more times per week, and the 2-hour post-prandial capillary glucose levels ≤ 180 mg/dL (10.0 mmol/L) after all meals 4 or more days per week will be determined the month prior to each quarterly visit throughout the study including monthly for the first three months after each transplant. Average daily insulin requirements will be obtained in both the transplant and the IIT groups. It will also be determined if the subject achieved the targeted level of metabolic control ($\text{HbA1c} \leq 6.5\%$).

Evidence of partial success will be considered for subjects who have a reduction in insulin requirements but who are not insulin independent. This will be assessed by comparing the pre-transplant insulin requirement expressed as insulin units per kg per day with the requirement preceding subsequent islet transplants and the insulin requirements at 6 months and 1, 2, and 3 years after the first and last transplant.

4.1.3.6.5 CLARKE SURVEY

Composite indices of hypoglycemia frequency, severity, and symptom recognition will be assessed by the Clarke survey⁹³. 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.

4.1.3.6.6 MEAN AMPLITUDE OF GLYCEMIC EXCURSIONS

The MAGE⁹⁴ 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 a.m. 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 excursion 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.

4.1.3.6.7 C-PEPTIDE TO GLUCOSE, CREATININE RATIO (CPGCR)

The c-peptide to glucose, creatinine ratio (CPGCR) will be determined in both the transplant and IIT groups using the fasting (basal) glucose and c-peptide, and a simultaneous SCr. 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. The CPGCR is calculated as $[\text{c-peptide (ng/mL)} * 100] / [\text{glucose (mg/dL)} * \text{creatinine (mg/dL)}]$. An index of islet graft function, this measure correlates well with both the 90-minute glucose levels during a MMTT and with the β -score⁹⁵.

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

The acute insulin response to glucose (AIR_{glu}), insulin sensitivity (SI), and disposition index (DI) will be determined using the FSIGT test. Special consideration will be taken for the IIT group in which the 75 ± 5 day timepoint will not be performed. The assessment will be limited to insulin sensitivity index. This assessment provides a composite measure of β -cell function, the disposition index (DI), which relates the effect of insulin sensitivity (SI) on first-phase insulin secretion (AIR_{glu}). Understanding the effect of insulin sensitivity on insulin secretory dynamics posttransplant 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 transplantation⁹⁶. These results require confirmation by longitudinal analysis.

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

4.1.3.6.9 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^{31, 97}.

4.1.3.7 GLOBAL TREATMENT FAILURE COMPOSITE OUTCOME MEASURE

A global treatment failure composite endpoint will be defined as the time to the first occurrence of any of the following:

- a. Death
- b. Stroke
- c. MI
- d. Above the ankle amputation

All of the above will be ascertained from the SAE reports and will be adjudicated if an adjudication committee is available.

- e. Renal Failure (Any one of the following):
 - i. Initiation of dialysis;
 - ii. Renal transplantation; or
 - iii. SCr $\geq$ 6.0 mg/dL (Correct any remediable conditions and repeat SCr at least 7 but no more than 20 days later. If confirmed, failure is the time of the initial draw.)
- f. Blindness: Visual acuity of 20/200 in both eyes.
- g. Poor diabetes control (if any of the following occur):
 - i. HbA1c > 9% and reduced less than 1% below the entry level, found 6 months or more following the initial transplant and confirmed on repeat examination at least 30 days and no more than 60 days later. If confirmed, failure is the time of the initial draw;
 - ii. Three consecutive monthly HbA1c values greater than the baseline value, with the first one being 6 months or more following the initial transplant; or
 - iii. Occurrence of two severe hypoglycemic events (requiring assistance from another person) within a three month period. Failure will occur at the time of the second hypoglycemic event.

4.1.3.8 QUALITY OF LIFE

Whether there is a difference in QOL in the IAK versus IIT group will be assessed by generic and disease-targeted measures.

4.1.3.8.1 **GENERIC MEASURES**

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

The SF-36® Health Survey, a 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)⁹⁸. 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. The current manual contains US population norms by gender and age group within gender. The publisher states that the next printing will contain disease-specific norms including diabetes and kidney disease.

b. EQ-5D (EuroQoL)

The EQ-5D is a utility measure that generates a descriptive profile and single index value for health status⁹⁹. 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 various general population samples, including one that has been developed in a nationally representative US sample¹⁰⁰. The second portion of the EQ-5D is a (0-100) visual analogue scale that is used to report overall health status and has been used in islet transplant candidates and recipients at the University of Minnesota. Advantages of this instrument include its brevity and potential application in cost-effectiveness research. The EQ-5D is a public domain instrument and projects may be registered and instruments obtained through the EQ-5D website, www.euroqol.org.

4.1.3.8.2 **DISEASE-TARGETED MEASURES**

Diabetes Distress Scale (DDS)

The DDS represents the latest iteration of the Problem Areas in Diabetes (PAID) scale^{93, 101}. This is a 17-item self-administered questionnaire culled from a longer battery of 28-items and its psychometric properties were recently described⁹³. 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. This instrument has been used in islet transplant candidates and recipients at the University of Minnesota.

4.1.3.9 COST OF CARE

The cost of care for IIT versus islet transplantation will be analyzed yearly post-initial transplant (see the Cost of Care Economic Evaluation in the CIT-06 Manual of Operations). See Appendix 1, SOE, for the cost of care timepoints and corresponding study instruments.

4.1.3.10 OTHER ORGAN TRANSPLANTS

The proportion of subjects that abandon study treatment due to other non-kidney or non-islet transplants will be assessed.

4.1.4 Safety Endpoints

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

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

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 islet transplant:

- The incidence of worsening retinopathy as assessed by blindness and changes in reported visual acuity

5. STUDY TREATMENT REGIMEN

Please refer to Section 1.6 and to applicable package inserts and product labeling for known and potential risks to human subjects associated with the study treatment regimen.

Table 4 Islet Transplant and Immunosuppression 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 <i>(initial transplant only)</i>																	
Daclizumab <i>(subsequent transplants only)</i>			X											X	X	X	X
Etanercept			X			X				X			X				

5.1 Investigational Agent: Allogeneic Islets

5.1.1 Formulation, Dosage, and Administration

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

Table 5 Composition of Final Drug Product [Product Code PHPI-A-01]

Component	Quantity per Batch
Purified Human Pancreatic Islets	$\geq 4.0 \times 10^3$ IEq/kg of recipient BW (total IEq/infusion)
CIT Transplant Media	≤ 600 mL (maximum total volume in Ricordi® infusion bags containing 200 mL each)
HSA, USP	2.5%
HEPES Buffer, USP	25 mM

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 (computed tomography) guidance. Alternatively access to a mesenteric or omental venous tributary of the portal vein can be obtained by mini-laparotomy under general anesthesia (transplant site preference or in the extremely rare circumstance that percutaneous access cannot be achieved).

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

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 standard immunosuppressive medications.

5.2.1.1 RABBIT ANTI-THYMOCYTE GLOBULIN (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 first dose will be administered over 12 hours and subsequent doses will be administered over 6 hours.

The dose will be limited to 3 mg/kg total (0.5mg/kg on day -2, 1.0 mg/kg on day -1 and 1.5mg/kg on day 0) in patients treated with depleting antibody induction therapy (hOKT3γ1 ala-ala, rabbit ATG, or Campath®) for their renal transplant within the last year. Patients previously treated with rabbit ATG will be tested for efficacy of rabbit ATG therapy at day 2 by assessment of T cell depletion by CD3 counts. If lack of efficacy is detected, Zenapax® can be substituted for rabbit ATG using the regimen detailed below for subsequent transplants.

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 repeated midway through the first ATG infusion only (*i.e.* on day -2)

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

If the subject is admitted when the vascular access team is not available or at a time when the placement of a PICC could delay the first rabbit ATG dose it may be administered IV via a peripheral line as follows:

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

5.2.2 Subsequent Allogeneic Islet Transplants

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

5.2.2.1 DACLIZUMAB (ZENAPAX®)

Daclizumab, manufactured by Roche, will be used instead of Thymoglobulin® for all subsequent islet transplants. Daclizumab (Zenapax®) is a humanized anti-CD25 monoclonal antibody in clinical use since 1997 for prophylaxis against acute organ rejection in subjects receiving a kidney allograft. Please refer to applicable product labeling and the Package Insert for its known and potential risks to human subjects.

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

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

If a third islet transplant is deemed necessary and performed more than 70 days after the second transplant (see Section 4.1), all five doses of daclizumab will be repeated.

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.

Methylprednisolone (Solu-Medrol®) will be administered at a dose of 1 mg/kg IV one hour prior to and repeated midway through the first ATG infusion only (*i.e.* on day -2).

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 (BACTRIM SS® OR SEPTRA SS®)

Trimethoprim/sulfamethoxazole will be administered at a dose of 80 mg/400 mg PO QD starting on Day +1 for 1 year post last 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 transplant, to be continued for 3 months after transplantation.

5.3.2.3 VALGANCICLOVIR (VALCYTE®)

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

5.3.3 Anticoagulation Prophylaxis / Hematological Agents

5.3.3.1 HEPARIN

Heparin will be administered at a dose of 70 U/kg BW of recipient, divided equally among the islet bags, given with islet transplant, followed by 3U/kg/hr IV for the next 4 hours. From the 5th through the 48th hr post-transplant heparin will be titrated to achieve and maintain 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).

5.3.3.3 ASPIRIN

Enteric coated aspirin will be administered at a dose of 81 mg PO qPM starting 48 hours posttransplant and continued as medically indicated.

5.3.3.4 PENTOXIFYLLINE (TRENTAL®)

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 Insulin Therapy

Glucose levels will be targeted to 80-120 mg/dL. Insulin (*e.g.*, Regular®, Lispro®, NPH®, or 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. Subjects will be considered insulin independent according to the definition of insulin independence summarized in Section 4.1.3.1.

5.3.5 Other Standard Therapies

Anti-hypertensive, anti-hyperlipidemia, and other approved therapies for pre-existing and new medical conditions will be provided per standard of care. Pre- and post-islet transplant

procedure drug regimens (e.g., pre-transplant sedation and anesthetic) will be given per standard of care.

5.4 Rescue Medications

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

5.5 Prohibited Medications

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

- Pramlintide acetate (Symlin®)
- Exenatide (Byetta®)
- Sulfonylureas (e.g., tolbutamide, tolazamide, chlorpropamide, glipizide, glyburide, glimepiride)
- Glitinides (e.g., repaglinide, nateglinide)
- Acarbose (Glucobay®, Precose®)
- Any medications in the macrolide antibiotic class
- Other investigational products
- Immunomodulatory agents
- Other anti-diabetic agents
- Dapsone
- > 10 mg Prednisone

5.6 Prophylactic Medications

- a. Ampicillin/sulbactam* (Unasyn®) will be given unless the subject is allergic to penicillin, in which case levofloxacin will be given. Ampicillin/sulbactam has possible side effects including severe allergic reaction, pain at the injection site (3%), diarrhea (3%), and rash (2%). Levofloxacin has possible side effects of nausea (7% in clinical trials), injection site reaction (6%), diarrhea (5%), headache (5%), and constipation (3%).
- b. Trimethoprim/sulfamethoxazole* (Bactrim SS® or Septra SS®) will be administered orally once per day for prevention of *Pneumocystis carinii* pneumonia (PCP) for the first year after the last transplant. Side effects of Bactrim include allergic reactions, nausea, vomiting, diarrhea, fulminant hepatic necrosis, and blood dyscrasias (leucopenia, agranulocytosis, aplastic anemia, and hemolytic anemia). Subjects who are allergic to sulfa drugs will receive pentamidine. 300mg of pentamidine will be given via nebulizer once a month after the transplant to prevent PCP. Pentamidine aerosol has the side-effects of metallic taste, fatigue, and decreased appetite.

