Multicenter Trial of the effect of AAT on Islet Transplant Engraftment and Durability after Renal Transplant

Study Sponsors: NIDDK

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Protocol Approval

Protocol Number:	**Version/Date:** 2.0 / 07MAR2017
IND: BB-IND 13804	**Principal Investigators:** Andrew Posselt, MD, PhD; Ali Naji, MD, PhD ; James Markmann, MD, PhD
Short Title: *Efficacy of AAT in Islet after Kidney Transplantation*	
Study Sponsors: 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
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I confirm that I have read the above protocol in the latest version. I understand it, and I will work according to the principles of 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, "Efficacy of AAT in 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 sponsor.

_____
Site Principal Investigator (Print)

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

Protocol Synopsis

Title	**Multicenter Trial of the effect of AAT on Islet Transplant Engraftment and Durability after Renal Transplant**
Short Title	Efficacy of AAT in Islet after Kidney Transplantation
Clinical Phase	Phase 2
IND Sponsor	Dr. James Markmann
IND Number	BB-IND 13804
Activation Date	Proposed 12/2016
Accrual Objective	20 transplanted (up to 28 transplant eligible)
Accrual Period	3 years
Study Duration	5 years (Accrual plus follow-up)
Study Design	Prospective, multi-center, trial assessing the benefit of alpha-1 antitrypsin (AAT) on islet transplantation in type 1 diabetic (T1D) kidney transplant recipients.
Treatment Description	Patients meeting the study entry criteria will receive 1-3 infusion(s) of in vitro cultured islets. Patients will receive three times a week AAT infusions in the peri-transplant period for three weeks. For the first islet transplant, patients will receive induction therapy with rabbit anti-thymocyte globulin (ATG, 6 mg/kg total in 5 doses) plus etanercept and will remain on their calcineurin-based maintenance immunosuppression regimen already in place for their renal allograft. Induction therapy will be basiliximab instead of ATG for 2nd and 3rd transplants, if applicable.

Primary Endpoints

The proportion of GLASSIA versus control subjects from CIT-06 achieving insulin independence at day 75 after the first infusion of single donor islets.

Secondary Endpoints

Insulin Utilization

- The proportion of GLASSIA treated versus control CIT-06 subjects who are insulin independent after 1 or more islet infusions at:
    - 1 year after the first islet infusion
    - 1 year after the last islet infusion
    - 2 years after the first islet infusion
    - 2 years after the last islet infusion

- Percent change from baseline insulin requirement comparing GLASSIA treated versus control CIT-06 subjects at Day 75, 1 year and 2 years following the first and last islet transplant(s)

Islet Mass

The relative functional engrafted islet mass comparing GLASSIA treated versus control CIT-06 subjects using FSIGT testing at:

- Day 75 after the first islet transplant
- 1 year after the first islet transplant
- 2 years after the first islet transplant

- Correlation of markers of early islet loss as assessed by the rapid release of thrombin-anti thrombin complexes (TAT), C3 and c-peptide, and insulin specific DNA with FSIGT and clinical markers of islet function (HbA1c, insulin independence rates, duration of islet function and insulin independence)

- The proportion of GLASSIA treated versus CIT-06 control subjects with both an HbA1c ≤ 6.5% AND an absence of severe hypoglycemic events:
    - From Day 28 to Day 365 after the first islet transplant
    - From Day 28 to Day 730 after the first islet transplant

- The proportion of GLASSIA treated versus control subjects with both an HbA1c < 7.0% AND free of severe hypoglycemic events:
    - From Day 28 to Day 365 after the first islet transplant
    - From Day 28 to Day 730 after the first islet transplant

- The proportion of GLASSIA treated versus control CIT-06 subjects A reduction in HbA1c of 1 point AND an absence of severe hypoglycemia from:
    - From Day 28 to Day 365 after the first islet transplant
    - From Day 28 to Day 730 after the first islet transplant

- Number of severe hypoglycemic events comparing GLASSIA treated versus control CIT-06 subjects at 1 year and 2 years following the *first* and *last* islet transplant(s)

- HbA1c comparing GLASSIA treated versus control CIT-06 subjects at 1 year and 2 years following the *first* and *last* islet transplant(s)

Glycemic Control

- The change in Clarke score from baseline in GLASSIA treated versus control CIT-06 subjects at:
    - 1 year after the first islet transplant
    - 2 years after the first islet transplant

- β-score comparing GLASSIA treated versus control CIT-06 subjects at 1 year and 2 years following the first and last islet transplant(s)

- Glucose variability and hypoglycemia duration derived from the CGMS comparing GLASSIA treated versus control CIT-06 subjects at 1 year and 2 years following the first and last islet transplant(s)

- Basal (fasting) and 90-min glucose and c-peptide derived from the mixed-meal tolerance test (MMTT) comparing GLASSIA treated versus control CIT-06 subjects at 1 year and 2 years following the first and last islet transplant(s)

- MAGE comparing GLASSIA treated versus control CIT-06 subjects at 1 year and 2 years following the first and last islet transplant(s)

- Glycemic lability index (LI) comparing GLASSIA treated versus CIT-06 control subjects at 1 year and