- c. *Valganciclovir* (Valcyte®) is an antiviral drug which will be given to prevent CMV infection starting on Day -2 at a dose of 450 mg PO QD increasing to 900 mg QD by Day 12 and continuing for 14 weeks posttransplant (irrespective of CMV status of donor or recipient). Valganciclovir has the possible side effects of neutropenia and thrombocytopenia, with related risks of infection and bleeding. Frequent cell counts will be performed and the valganciclovir dose adjusted accordingly. Other infrequent (~2%) side effects include low red blood cell count, fever, rash, and an increase in liver enzymes.
- d. *Enteric coated aspirin* will be administered at a dose of 81 mg PO qPM starting 48 hrs posttransplant and continued as medically indicated.
- e. *Pentoxifylline* (Trental®) is indicated as an adjunctive treatment for subjects with claudication and peripheral vascular disease because of its ability to improve blood viscosity. In experimental islet transplantation in mice, daily administration of pentoxifylline for the first four weeks post-transplant led to improved islet graft function and an improved response to challenge with a glucose load¹⁰². Subjects will be given pentoxifylline 400 mg tid from day -2 to day 7.

Adverse effects include flushing (2.3%), nausea and emesis (~30%), headache, and dizziness. Mild reductions in blood pressure may also occur in some subjects. Concomitant administration of pentoxifylline with theophylline containing drugs can result in increased theophylline levels in some subjects. Its use is contraindicated in subjects with recent cerebral or retinal hemorrhages as it may increase the risk of bleeding.

5.7 Assessment of Compliance with Study Treatment

Assessment of subject compliance will be determined by the completion of the scheduled study visits and required documentation that the specific subject is responsible for (e.g., Blood Glucose Logs, AEs, 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 5.8.3 for a description of possible indications for premature discontinuation of study treatment.

5.8 Modification or Discontinuation of Study Treatment

5.8.1 Modification of Protocol Specific Drug(s)

Modification of certain protocol specific drugs, including etanercept, daclizumab, and rabbit ATG will be allowed in the event of complications. Details of the approved modifications are listed in Section 5.8.2.

5.8.2 Modification of Standard Immunosuppression

Should an islet product become unsuitable for transplantation subsequent to recipient randomization and treatment with induction immunosuppression, the subject will remain on maintenance therapy required for their renal transplant. This will provide sufficient time for organ procurement, islet isolation, and infusion of an islet product without any additional

induction treatment of the recipient. An emergency request will be placed through UNOS to ensure that the next available pancreas for islet transplantation is directed to the selected manufacturing site. All CIT investigators will also be notified of this situation. Should an islet product not be available the subject will continue maintenance immunosuppression, but induction with Daclizumab will be administered at the time of the islet transplant. If an adequate islet product does not become available within a 90 day period, the patient will continue the immunosuppression regimen indicated for their kidney transplant, but the case will be considered an IAK treatment failure. The number of cases considered a treatment failure will be documented in the electronic case report forms (eCRFs).

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 and renal grafts. In the event that the immunosuppression regimen is not tolerated, the site PI may elect to prescribe an alternative immunosuppression regimen. The intent would be for the alternative regimen to be temporary in nature where possible and any such decision made with the primary interest of maintaining the function of the renal allograft. Any non-protocol directed study treatment modification that the site PI determines is necessary should be reported as a protocol deviation.

5.8.2.1 RABBIT ANTI-THYMOCYTE GLOBULIN-INDUCED ANAPHYLAXIS

In rare instances, anaphylaxis has been reported with Thymoglobulin® use. In such cases, the infusion should be terminated immediately. Medical personnel should be available to treat subjects who experience anaphylaxis. Emergency treatment such as 0.3 mL to 0.5 mL aqueous epinephrine (1:1000 dilution) 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.

5.8.2.2 RABBIT ANTI-THYMOCYTE GLOBULIN-INDUCED CYTOKINE RELEASE

Thymoglobulin® infusion may cause cytokine release-related fever and chills. To minimize these, the first dose should be infused over a minimum of 6 hours into a high-flow vein. Also, premedication with corticosteroids (solumedrol, 1 mg/kg IV), 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.

5.8.2.3 NEUTROPENIA

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

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

- Reduce ATG by 50%.
- Test for CMV and if negative hold valganciclovir.

- Reduce trimethoprim/sulfamethoxazole to 80 mg/400 mg 3 times per week or hold trimethoprim/sulfamethoxazole.
- Review and obtain current sirolimus trough levels and consider dosage adjustment if trough levels are > 12 ng/dL (please refer to Appendix 1, SOE, for additional information).
- 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 CBC with differential, subject symptoms, and measured temperatures.

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

- Obtain Infectious Disease Consult.
- Obtain CMV antigenemia or PCR for CMV.
- Hold rabbit ATG.
- Hold valganciclovir and trimethoprim/sulfamethoxazole.
- Review and obtain current sirolimus trough levels and consider dosage adjustment if trough levels are > 12 ng/dL (please refer to Appendix 1, SOE, for additional information).
- 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 less than 500 cells/ μ L and the subject is afebrile, then the following will be done:

- Hold rabbit ATG.
- Hold administration of trimethoprim/sulfamethoxazole and/or valganciclovir.
- Review and obtain current sirolimus trough levels and hold dose if trough levels are > 12 ng/dL (please refer to Appendix 1, SOE, for additional information).
- 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 with differential, subject symptoms, and measured temperatures.

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

- The subject will be hospitalized under neutropenic precautions and an 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 levels are > 12 ng/dL (please refer to Appendix 1, SOE, for additional information).
- 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.

5.8.2.4 THROMBOCYTOPENIA

If the subject is found to have a platelet count (PLT) of $< 50 \times 10^9/L$, rabbit 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 resume 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 rabbit ATG dose by 50% until PLT is $> 75 \times 10^9/L$.

5.8.2.5 NEPHROTOXICITY

A sustained 33% increase in SCr or a 33% decrease in GFR warrants a prompt referral to a nephrologist for evaluation. Additionally, significant changes in renal function should be reported to the patient's physician managing the renal transplant. If it is thought that the decrease in renal function is attributable to CNI immunosuppressive therapy, the physician managing the renal transplant should consider ONE of the therapeutic alternatives shown in Table 6:

Table 6 Immunosuppressive Medication Modifications

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 ^{103, 104} .
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

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

5.8.3 Premature Discontinuation of Study Treatment (Transition to “Off-Protocol” Treatment)

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

1. The subject is unwilling or unable to comply with the protocol.
2. The investigator believes that the study treatment is no longer in the best interest of the subject.
3. The renal allograft is lost and the subject elects to terminate chronic immunosuppression.
4. Graft Failure: Islet allograft failure will be defined as absence of insulin production by transplanted islets, as evidenced by absence of c-peptide. This will be determined by (1) undetectable (defined as a level below the limits of detection in the assay) c-peptide on random testing, followed by (2) undetectable c-peptide at baseline and at 60 and 90 minutes after MMTT. C-peptide levels obtained in the course of the MMTT will be run at the core lab in Seattle, WA. All study treatments not related to the care of the renal transplant will be tapered and discontinued, at the discretion of the site PI, when it is determined that graft failure has occurred.
5. 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.

6. CRITERIA FOR PREMATURE TERMINATION OF THE STUDY

6.1 Participant Withdrawal Criteria

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

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

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

6.2 Study Stopping Rules

6.2.1 Protocol Suspension and Review

Study enrollment at all participating clinical sites will be suspended pending expedited review of all pertinent data by the institutional review board (IRB), the National Institute of Allergy and Infection Disease (NIAID), the National Institute of Diabetes and Digestive 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 (PNF) occurs in 3 or more consecutive subjects at 2 or more participating clinical sites. PNF is defined by undetectable c-peptide levels (1-3 hours post-prandial) by Day 7 post-transplant.
3. There are 6 consecutive study subjects with a c-peptide less than 0.3 ng/mL (on random testing, at baseline and 1-3 hours post-MMTT) at 75 days post-transplant.
4. Any event(s) which in the opinion of the Medical Monitor or Protocol Chair indicates the need for DSMB review.
5. The DSMB recommends termination of protocol enrollment and further transplants on a study-wide basis based on a review of the data and finding evidence that such action is necessary. Statistical guidelines for terminating the study based on monitoring guidelines are provided in Section 10.5.

After the protocol is placed on hold, no additional transplants within the trial will be performed at any participating clinical site until the CIT Steering Committee and DSMB meet either in

person or by conference call to review in depth the results and circumstances surrounding the islet functional failure or SAE and 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 PNF transplants defined by undetectable c-peptide levels (1-3 hours post-prandial) between 3-7 days post-transplant.

After any site is placed on hold, no additional transplants will be performed at that site until the CIT Steering Committee and DSMB meet either in person or by conference call to review in depth the results and circumstances surrounding the islet functional failure or SAE and 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 pivotal protocol also at other affiliated sites testing the same protocol.

In all cases of PNF who no longer have a functioning renal allograft, subjects will be asked to temporarily continue immunosuppression for 3 months 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

A detailed description of activities at each study visit is provided in the SOE in Appendix 1.

7.1 Enrollment and Screening

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

1. age, gender, height, weight, age of diabetes onset, insulin type and requirements;
2. completed questionnaire inquiring for the presence of contraindicated medical conditions;
3. recent (last 6 months) laboratory reports for HbA1c, urinary albumin, kidney and liver function, and fasting lipids.

If review of these materials indicates that the potential subject is not eligible, she/he will be notified by a study coordinator and explained why this is so (*e.g.* age > 68 years); if review indicates that the potential subject may be eligible, she/he will be contacted by a study coordinator to schedule the screening visit.

At the screening visit, the screening and IIT details and procedures will be discussed in lay terms to each potential research subject. If the potential subject remains interested, she/he will review the screening informed consent document with the coordinator, and an investigator will join the discussion and answer any questions. Once satisfied that all questions have been answered, the potential subject will either decline to participate or sign the screening informed consent document. The potential subject will sign an informed consent form (see Section 13.2) before undergoing any screening study procedures. This may occur at a subsequent visit if the potential subject desires, in order to think further about what participation means and/or to consult with family and/or friends. If at any time the suitability of diabetes care is questioned, the potential subject will be referred for further assessment and management.

Once informed consent has been obtained, eligibility will be confirmed through the performance of the screening visit procedures detailed in Appendix 1, SOE, and from additional reports required from each subject's diabetologist, retinologist, dentist (to exclude infection), and gynecologist (indicating results of Pap smear and age-appropriate mammography). A psychosocial evaluation may be requested if either a coordinator or investigator is unsure whether a potential subject may be mentally unfit to undergo the procedure or to determine whether a psychosocial problem may be responsible for the instability of diabetes; such an evaluation would be performed by an experienced transplant social worker and/or psychiatrist.

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 screening 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 7 Timeframes for local screening assessments

Local Screening Assessments	Allowable timeframe prior to the date of consent
PAP Smear; Mammogram; Retinopathy evaluation; Physical exam; CXR; Abdominal US; electrocardiogram (ECG); Cardiac Stress Test; purified protein derivative (PPD); PSA; Serology; EBV IgG; Coagulation	Within one year
CBC; Chemistry; Lipids	Within 6 months
PRA by flow cytometry	Within 3 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.

Once all eligibility criteria (inclusion and exclusion) are met, subjects will be referred to a study diabetologist for IIT, which will be administered per the guidelines in Appendix 3. Subjects will return to the clinic at least quarterly from the time of enrollment until randomization.

7.2 Waitlist/Baseline

After completion of at least 4 months of intensive insulin therapy (see Appendix 3, Diabetes Management Plan), and the subject is deemed eligible to continue in the study, he/she will be listed for an islet transplant. During the period when subjects are awaiting randomization, they will return to the study site for clinic visits every 3 months. HbA1c sampling between the required 3 month interval visits may be drawn locally, sent from the local lab to the study site and then shipped to the central lab for testing. Waitlist assessments will be repeated at pre-defined intervals as detailed in Appendix 1. Results from repeat assessments done closest to randomization will be used as the subject's baseline values. All one-time baseline assessments should be completed on Day -2, whenever possible, but always prior to the start of immunosuppression.

If after being placed on the waitlist and in the absence of severe hypoglycemic events, more than one HbA1c test result in the last three consecutive monthly tests is less than 7%, the patient will be withheld from randomization if a donor match is found. If while on the waiting list the patient has an HbA1c consistently below 7% for 12 successive months and is free of severe hypoglycemic events during such time, the patient will be discontinued from participation in the trial and will be referred for clinical care.

As in any other transplant situation, medical conditions that arise (*e.g.*, new serious infection, malignancy, compliance issues, etc.) will automatically trigger a re-evaluation to determine if the subject remains qualified for the protocol. Only qualified subjects may proceed to donor organ matching and transplant.

7.3 Randomization

Once a suitable islet preparation becomes available, subjects having a blood type compatible with the donor and crossmatch negative (obtained ≤ 30 days prior to randomization) will be

selected from the pool of waitlist subjects. Eligible subjects will be randomized on Day -2 relative to the transplant between an islet or continuing IIT. If the islet transplant preparation proves unsuitable after randomization to islet transplantation and whether or not induction therapy has been administered, the subject will be transplanted with the next available islet preparation from a compatible donor as outlined in Section 5.8.2.

Randomized treatment assignments will be developed by DCC statisticians and stored on the DCC server. The DCC will maintain a central web-based randomization system. When a usable islet preparation is available, the study site will access the appropriate web page. At that time the system will verify that the subject is indeed eligible. The system will then provide the investigator with the appropriate treatment assignments. The date and time of the randomization will be recorded in the database. Once a treatment assignment has been provided, the subject becomes a part of the intention-to-treat population and will be included in the primary analysis for the study. The basic principle of intention-to-treat requires that once a subject is randomized she/he must be included in the analysis and assigned to the treatment to which she/he was randomized. In the event that the islet transplant does not occur after randomization, the subject will retain his/her randomization assignment for the remainder of the study, therefore, a subject will be randomized only once. The islet randomized subject would then be transplanted with the next available islet preparation from a compatible donor as outlined in Section 5.8.2. A parallel and equivalent back-up randomization procedure is available in the unlikely event that the web-based system is not available.

7.4 Islet Transplant and Study Treatment Visits

Subjects randomized to islet transplantation will be invited to the study center upon determination of preliminary islet suitability based on count and quality. After preliminary testing detailed in Appendix 1, SOE (- 2 days relative to transplant), the subject will be admitted for induction therapy with rabbit ATG. This can be administered via central vein catheter, PICC line, or peripheral IV ⁵⁷. A period of in vitro culture is considered essential to the protocol because of the use of rabbit ATG induction immunosuppression may cause a transient cytokine release associated with the initial doses. The period of in vitro culture will allow time for any cytokine release to dissipate after treatment of the subject with ATG and will also permit time for microbiological and potency assessment of the islet preparation prior to transplant.

Following induction therapy, subjects will remain on a calcineurin-based maintenance immunosuppression regimen. This can consist of tacrolimus alone or in conjunction with sirolimus, mycophenolate mofetil, myfortic, or azathioprine; or cyclosporine in conjunction with sirolimus, mycophenolate mofetil, or myfortic. Subjects on CsA alone will be excluded. Up to 10 mg/day of steroids are also acceptable in conjunction with either regimen. For subjects on tacrolimus plus sirolimus, combined levels (*i.e.*, tacrolimus plus sirolimus) should target 12-20 (tacrolimus target level 3-10 ng/mL and sirolimus target level 3-15). For subjects receiving tacrolimus plus mycophenolate mofetil, tacrolimus levels should target 6-10 and the mycophenolate mofetil dose should target 1-2 gm/day. Mycophenolate sodium is an acceptable alternative to mycophenolate mofetil. For subjects on CsA plus either sirolimus, mycophenolate mofetil, or myfortic, combined trough levels should target 100-300 ng/mL or

the 2 hour level should target 350-500 ng/mL for the first 3 months post-transplant and 200-350 ng/mL thereafter. Drug target levels can be adjusted at the discretion of the treating transplant physician based on subject care needs including graft rejection, drug-related graft injury, drug-related side effects, or infection.

Subjects randomized to the control group will be invited to the study center for preliminary baseline testing outlined in Appendix 1, Schedule of Events (± 4 days relative to Day = 0). These patients will be managed according to the custom of the study center. Control group subjects should continue their IIT initiated upon study enrollment. Clinical evaluations should continue at least quarterly, corresponding with scheduled follow-up visits as much as possible for convenience to the patient. Additional visits should occur as necessary for diabetes education and nutrition as outlined in Appendix 3. The subject's insulin dose should continue to be monitored and adjusted to sustain target glucose and HbA1c levels. Study outcomes will be completed by the control group as indicated in Appendix 1.

7.5 Follow-up Visits

Subjects randomized to IIT will undergo a 36-month follow-up period according to Appendix 1, SOE. For logistical purposes, the Month 12, Month 24, and Month 36 visits are equivalent to the Y1 post-initial transplant, Y2 post-initial transplant, and Y3 post-initial transplant visits, respectively, for IIT randomized subjects only.

Subjects randomized to the islet transplant will undergo a minimum 36-month follow-up period following the last islet transplant to include timepoints relevant to the initial transplant. The timing of all follow-up assessments will "reset" with additional transplants; i.e., the day of the 2nd transplant becomes day 0 and the subsequent assessments are conducted in relation to this day. Please refer to the Appendix 1, SOE, for the clinical time points of specific follow-up study procedures.

7.6 Criteria and Timing for Subsequent Islet Transplants

7.6.1 Second Islet Transplant Criteria

Conditions required for consideration of a subsequent islet transplant should include:

1. Subject is at least 30 days post-initial transplant.
2. Subject received $\geq 5,000$ IEq/kg with the first transplant, but failed to achieve or maintain insulin independence.
3. Subject has been compliant with study monitoring and prescribed immunosuppressive therapy.
4. 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.
5. Subject has no unresolved SAEs.
6. No evidence of PTLT, requiring complete withdrawal from immunosuppressive therapy.

7. No evidence of hypersensitization, allergic responses, or other potentially serious drug reactions to medications required by the protocol.
8. Stable renal function as defined as being a creatinine of no more than one third greater than the average creatinine determination performed in the 3 previous months, until rejection, obstruction or infection is ruled out.

7.6.2 Third Islet Transplant Criteria

Conditions required for consideration of a third islet transplant should include the following:

1. The subject is at least 30 days post-second islet transplant.
2. The subject received greater than 4,000 IEq/kg following the second transplant, but remains dependent on insulin for longer than one month after the second transplant.
3. There is evidence of partial graft function at one month (defined by at least a 50% reduction in daily insulin requirement compared to the pre-transplant baseline, and a basal or stimulated c-peptide level ≥ 0.5 ng/mL).
4. The Protocol Chair, Site Principal Investigators (PIs), and the Steering Committee have determined that there were no relevant protocol deviations at the site.
5. The subject has been compliant with study monitoring and prescribed immunosuppressive therapy.
6. 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.
7. Subject has no unresolved SAEs.
8. No evidence of PTLT, requiring complete withdrawal from immunosuppressive therapy.
9. No evidence of hypersensitization, allergic responses, or other potentially serious drug reactions to medications required by the protocol.
10. Stable renal function as defined as being a creatinine of no more than one third greater than the average creatinine determination performed in the 3 previous months, until rejection, obstruction or infection is ruled out.

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

7.7 Visit Windows

The post-randomization weekly visits must occur on the scheduled day ± 3 days. The 75 day visit must occur at day 75 ± 5 days. Monthly visits must occur ± 10 days. For example, a subject receiving a transplant on the 15th of one month should have follow-up visits between the 5th and 25th of subsequent months. All other visits are scheduled on a quarterly basis from the last

transplant, and should occur within 14 days (plus or minus) of the calendar date for the transplant. A subsequent transplant resets this schedule. Pre-transplant visit windows would follow the same weekly, monthly, or quarterly post-transplant windows when applicable.

7.8 Study Treatment Assignment Procedures

7.8.1 Blinding and Randomization

This is an open-label study; therefore, no blinded treatment codes are required for the assignment of study drug. Study treatment will be assigned by randomization at the time that a viable transplant becomes available. These assignments will be maintained in secure files at the DCC.

8. SAFETY MONITORING

AEs that are classified as serious according to the definition set forth by the health authorities must be reported promptly to NIDDK/NIAID, Clinical Research Organization (CRO)/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 most current version of the *CIT-TCAE*. This document, created by the Clinical Islet Transplantation (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 participant at immediate risk of death from the reaction as it occurred.
3. In-patient hospitalization or prolongation of existing hospitalization. Please note that hospital admissions for the purpose of conducting protocol-mandated procedures do not need to be reported as SAEs, unless the hospitalization is prolonged due to complications.
4. Persistent or significant disability.
5. Congenital anomaly or birth defect.
6. An event that required intervention to prevent permanent impairment or damage. An important medical event that may not result in death, be life-threatening, or require hospitalization may be considered an SAE when, based on appropriate medical judgment, it may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.
7. Other conditions specified in the protocol.

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

Regardless of the relatedness of the AE to the investigational agent, 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 hypoglycemic events will be collected after the screening consent has been obtained and until the subject initiates 4 months of IIT. All AEs will be collected once the subject initiates 4 months of IIT. All AEs will continue to be collected until study completion, or for 30 days after the subject prematurely withdraws from the study. AEs will be followed until the time the event is resolved, stabilized, or the subject completes or withdraws from the study, whichever comes first.

AEs may be discovered through any of these methods:

- Observing the subject.
- Questioning the subject, which should be done in an objective manner.
- Receiving an unsolicited complaint from the subject.
- An abnormal value or result from a clinical or laboratory evaluation (*e.g.*, a radiograph, an ultrasound, or an ECG) can also indicate an AE. If this is the case, then the evaluation that produced the value or result should be repeated until the value or result returns to normal or can be explained and the subject’s safety is not at risk. If an abnormal value or result is determined by the investigator to be clinically significant, it must be reported as an AE.

8.2.2 Recording Procedure

Throughout the study, the investigator will record all AEs on the appropriate AE 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 most current version of the *CIT-TCAE*. This document provides a

common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all AEs.

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

Grade 1 = Mild AE.

Grade 2 = Moderate AE.

Grade 3 = Severe and undesirable AE.

Grade 4 = Life-threatening or disabling AE.

Grade 5 = Death.

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

Table 8 General Severity Definition of Adverse Event

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

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

8.2.3.2 DEFINITION OF ATTRIBUTION

The relatedness, or attribution, of an AE to islet transplantation, which includes the transplant procedures 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 and/or immunosuppression

and/or infection prophylaxis and/or IIT will be defined by using the descriptors provided below.

Table 9 Attribution of Adverse Events

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

For additional information and a current, 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 screening consent to participate in the study 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.

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 the IND 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 NIDDK/NIAID Medical Officer.

Standard reporting (*i.e.*, must 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 calendar days; fatal or life-threatening events must be reported within 7 calendar 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 SAEs will be reported to the DCC by the sites on at least a monthly basis.

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

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

8.3.3.4 NOTIFYING THE DATA AND SAFETY MONITORING BOARD

The NIDDK/NIAID will provide the DSMB with listings of all SAEs on an ongoing basis, and 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 be informed immediately of any pregnancy and should instruct a pregnant subject to stop taking study medication not related to the care of the renal transplant. The investigator should report all pregnancies within 24 hours (as described in section 8.3.3.2) using the SAE report form. The investigator should counsel the subject and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the subject should continue until the conclusion of the pregnancy, and a follow-up SAE report form detailing the outcome of the pregnancy should be submitted to the DCC.

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. The Investigator's Brochure for the islets will be amended as needed by the IND sponsor. Until these documents are updated, expedited reporting will be required for additional occurrences of a reaction.

9. MECHANISTIC ASSAYS

Timepoints are listed relative to each islet transplant; therefore the clock is restarted with each subsequent transplant. Exceptions are noted in each respective section. For logistical purposes, the day 75 timepoint is equivalent to the 3 month evaluation.

9.1 Metabolic Testing

9.1.1 Study Endpoints

Designated primary and secondary endpoints will be employed to compare the treatment group to the control group. Because the assessment of islet graft function is dependent on complex physiologic relationships between the graft and its recipient, no single test adequately addresses the viability of the transplant. Therefore, HbA1c and the number of episodes of severe hypoglycemia will be used as clinically relevant measures of islet graft function for the primary endpoint, and additional stimulatory tests of islet graft function utilizing MMTT, glucose (FSIGT) challenges, and the CPGCR will be used to assess secondary endpoints. Also, the effect of islet graft function on glycemic control (HbA1c), glycemic lability (MAGE and LI), hypoglycemia (Clarke and HYPO scores), glucose variability (CGMS[®]), QOL, cardiovascular impact, renal impact, diabetic neuropathy, and cost of care will be assessed as additional secondary endpoints.

9.1.2 Metabolic Assessments

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

9.1.2.1 INSULIN REQUIREMENT

Subjects will record their daily insulin dose on self-monitoring diaries. Subjects should be given exogenous insulin as needed to maintain fasting capillary glucose level ≤ 140 mg/dL (7.8 mmol/L) at a minimum of 4 out of 7 days per week; 2-hour post-prandial capillary glucose levels should not exceed 180 mg/dL (10.0 mmol/L) more than 3 times per week. Average daily insulin requirements will be obtained in both the transplant and the IIT groups. It will also be determined if the subject achieved the targeted level of metabolic control ($\text{HbA1c} \leq 6.5\%$).

9.1.2.2 GLYCEMIC CONTROL

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

9.1.2.3 GLYCEMIC LABILITY

Glycemic lability will be assessed in both the transplant and IIT groups by both the MAGE⁹⁴ and the LI⁸⁷.

The MAGE requires 14 – 16 capillary BG measurements over two consecutive days taken before and 2 hours after breakfast, lunch, and dinner, and at bedtime with an optional measurement at 3 AM. A glycemic excursion is calculated as the absolute difference in peak and subsequent nadir (or vice versa) glucose values, with the direction (peak to nadir versus nadir to peak)

determined by the first quantifiable excursion in the two day period. All excursions > 1 S.D. of the 7 – 8 glucose readings for the day in which they occurred qualify for the analysis, where they are summed and divided by the number of qualified excursions to give the MAGE in mmol/L (or mg/dL) glucose. A MAGE > 11.1 mmol/L (200 mg/dL) is indicative of marked glycemic lability.

The LI requires 4 or more daily capillary BG measurements over a 4 week period and is calculated as the sum of all the squared differences in consecutive glucose readings divided by the hours apart the readings were determined (range 1 to 12 hours) in (mmol/L²/hr wk⁻¹). A LI greater than or equal to the 90th percentile (433 mmol/L²/hr wk⁻¹) of values derived from an unselected group of T1D subjects is evidence for severe glycemic lability.

9.1.2.4 HYPOGLYCEMIA

All measures will be performed in both the transplant and IIT groups.

Episodes of severe hypoglycemia will be documented every month as defined by 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 BG level < 54 mg/dL (3.0 mmol/L) or prompt recovery after oral carbohydrate, IV glucose, or glucagon administration.⁶

In addition, composite indices of hypoglycemia frequency, severity, and symptom recognition will be assessed by both the Clarke survey⁹³ and the HYPO score⁸⁷.

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 subjects indicates severe problems with hypoglycemia.

9.1.2.5 MIXED-MEAL TOLERANCE TEST

Basal (fasting) and stimulated glucose and c-peptide levels will be determined using the MMTT.

The MMTT will be performed following transplantation only in the group receiving islets. The IIT arm will have pre-transplant values carried forward, and so not be subjected to repeated marked hyperglycemia that develops when this test is performed in subjects without functioning β -cells. 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 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, basal glucose and c-peptide levels will be drawn. Immediately after, the subject will receive 6 mL per kg BW (to a maximum of 360 mL) of Boost® High Protein Drink (or a nutritionally equivalent substitute) to consume in 5 minutes starting at time = 0. Then, at time = 90 minutes, stimulated glucose and c-peptide levels will again be drawn.

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

9.1.2.6 B-SCORE: A COMPOSITE INDEX OF POST-TRANSPLANT GRAFT FUNCTION

The β -score will be determined in both the transplant and IIT groups from the HbA1c, insulin requirements, fasting (basal) serum glucose, and basal or stimulated c-peptide as developed by Ryan et al⁸⁶. The score may range from 0 (no graft function) to 8, with all subjects reported with a score of 8 also having 90-minute serum 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 TO GLUCOSE, CREATININE RATIO

The c-peptide to glucose, creatinine ratio (CPGCR) will be determined in both the transplant and IIT groups using the fasting (basal) serum glucose and c-peptide, and a simultaneous SCr. 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^{105, 106}. The CPGCR is calculated as $[\text{c-peptide (ng/mL)} * 100] / [\text{glucose (mg/dL)} * \text{creatinine (mg/dL)}]$. An index of islet graft function, this measure correlates well with both the 90-minute serum glucose levels during a MMTT and with the β -score⁹⁵.

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

The AIR_{glu} , insulin sensitivity (SI), and disposition index (DI) will be determined using the FSIGT test. Special consideration will be taken for the IIT group in which the 75 ± 5 day timepoint will not be performed. This assessment provides a composite measure of β -cell function, the disposition index (DI), which relates the effect of insulin sensitivity (SI) on first-phase insulin secretion (AIR_{glu}). Understanding the effect of insulin sensitivity on insulin secretory dynamics posttransplant 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 transplantation⁹⁶. These results require confirmation by longitudinal analysis.

The insulin-modified FSIGT test¹⁰⁷ involves blood sampling at baseline ($t = -10, -5,$ and -1 min) and at $t = 1, 2, 3, 4, 5, 7, 10, 12, 14, 16, 18, 20, 22, 25, 30, 40, 50, 70, 100, 140,$ & 180 minutes post-injection of glucose at $t = -30$ seconds with an injection of insulin at $t = 20$ min. Each blood sample collected for insulin, c-peptide, and glucose determination will be drawn according to

University of Washington (Seattle, WA) SOP and will be shipped frozen to U of W for measurement in the core laboratory. Aliquots not utilized by U of W will be deposited with the NIDDK repository. 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 (S_C), a measure of insulin-independent glucose disposal, and insulin sensitivity (S_I), a measure of insulin-dependent glucose disposal, are derived from Bergman's minimal model using MinMod Millenium® software, and further allow for determination of the disposition index ($DI = AIR_{glu} \cdot S_I$).

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^{31, 97}.

9.1.2.10 QUALITY OF LIFE

Whether there is a difference in QOL in the IAK versus IIT group will be assessed by generic and disease-targeted measures.

9.1.2.10.1 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)⁹⁸. 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).

EQ-5D (EuroQoL)

This instrument is a utility measure that generates a descriptive profile and single index value for health status⁹⁹. 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 various general population samples, including one that has been developed in a nationally representative US sample¹⁰⁰. The second portion of the EQ-5D is a (0-100) visual analogue scale that is used to report overall health status and has been used in islet transplant candidates and recipients at the University of Minnesota. Advantages of this instrument include its brevity and potential application in cost-effectiveness research. The EQ-5D is a public domain instrument and projects may be registered and instruments obtained through the EQ-5D website, www.euroqol.org.

9.1.2.11 DISEASE-TARGETED MEASURES

Diabetes Distress Scale (DDS)

The DDS represents the latest iteration of the PAID scale^{93, 101}. 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)⁹³. 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.

Hypoglycemia Fear Scale

The Hypoglycemia Fear Survey (HFS) is a 23-item self-administered survey for measuring the fear experienced with respect to hypoglycemia^{88, 89}. The HFS measures hypoglycemia avoidance behavior and worry about hypoglycemia. Different versions of the instrument can be found in the literature, varying in length from 15 to 33 items. We have used the 33-item recommended by Daniel Cox. Coefficient alpha for the behavioral and the worry scales were found to exceed 0.90.

9.1.3 Measures of Renal, Neurological, and Cardiovascular Outcomes

9.1.3.1 RENAL CHANGES

Renal allograft biopsies will be performed for each treatment arm. The investigators at each site have agreed that protocol driven renal biopsies should be done for clinical purposes. This will be done following the first islet transplant in patients randomized to each treatment arm from each pair of randomized patients. Work by Nankivell and others^{90, 91} have demonstrated a high frequency (>50%) of chronic allograft nephropathy already present three years following a transplant, often due to CNI nephrotoxicity. These possibilities may require changes in immunosuppressive therapy, and therefore warrants a repeat biopsy 3 years following an islet transplant.

Both groups will have careful control of systemic blood pressure (see Blood Pressure Protocol in the Manual of Operations).

A secondary endpoint of this study is the change over 3 years in composite index of diabetic glomerulopathy structural parameters. Secondary endpoints include change over 3 years in

individual structural parameters including glomerular basement membrane (GBM) width, as well as mesangial [Vv (Mes/glom)] and mesangial matrix [Vv (MM/glom)] fractional volume. Other structural variables to be measured include mesangial cell fractional volume [Vv (MC/glom)] index of arteriolar hyalinosis, % global glomerular sclerosis and cortical interstitial fractional volume [Vv (Int/cortex)]. Baseline biopsies will be blindly evaluated by light (LM) and electron (EM) microscopy for the presence of transplant glomerulopathy or other glomerular lesions that could interfere with the interpretation of changes due to diabetes and for the presence of processes such as BK virus nephropathy that would limit the likelihood of 3 further years of graft survival.

If upon initial biopsy either member of a pair show one of these conditions that pair will be excluded from this component of the study. Otherwise, they will be included in the renal study and all measures will be performed on the follow-up biopsy regardless of presence or absence of these confounding conditions.

9.1.3.2 NEUROLOGICAL CHANGES

A standardized neurological history and exam and nerve conduction studies will be conducted for each treatment group. Neurological function will be assessed in each treatment group using the neurologic score, motor index, sensory index and autonomic index described by Kennedy et al ⁹².

9.1.3.3 CARDIOVASCULAR CHANGES

Carotid intima-medial thickness will be measured by standardized carotid duplex examination. Changes in carotid intimal thickness will be assessed in each treatment group. Atherogenic profile consisting of a fasting lipid panel (triglycerides, total cholesterol, HDL, and LDL (calculated)), c-reactive protein, serum amyloid A, and Apolipoproteins A1 and B will be assessed in each treatment group. Each blood sample collected for the atherogenic profile will be drawn and shipped frozen to U of W for measurement in the core laboratory.

9.2 Immunologic Testing

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

While methods for determination of allo and autoantibody have been extensively studied and are fairly well-established, reliable, reproducible and validated methods for assessment of T cell immunoreactivity to allo and/or autoantigens do not exist. For the most part, these techniques

are time-consuming, technically demanding and require large blood volumes and significant staff time for set up and analysis of the resultant data. Several methods are undergoing testing in multiple T1D consortia (*e.g.*, ELISPOT, tetramer staining, T cell proliferation assays) to determine which tests provide the most reliable data with regards to distinguishing between subjects with T1D vs normal controls (for autoantigen) and to improve techniques for assessing recipient antidonor 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 UPenn 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 proinflammatory or procoagulant markers on islets with recipient response in the early posttransplant period, ethylenediaminetetraacetic acid (EDTA) anti-coagulated blood will be collected for assessment of thrombin-antithrombin (TAT), C3a and c-peptide levels.

9.2.1.5 ARCHIVED SERUM, CELLS AND RIBONUCLEIC ACID (RNA)

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

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

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

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

10. STATISTICAL CONSIDERATIONS AND ANALYTICAL PLAN

10.1 Analysis Samples

All efficacy analyses will be based on the intention-to-treat principle. Once a subject has been randomized she/he will be analyzed in the treatment group assigned by the randomization. All subjects will also be included in the safety analysis. While it is highly unlikely that a subject will not receive her/his assigned treatment, the safety analysis will be based on the treatment received rather than the treatment assigned by the randomization.

10.2 Study Endpoint Assessment

10.2.1 Primary Endpoint

The primary endpoint (as defined in Section 4.1.1) is the proportion of subjects with both an $HbA1c \leq 6.5\%$ and an absence of severe hypoglycemic events at 1 year or a reduction in HbA1c of 1 point and an absence of severe hypoglycemia at 1 year.

The proportion of subjects experiencing favorable outcomes at one year in the transplanted group will be compared to the proportion having favorable outcomes in the control group. We will use the likelihood ratio test for treatment from a logistic regression with terms (dummy variables) for treatment and clinical center. The decision for efficacy will be based on the one degree of freedom chi-square test for treatment. The comparison will be declared significant if the p-value is less than 0.05. The effect size will be estimated by the odds ratio.

The primary endpoint should be available for all enrolled subjects. An exception will be if a death occurs or if the subject withdraws consent to be followed before their one year post final transplant follow-up visit. In those cases the endpoint will be classified as failure 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 their final transplant, in which case that later value will be imputed. All imputations will be reported with the primary analysis.

10.2.2 Secondary Endpoints

The secondary endpoints are defined in sections 4.1.2 and 4.1.3. Specific analysis plans will be presented in the formal statistical analysis plan (SAP).

By examining multiple secondary endpoints it is likely that some variables will be found significantly different between the two treatment groups, but these findings may be Type 1 errors. Appropriate qualifiers will be reported with any significant secondary findings.

Secondary endpoints are grouped into two groups: key secondary endpoints and other secondary endpoints. The key endpoints are grouped together because they represent the key beneficial outcomes which the investigators predict the islet transplantation will affect. By identifying these endpoints in the protocol we expect that significant differences in these endpoints will provide stronger evidence for a causal relationship than would result from observed significant differences in a large group of secondary endpoints. However, because there are still a large number of key secondary endpoints, if we adjust for the many multiple

comparisons then there will be little chance of observing a statistically significant difference. Therefore, we will not adjust for multiple comparisons even for the key secondary endpoints.

In general, continuous variables such as QOL scores, HbA1c levels, and c-peptide response will be analyzed by a t-test. If there is compelling evidence that the normal distribution does not hold, then a logarithmic or square root transformation will be done before using the t-test. If lack of normality persists, non-parametric tests will be used.

Regression models for continuous longitudinal data (mixed models) will be used to examine the differences between groups in each of the response variables where measurements are repeated over time. Models will include terms for treatment, time, and the interaction of treatment and time. Similar models will be used to model dichotomous variables over time. Survival analysis models will be used to compare time to becoming insulin dependent and to identify risk factors through Kaplan-Meier estimates and Cox regression models.

10.3 Subject and Demographic Data

10.3.1 Baseline Characteristics and Demographics

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

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

Statistical presentation for baseline and demographic characteristics will be further summarized and defined in the statistical analysis plan (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 relevant groups such as gender, age, race, etc. Any others will be specified in the statistical analysis plan SAP.

10.3.4 Study Completion

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

10.4 Sample Size and Power Calculations

10.4.1 Power for Primary Endpoint

Sample sizes were calculated for the primary endpoint as a composite dichotomous variable as planned in the study: “HbA1c $\leq$ 6.5% and an absence of severe hypoglycemic events at 1 year after the first islet transplant or a reduction in HbA1c of 1 point and an absence of severe hypoglycemia at 1 year after the first islet transplant “. For simplicity we will call this outcome a “good outcome”.

The DCCT trial provided the consortium data on 711 patients who were under intensive monitoring of HbA1c for at least 16 months. For our purposes, we assumed that enrolled patients for this trial will undergo the same monitoring process as for the DCCT and that a patient would be eligible for randomization if her/his average value of HbA1c at months 3 and 4 is greater than 7.0%. Using this criterion, 390 of the 711 DCCT subjects would have qualified for randomization. Among those DCCT patients who would be eligible for randomization the observed good outcome rate based on 12 months follow-up was 27% (101/373, se=2.2). Since data on hypoglycemic events was not available in the DCCT data, this good outcome rate overestimates the true rate in the IIT group. The projected sample size will therefore be conservative.

The following table provides calculations that use 27% as an estimate of the true good outcome rate in the IIT group. The alternative values were selected by using 23% (27%-2 times standard error), 25% (27%- standard error), 29% (27% + standard error) and 32% (27% + 2 times standard error). The table provides minimal detectable differences assuming that the true rate of good outcome in the IIT group is larger than 20%. If the true good outcome rate is 32% in the IIT group and the study randomized 65 subjects per group then there would be 90% power to detect a difference of 32% versus 60% and 80% power to detect a difference of 32% versus 56%. If the true good outcome rate in the IIT group is 23% then with 65 subjects per group the study would have 90% power to detect a difference of 23% versus 50% and 80% power to detect a difference of 23% versus 46%. If the true good outcome rate in the IIT group is 25% then with 65 subjects per group the study would have 90% power to detect a difference of 25% versus 52% and 80% power to detect a difference of 25% versus 48%.

We have conservatively selected a sample size of 65 subjects per group and assumed that the true favorable outcome rate in the IIT group will not be greater than 27%. Thus the study will have 90% power to detect a difference of 54% in the islet transplant group and 27% in the IIT group and 80% power to detect a difference of 27% versus 51%.

Table 10 Table of Minimal Detectable Difference

Desired Power	Number in Each Group	True Rate in Standard Medical Care Group (%)				
		23%	25%	27%	29%	32%
90%	35	60.4	62.9	65.2	67.4	70.4
	45	55.7	58.2	60.5	62.7	65.9
	55	52.2	54.7	57.1	59.4	62.7
	65	49.6	52.0	54.4	56.8	60.3
	75	47.9	50.1	52.3	54.4	57.6
	85	46.4	48.8	51.0	53.1	56.2
80%	35	55.1	57.6	60.1	62.4	65.6
	45	50.9	53.4	55.9	58.2	61.5
	55	48.0	50.4	52.8	55.2	58.6
	65	45.9	48.2	50.5	52.8	56.4
	75	44.5	46.7	48.8	50.9	54.0
	85	43.1	45.4	47.7	49.8	52.9

10.4.2 Power for Secondary Endpoints

The following tables describe minimal detectable difference for selected sets of secondary endpoints. The sample size for the proposed study was selected because it provides 90% power to detect a difference in the proportion of subjects with HbA1c $\leq 6.5\%$ and free of hypoglycemic events of 32% in the standard medical treatment group and 60% in the islet transplanted group.

The secondary outcomes listed in the table are all ordinal scale variables. With 65 subjects in each group a two-sided 5% level t-test will have 80% power to detect a difference of 0.5 standard deviations or more. The table that follows displays data for QOL outcomes, metabolic outcomes, and renal outcomes. The table displays estimates of the means and standard deviations for transplanted subjects at 12 months. The minimal detectable differences are computed as 0.5 times the standard deviation at 12 months for that outcome. Note that these calculations do not take into account the large number of comparisons that will be made in the analysis of the secondary endpoints.

Table 11 Table of Minimal Detectable Differences for QOL Outcomes

Outcome Category	Outcome	Mean	Standard Deviation	Minimal Detectable Difference*
SF-36 Health Survey	Physical Functioning	96	6	3.0
	Role-Limitations Physical	83	22	11.0
	Role-Limitations Emotional	82	18	9.0
	Bodily Pain	74	14	7.0
	Social Functioning	84	15	7.5
	Vitality	54	17	8.5
	Mental Health	60	13	6.5
	General Health	75	16	8.0
	Physical Component Summary	56	5	2.5
	Mental Component Summary	43	8	3.0
EQ-5D(VAS)		83	13	4.5
Hypoglycemia Fear Survey	Behavior	6	11	5.5
	Worry	5	12	6.0
Diabetes Distress Scale	Emotional Burden	24	25	9.0
	Physician-Related Distress	0	0	NA
	Regimen-Related Distress	14	22	11.0

Table 12 Table of Minimal Detectable Differences for Metabolic and Renal Outcomes

Outcome Category	Outcome	Mean	Standard Deviation	Minimal Detectable Difference
Metabolic Control	HbA1c	6	0.7	0.35
	c-Peptide	2	0.7	0.35
	MMTT	133	55	27.5
	Hypo Score	850	750	375
	MAGE	8	4	2
	LI	223	200	100
Renal One year	GBM	533	149	75
	Glomerular mesangial matrix (Vv)	0.19	.08	0.04
	Surface density of peripheral GBM (Sv)	0.1	0.03	0.015
	Composite	-76.5	22	11
	GFR	103	30	15
Renal Five Years	GBM	475	181	90.5
	VV	0.13	0.04	0.02
	Slope of GFR	-0.194	1.053	0.527

10.5 Interim Analyses to Ensure Patient Safety

We will propose a strategy to the DSMB that monitors for both efficacy and futility. This strategy would recommend termination of randomization into the islet transplantation group in any of the following circumstances:

- There is strong evidence that the islet transplantation is effective (the rate of favorable outcome in the islet group is significantly better than the rate in the comparison group).
- There is strong evidence that the islet transplantation is harmful (the rate of favorable outcome is significantly better in the comparison group than the rate in the islet group).
- There is reasonable evidence that the study will never reject the null hypothesis (futility).

Interim monitoring of the primary endpoints will use an overall level of significance of 0.05. Boundaries similar to those of O'Brien and Fleming will be used for both efficacy and futility¹⁰⁸. Such boundaries are conservative in that it is unlikely that the boundary is crossed unless the differences are real. The spending function of Lan and DeMets will be used if necessary¹⁰⁹. This method preserves the interpretability of the final results, p-values, and confidence intervals so that the final results will not have to be adjusted for the interim analyses. An intention-to-treat principle will be used in all analyses.

Should the effect be found to cross the monitoring boundary before the end of the study, the DSMB will advise on whether enrollment should be terminated early. Subjects already enrolled

in the study will continue with their assigned treatment unless the DSMB recommends otherwise.

The following table describes a possible monitoring plan. The computations assumed four equally spaced analyses and that the planned total sample size would be 130 randomized subjects. The stopping boundaries were computed using the SeqTrial module in SPlus. Table 12 describes the plan.

Table 13 Data Safety Monitoring Plan

Analysis	Boundary Values				Maximum p-value to reject null	Minimum p-value to accept null
	Conclude Islet Worse	Accept Null Low	Accept Null High	Conclude Islet Better		
	Observed Differences in Proportions (islet- comparison)					
1 (N=32)	-0.3719	--	--	0.6719	0.00001	N/A
2 (N=65)	-0.3359	-0.0009	0.0009	0.3359	0.0046	0.9938
3 (N=98)	-0.2240	-0.1123	0.1123	0.2240	0.0206	0.2458
4 (B=130)	-0.1680	-0.1680	0.1680	0.1680	0.0450	0.0450

This table can be interpreted as follows. At the first interim analyses one would do the following:

- Reject the null hypothesis and conclude that islet subjects are doing worse than IIT subjects if the observed difference in favorable outcome rates is more than 0.3719 in favor of the IIT group.
- Reject the null hypothesis and conclude that islet subjects are doing better if the test observed difference in favorable outcome rates is more than 0.2719 in favor of the islet transplanted group.

At the second interim analysis one would do the following:

- Reject the null hypothesis and conclude that islet subjects are doing worse if the observed difference is greater than or equal to 0.2922 in favor of the IIT group.
- Reject the null hypothesis and conclude that islet subjects are doing better if the observed difference is greater than or equal to 0.2922 in favor of the islet group.
- Conclude that it will be impossible to reject the null hypothesis (futility) in case the observed difference between islet and IIT subjects is less than or equal to 0.0009.
- Otherwise continue to randomize subjects.

At the final (fourth) analyses one would do the following:

- Reject the null hypothesis and conclude that islet subjects are doing worse if the observed difference is greater than or equal to 0.1680 in favor of the IIT group.

- b. Reject the null hypothesis and conclude that islet subjects are doing better if the observed difference is greater than or equal to 0.1680 in favor of the islet group.
- c. Conclude that there is not sufficient evidence to show that one treatment is better than the other if the difference between favorable outcome rates is less than 0.168.

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 statistical analysis plan (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 clinical study report as appropriate.

11. IDENTIFICATION AND ACCESS TO SOURCE DATA

11.1 Identifying Source Data

The investigator is required to keep accurate records to ensure that the conduct of the study is fully documented (see Section 12). The results of all clinical and 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 the SAEs will be reported on an SAE report form as well as on individual CRFs. 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 sites 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) 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 subject's 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*¹¹⁰, 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 and submitted to the applicable Health Authorities. Any amendments to the protocol or to the consent materials must also be approved by the EC or IRB, and NIAID/NIDDK 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 subject's appropriate language and back-translated into English for review by the Sponsor.

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

13.3 Privacy and Confidentiality

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

14. PUBLICATION POLICY

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

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Appendix 1 Schedule of Events

Time points (days relative to transplant)	SCR	IIT ≥ 4 months	WL ¹	BL ²	0 ³	3	7	14	21	28	56	75	180	270	365	365 post-initial Tx	450	540	630	730	730 post-initial Tx	810	900	990	1095	1095post-initial Tx	
Visit Number	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	Y1	16	17	18	19	Y2	20	21	22	23	Y3	
Visit Windows (specified in days)	N/A	N/A	N/A	≤ 4	N/A	N/A	± 3				± 10	± 5	± 14														
Equivalent Wk/Mo/Yr	N/A	N/A	N/A	N/A	N/A	N/A	W1	W2	W3	W4	M2	M2.5	M6	M9	M12	Varies	M15	M18	M21	M24	Varies	M27	M30	M33	M36	Varies	
GENERAL ASSESSMENTS																											
Informed Consent ⁴	X		X																								
Medical & Diabetes Hx and Demographics	X																										
Evaluation of Inclusion / Exclusion	X			X																							
PAP Smear (females) ⁵	X		X-yrly													X					X					X	
Mammogram(females>35) ⁵	X		X-yrly													X					X					X	
Retinopathy Exam ⁵	X		X-yrly									X	X			X					X					X	
Colorectal cancer screen (subj ≥ 50) ⁶	X																										
Physical Exam ⁷	X	X	X	X ⁷	X ⁸	X ⁸	X ⁸	X ⁸	X ⁸	X	X	X	X	X	X	X ⁷	X	X	X	X	X ⁷	X	X	X	X	X ⁷	
QOL			X-q3mo									X	X		X	X				X					X		
Chest X-Ray	X ⁹			X ⁸											X ⁸					X ⁸					X ⁸		
Abdominal US (or MRI if clinically indicated)	X					X ¹⁰																					
ECG	X		X-yrly	X											X					X					X		
Review Medications ¹¹	X	X	X	X	X ⁸	X ⁸	X ⁸	X ⁸	X ⁸	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
AE/Hypoglycemic Events		X	X	X	X ⁸	X ⁸	X ⁸	X ⁸	X ⁸	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
PPD	X		X-yrly												X					X					X		
LOCAL LABORATORY ASSESSMENTS																											
Coagulation (PT, PTT, INR)	X			X ⁸																							
PRA by flow cytometry			X-q3mo													X					X					X	

Time points (days relative to transplant)	SCR	IIT ≥ 4 months	WL ¹		BL ²	0 ³	3	7	14	21	28	56	75	180	270	365	365 post-initial Tx	450	540	630	730	730 post-initial Tx	810	900	990	1095	1095post-initial Tx
Visit Number	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	Y1	16	17	18	19	Y2	20	21	22	23	Y3	
Visit Windows (specified in days)	N/A	N/A	N/A	≤ 4	N/A	N/A	± 3				± 10	± 5	± 14														
Equivalent Wk/Mo/Yr	N/A	N/A	N/A	N/A	N/A	N/A	W1	W2	W3	W4	M2	M2.5	M6	M9	M12	Varies	M15	M18	M21	M24	Varies	M27	M30	M33	M36	Varies	
LOCAL LABORATORY ASSESSMENTS, <i>continued</i>																											
Serology (Hep B, Hep C, HIV, HTLV) ¹²	X		X-yrly													X					X					X	
PSA (males >50 or family history of prostate cancer)	X		X-yrly													X					X					X	
Lipids	X																										
CBC (WBC + Diff & Plat)	X		X-q6mo		X ¹³		X ⁸	X ⁸	X ⁸	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Chemistry ¹⁴	X		X-q6mo	X	X ¹³					X-q1mo ¹⁵							X	X	X	X		X	X	X	X	X	
Pregnancy test (females) ¹⁶	X		X-yrly	X												X					X					X	
Urinalysis	X																										
Blood type & HLA (SCR report may be from kidney transplant; repeat test at each islet transplant)	X			X																							
Crossmatch (repeat test at each transplant) ¹⁷				X																							
Fasting & post-prandial c-peptide ¹⁸						X ⁸	X ⁸																				
Glucose immediately post-transplant ¹⁹					X																						
EBV IgG	X																										
CMV IgG, CMV IgM (repeat if test was negative)			X-yrly													X					X					X	
CENTRAL LABORATORY / METABOLIC ASSESSMENTS																											
First morning spot urine (albumin & creatinine)	X		X-q3mo									X	X	X	X	X	X	X	X	X		X	X	X	X	X	
CMV, EBV by PCR ²⁰			X-yrly	X ²¹						X	X	X	X														
HbA1c	X	X-q1mos ¹⁵								X	X	X	X	X	X ²²	X	X	X	X	X	X	X	X	X	X	X	

Time points (days relative to transplant)	SCR	IIT ≥ 4 months	WL ¹	BL ²	0 ³	3	7	14	21	28	56	75	180	270	365	365 post-initial Tx	450	540	630	730	730 post-initial Tx	810	900	990	1095	1095post-initial Tx	
Visit Number	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	Y1	16	17	18	19	Y2	20	21	22	23	Y3	
Visit Windows (specified in days)	N/A	N/A	N/A	≤ 4	N/A	N/A	± 3				± 10	± 5	± 14														
Equivalent Wk/Mo/Yr	N/A	N/A	N/A	N/A	N/A	N/A	W1	W2	W3	W4	M2	M2.5	M6	M9	M12	Varies	M15	M18	M21	M24	Varies	M27	M30	M33	M36	Varies	
CENTRAL LABORATORY / METABOLIC ASSESSMENTS, continued																											
BKV by PCR ²⁰ (if positive urine, test blood)	X		X-yrly	X ²¹																							
Basal c-peptide ²⁰							X	X	X																		
Fasting serum gluc, c-pep & SCr ²⁰	X		X		X-q1mo ¹⁵											X	X	X	X	X	X	X	X	X	X	X	X
90 min c-pep/ glucose (MMTT) ²³	X											X	X	X	X	X	X	X	X	X		X	X	X	X		
Insulin modified FSIGT ²⁴			X-yrly									X			X	X											
Atherogenic profile ²⁵			X													X					X					X	
LOCAL METABOLIC ASSESSMENTS																											
Glycemic Stability (CGMS)			X-yrly									X	X		X	X		X		X	X				X	X	
BSR eCRFs ²⁶			X-q3mo							X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
CALCULATED METABOLIC ASSESSMENTS																											
MAGE			X-q3mo							X	X	X	X	X	X	X	X	X	X	X	X		X		X	X	
LI			X-q6mo							X	X	X	X	X	X	X	X	X	X	X	X		X		X	X	
HYPO			X-q6mo									X	X	X	X	X	X	X	X	X	X		X		X	X	
Clarke Survey		X	X-q6mo										X		X	X		X		X	X		X		X	X	
CALCULATED METABOLIC ASSESSMENTS continued																											
Beta Score			X							X	X	X	X	X	X	X	X	X	X	X	X		X		X	X	
c-peptide glucose creatinine ratio (CPGCR)	X		X		X-q1mo ¹⁵											X	X	X	X	X	X		X		X	X	
CARDIOVASCULAR ASSESSMENTS																											
Carotid IMT			X-q6mo																							X	
Cardiac Persantine thallium or Stress echo or equivalent	X																										

Time points (days relative to transplant)	SCR	IIT ≥ 4 months	WL ¹	BL ²	0 ³	3	7	14	21	28	56	75	180	270	365	365 post-initial Tx	450	540	630	730	730 post-initial Tx	810	900	990	1095	1095post-initial Tx	
Visit Number	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	Y1	16	17	18	19	Y2	20	21	22	23	Y3	
Visit Windows (specified in days)	N/A	N/A	N/A	≤ 4	N/A	N/A	± 3				± 10	± 5	± 14														
Equivalent Wk/Mo/Yr	N/A	N/A	N/A	N/A	N/A	N/A	W1	W2	W3	W4	M2	M2.5	M6	M9	M12	Varies	M15	M18	M21	M24	Varies	M27	M30	M33	M36	Varies	
RENAL ASSESSMENTS																											
Renal biopsy ²⁷										X																X	
GFR ²⁸	X		X-yrly							X						X					X					X	
NEUROLOGICAL ASSESSMENTS																											
Neurologic History & Exam			X-q6mo													X					X					X	
Nerve Conduction Studies			X-q6mo													X					X					X	
Neurologic Function Testing			X-q6mo													X					X					X	
COST OF CARE ASSESSMENTS																											
Feeling Thermometer														X			X	X	X	X		X	X	X	X		
QWB-SA			X									X	X		X					X					X		
Staff Billing Log	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Subject Resource Questionnaire			X-q3mo									X	X	X	X		X	X	X	X		X	X	X	X	X	
MAINTENANCE IMMUNOSUPPRESSION LEVELS																											
Immunosuppression levels				X	X ⁸	X ⁸	X ⁸	X ⁸	X ⁸	X	X	X	X	X	X		X	X	X	X		X	X	X	X		
MECHANISTIC ASSAYS																											
Alloantibody	X		X									X	X	X	X	X	X	X	X	X			X		X		
Autoantibody (GAD, IA-2, IAA)			X									X	X	X	X	X	X	X	X	X			X		X		
TAT, C3a, c-peptide ²⁹				X	X																						
ARCHIVED SAMPLES																											
Serum			X									X	X	X	X	X	X	X	X	X			X		X		
PBMC & Plasma			X									X	X	X	X	X	X	X	X	X			X		X		
RNA			X									X	X	X	X	X	X	X	X	X			X		X		

Note: Assessments highlighted in the SOE table are not required for IIT randomized subjects. For logistical purposes, the Month 12, Month 24, and Month 36 visits are equivalent to the Y1 post-initial transplant, Y2 post-initial transplant, and Y3 post-initial transplant visits, respectively, for IIT randomized subjects only.

For example, assessments required for M12 and Y1 will be completed one time only.

- 1 WL = Waiting List. Testing is to be initiated once the subject has been placed on WL. Repeat assessments as indicated (i.e. yrly, q3mo), while the subject is on the WL.
- 2 BL = Baseline. Baseline window for islet randomized patients is -2 days relative to transplant. Baseline window for IIT randomized patients is ± 4 days relative to Day = 0.
- 3 Day 0 is the day of islet transplant applicable to both treatment groups. This SOE applies to the 1st, 2nd, and 3rd islet transplant; restarting at Day 0 for each subsequent transplant.
- 4 *Informed Consent #1* includes information specific to screening procedures, but generic to CIT06 study procedures. *Informed Consent #2* includes information specific to CIT06 study procedures. IC#2 must be reviewed and signed before placing on the waiting list.
- 5 Patient to provide reports from appropriate practitioners on a yearly basis throughout study participation.
- 6 FOBT (take-home multiple sample method) or FIT w/in last yr, flexible sigmoidoscopy w/in last 5 yrs, double-contrast barium enema w/in last 5 yrs, colonoscopy w/in last 10 yrs. All positive tests should be followed up with colonoscopy.
- 7 Cardiovascular history and physical to be conducted at BL, Y1, Y2, Y3, in addition to patient physical.
- 8 Assessment does not need to be performed by IIT randomized subjects.
- 9 Chest x-ray report and films will be accepted if taken within one month prior to screening. Baseline report and films will be accepted if taken within one year of Day 0.
- 10 Abdominal US to be performed within 3 days post-transplant in islet randomized subjects only.
- 11 At screening, review medications: immunosuppressive, contraceptives, ACEs, ARBs, anti-hypertensives, and lipid lowering agents. Review changes at subsequent visits.
- 12 Serology includes: HBc Ab, HBs Ab, HBs Ag, HCV Ab, HIV, and HTLV-I/II. Do not repeat Hep B tests if HBs Ab was previously positive.
- 13 Test repeated Days -1, 0, +1, +2 in islet transplanted subjects only.
- 14 Chemistry includes: Na⁺, albumin, Mg²⁺, Cl⁻, K⁺, alk phosphatase, total bilirubin, CO₂, creatinine, ALT(SGPT), BUN, gamma GT, glucose, ST (SGOT), Ca²⁺, phosphorus
- 15 Test to be performed monthly during time period blocked in chart. Visit window for monthly visits is ± 10 days.
- 16 Complete urine or serum pregnancy at screening. Complete serum pregnancy test w/in 72 hours prior to transplant in islet transplant patients only. If urine tests positive at anytime, confirm by serum pregnancy.
- 17 Sample used for crossmatch may be obtained up to 30 days prior to the start of induction therapy, as long as there is no evidence of infections or transfusions since the time the sample was drawn. Crossmatch should be repeated for subsequent islet transplants in islet randomized subjects only.
- 18 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 in islet randomized subjects only.
- 19 Finger stick glucose should be done locally and drawn every hour for the first 6 hours immediately post-transplant in islet randomized subjects only.
- 20 If blood drawn locally, sample should be sent from local lab to study site and then shipped to the central laboratory (Univ of Washington for fasting glucose/c-peptide and SCr; Univ of Alberta for EBV, CMV, BKV by PCR). Testing should be conducted for both treatment groups.
- 21 CMV, EBV, and BKV by PCR immediately prior to the transplant in both treatment groups. Post-transplant testing may be more frequent per standard of care.
- 22 Additional HbA1c test to be performed at month 11 post-transplant in both treatment groups.
- 23 MMTT should include 60 and 90 minute c-peptide and glucose measurements for the screening visit and as necessary when determining islet graft failure. MMTT is not to be conducted after Day 0 on IIT randomized patients.
- 24 FSGT to be performed on the IIT randomized subjects at WL and 365d post-initial transplant
- 25 Atherogenic profile consisting of fasting lipid panel (TG, TC, HDL, LDL), C reactive protein, serum amyloid A, Apolipoprotein A1 and Apolipoprotein B. If blood drawn locally, sample should be sent from local lab to study site and then shipped to the central laboratory (Univ of Washington).
- 26 BSR eCRF is completed using information gathered from subject blood sugar logs, glucometer download data, and insulin requirements (total units/24hrs). BSR eCRF and associated documents will be completed by both treatment groups throughout the study.
- 27 Renal biopsy to be performed 4 ± 2 weeks post-initial transplant and 36 ± 2 months after the initial biopsy in both treatment groups. Biopsy to correspond with initial islet transplant only.
- 28 Post-initial transplant GFR performed 4 ± 2 weeks after each islet transplant in both treatment groups to correspond with an already scheduled visit and again yearly post-initial transplant.
- 29 TAT, C3a, & c-peptide (Innate Immunity): pre-induction AND pre-transplant, 15, 60, 180 min post-tx.

Appendix 2 Reduced Follow-up Schedule of Events

Subjects prematurely discontinued from study treatment according to the criteria stated in section 5.8.3 will remain in the study until normal termination, but for the purpose of monitoring safety and efficacy parameters should be followed according to the reduced follow-up schedule. The day on which the study treatment is discontinued is considered "Day 0". The last reduced follow-up visit will vary depending on when the subject discontinues study treatment and should be done at 3 years after the subject's final transplant.

Days post -discontinuation of study treatment	28	56	75	180	270	365	1 year post-final transplant	2 years post-final transplant	3 years post-final transplant
Visit Windows (specified in days)	± 7						±14		
Equivalent Month	1	2	2.5	6	9	12	Varies	Varies	Varies
ASSESSMENTS FOR ALL SUBJECTS ON REDUCED FOLLOW-UP									
Assess SAEs and hypoglycemic events ¹	X	X	X	X	X	X	X	X	X
PRA by flow cytometry				X			X	X	X
HbA1c							X	X	X
SCr							X	X	X
QOL questionnaires via mail							X	X	X

¹ If subject does not come to the study site for the visit, attempt to obtain information via a phone contact.

Appendix 3 Protocol for Diabetes Management and Education

OUTLINE

1. Pre-transplant management (initiate at least 4 months prior to planned islet transplant)
 - a. History, physical and initial evaluation (endocrinologist & ARNP)
 - b. Implementation of an appropriate basal/bolus insulin therapy [multiple daily insulin injections (MDI) or CSII]
 - c. Subject education (diabetes educator)
 - i. Initial education assessment and survival skills review and reinforcement
 - ii. Basal/bolus therapy and carbohydrate counting
2. Islet transplant vs control group
3. Post-transplant management
 - a. Islet transplant
 - i. Management as per transplant protocol
 - b. Control group
 - i. Continue and reinforce pre-transplant diabetes management

PROTOCOL

1. Pre-transplant management (initiate at least 4 months prior to randomization)
 - a. History & physical and initial evaluation (endocrinologist & ARNP)

At least four months prior to planned islet transplant, study subjects will undergo a complete diabetes evaluation by an endocrinologist. The evaluation will abide by the recommendations of the ADA, as outlined in their yearly report¹. The initial evaluation will focus on assessing their glycemic control, including A1C level, self-monitoring BG results and hypoglycemia frequency and severity. Insulin therapy will be reviewed for effectiveness and appropriateness and glycemic targets will be established with the goal of optimizing glycemic control and minimizing complications of hypoglycemia. In addition, an assessment of diabetes-related complications and co-morbid conditions will be completed, and appropriate recommendations for management of hypertension/nephropathy, lipid abnormalities, and cardiovascular prevention provided to study subjects. Referrals for dilated retinal exam, foot care, cardiovascular risk prevention, and renal management will be issued as recommended by the ADA. In

¹ ADA Position Statement. Standards of Medical Care in Diabetes. Diabetes Care 2005;28:S4-S36.

addition, subject will be referred to the certified diabetes educator for evaluation of their diabetes knowledge, nutritional habits and problem solving skills (see section 1.c. of Appendix 3). At the end of the initial evaluation with the endocrinologist, study subjects will have been assigned a complete diabetes management plan for optimization of diabetes control. They will be scheduled for follow-up visits with the endocrinologist/ARNP team monthly for the first four months and every three months thereafter.

b. Implementation of an appropriate basal/bolus insulin therapy MDI or CSII

After reviewing a subject's insulin schedule a determination will be made regarding the efficacy and appropriateness of both the type of insulin used and the distribution of insulin administration. All subjects in the study will be placed on physiologic insulin replacement through a basal/bolus approach, either via MDI or CSII^{2,3}. MDI will be accomplished through the injection of appropriate basal insulin with a rapid acting insulin analogue administered before meals according to planned carbohydrate intake and ambient BG levels. Subjects on CSII will replace basal insulin requirements through an hourly basal rate (with a rapid-acting insulin analogue), and use a similar insulin dosage algorithm to cover meal-related needs and correct hyperglycemia. Subjects will be expected to perform self BG monitoring at least 4 times/day and to utilize an insulin regimen consisting of at least 3 insulin injections/day (the type of insulin used should be tailored to the individual subject and include currently available insulin analogs). All subjects will be taught how to estimate the carbohydrate content of their meals (in grams of carbohydrates or carbohydrate exchanges) and will be given an insulin-to-carbohydrate ratio to assist them with dosing the appropriate amount of insulin for a specific meal. In addition, all subjects will be given target BG ranges, along with an insulin correction factor (CF) and will be taught how to administer insulin to correct BG values above the upper target range. This will include a target HbA1c of $\leq 6.5\%$, fasting glucose level of < 140 mg/dL and 2-hour postprandial glucose levels of < 180 mg/dL. Target levels can be adjusted as needed based on the development of hypoglycemic episodes. Intensive subject education, which is paramount if optimal diabetes management is to be successful, will be provided as described below (see section 1.c. of Appendix 3).

c. Subject education (diabetes educator)

Study subjects will be referred to a certified diabetes educator for an initial assessment of their diabetes knowledge base, self-monitoring skills, carbohydrate counting ability and

² Meneghini LF. Clinical Endocrinology Update 2003 Syllabus. **Insulin Pump Therapy**; Published by The Endocrine Society, 2003

³ Meneghini LF. Physiologic Insulin Replacement: Insulin Pump Therapy (CSII) vs. Multiple Daily Insulin Injections (MDI). **American Journal of Diabetes** 2004, Vol. 1, No. 1

problem solving skills. The diabetes educator will develop an education plan for each subject and focus on reinforcing areas of weakness for each particular study subject. Subjects with deficiencies in basic diabetes management skills will receive further reinforcement regarding self-monitoring of BG, insulin administration and related techniques and basic nutrition guidelines. Subject will be taught how to correctly estimate the carbohydrate content of various food items and meal, through the use of nutrition labels, reference books, weight scales, and volume measures. Areas of deficiency will be reinforced, and if needed follow-up with a registered nutritionist will be arranged for the appropriate study subjects.

2. Intervention (islet transplant) versus control group (IIT)

Subjects in the study will be randomly assigned to either the intervention group or the control group. The intervention group will undergo islet transplantation according to the study's pre-defined protocol. Following transplantation, diabetes management in the intervention group will be implemented by a diabetologist associated with the islet transplant team. Subjects assigned to the control group will continue to be followed and managed by their initial endocrinologist/ARNP team.

3. Post-transplant management

- a. Islet transplant: Subjects in the intervention group will be followed by a diabetologist associated with the islet transplant team every three months until the completion of the study (See Appendix 1 for SOE). Glycemic control will be assessed and reviewed at every visit, and adjustments made to their glycemic therapies with the aim of achieving near-normal glycemic control, while avoiding unacceptable hypoglycemia. The goals of therapy will continue to be defined according to current recommendations outlined in the ADA's position statement for the management of diabetes. Similar guidelines for insulin therapy will also be applied to subjects with partial function following islet transplantation.
- b. Intensive insulin therapy: Subjects in the control group will continue to be followed and managed according to pre-transplant management every three months until the completion of the study (See Appendix 1 for SOE). Glycemic control will be assessed and reviewed at every visit, and adjustments made to their glycemic therapies, with the aim of achieving near-normal glycemic control, while avoiding unacceptable hypoglycemia. In the course of these clinic visits, concepts of basal/bolus insulin management will be reinforced in order to optimize glycemia and minimize hypoglycemia. Study subjects will be referred for booster education session as needed in order to achieve the best level of glycemic control possible. In essence, pre-transplant diabetes management will continue to be reinforced.

1 Appendix 4 Study Contacts

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Appendix 5 Cardiac Definitions

Definition of a Cardiovascular Death

Unexpected death: Unexpected death presumed to be due to ischemic cardiovascular disease, occurring within 24 hours of the onset of symptoms without confirmation of cardiovascular disease, and without clinical or post mortem evidence of other etiology.

Fatal MI: death within 7 days of the onset of documented MI.

Congestive heart failure (CHF): death due to clinical, radiological or postmortem evidence of CHF without clinical or postmortem evidence of an acute ischemic event (cardiogenic shock to be included).

Death after invasive cardiovascular interventions: death associated with the intervention, *i.e.*, within 30 days of cardiovascular surgery, or within 7 days of cardiac catheterization, arrhythmia ablation, angioplasty, atherectomy, stent deployment, or other invasive coronary or peripheral vascular intervention.

Documented arrhythmia: death due to bradyarrhythmias or tachyarrhythmias not associated with an acute cardiac ischemic event.

Death following non-cardiovascular surgery: death due to cardiovascular causes within 30 days of surgery.

Stroke: death due to stroke occurring within 7 days of the signs and symptoms of a stroke.

Other cardiovascular diseases: death due to other vascular diseases including pulmonary emboli and abdominal aortic aneurysm rupture.

Presumed cardiovascular death: Suspicion of cardiovascular death with supporting clinical evidence that may not fulfill criteria otherwise stated. Example: Patient admitted with typical chest pain of 3 hours duration and treated as an MI, but without ECG and enzymatic documentation to meet usual criteria.

Definition of a MI

The definitions for MI are presented below. If necessary for a definition, prolonged ischemic symptoms must last 20 minutes, and the cardiac enzymes of interest are Troponin T or I and/or serum CK-MB mass.

Q-wave MI: Diagnosis based on the occurrence of a compatible clinical syndrome with prolonged ischemic symptoms, associated with the development of new significant Q waves. Diagnostic elevation of cardiac enzymes will include increase in CK-MB mass to a level > twice the ULN, and/or and increase in Troponin T or I to a level that indicates myonecrosis in the laboratory performing the study.

Non Q-wave MI: Diagnosis based on the occurrence of a compatible clinical syndrome with prolonged ischemic symptoms, associated with elevation of serum enzymes, as for Q-wave MI. Only in the case that both Troponin and CK-MB mass measurements are not available, would the elevation of total CK to greater than or equal to twice the ULN qualify for diagnosis.

Silent (unrecognized) MI: Development of new significant Q waves without other evidence of MI (the date of event will be assigned halfway between the date of discovery and last normal ECG).

Probable non Q-wave MI: Diagnosis based on the occurrence of a compatible clinical syndrome with prolonged ischemic symptoms, without documentation of cardiac enzyme elevation, but associated with the development of new and persistent significant ST-T changes (>24 hr in duration).

MI after cardiovascular invasive interventions: Diagnosis based upon the occurrence of CK-MB (or Troponin) elevations to a level increased 3-5 times normal for the laboratory performing the studies, occurring within 7 days of cardiac catheterization, arrhythmia ablation, angioplasty, atherectomy, stent deployment or other invasive coronary, carotid or peripheral vascular intervention.

MI after coronary bypass graft surgery: Diagnosis based upon the occurrence of CK-MB (or Troponin) elevations to a level increased $\geq$ 5-10 times normal for the laboratory performing the studies, occurring within 30 days of cardiac surgery.

MI after non-cardiovascular surgery: MI (as defined above, occurring within 30 days of non-cardiovascular surgery).

Definition of a Stroke/CVA

Definite ischemic stroke: CT or magnetic resonance imaging (MRI) scan within 14 days of onset of a focal neurological deficit lasting more than 24 hours with evidence of brain infarction (mottled cerebral pattern or decreased density in a compatible location), no intraparenchymal hemorrhage by CT/MRI, no significant blood in the subarachnoid space by CT/MRI or by lumbar puncture, or autopsy confirmation. A nonvascular etiology must be absent.

Definite primary intracerebral hemorrhage: Focal neurological deficit lasting more than 24 hours. Confirmation of intraparenchymal hemorrhage in a compatible location with CT/MRI scan within 14 days of the deficit onset, or at autopsy, or by lumbar puncture.

Subarachnoid hemorrhage: Sudden onset of a headache, neck stiffness, loss of consciousness. There may be a focal neurological deficit, but neck stiffness is more prominent. Blood in the subarachnoid space by CT/MRI or lumbar puncture or intraventricular by CT/MRI.

Stroke of unknown type etiology: Definite stroke of unknown etiology when CT, MRI, or autopsy is not done. Information is inadequate to diagnose ischemic (infarction), intracerebral hemorrhage, or subarachnoid hemorrhage.

Non-fatal stroke after cardiovascular invasive interventions: Stroke associated to the intervention within 30 days of cardiovascular surgery, or within 7 days of cardiac catheterization, arrhythmia ablation, angioplasty, atherectomy, stent deployment or other invasive coronary or peripheral vascular interventions.

Non-fatal stroke post non-cardiovascular surgery: Stroke occurring within 30 days of non-cardiovascular surgery.

Other Cardiovascular Outcomes

All cardiovascular revascularization procedures, including:

- PTCA (balloon)
- PTCA with stent
- CABG
- Carotid angioplasty with stent
- Carotid endarterectomy
- Peripheral angioplasty with or without stent
- Peripheral vascular surgery (including aortic aneurysm repair)
- Limb amputation: including partial or digit amputation due to vascular disease.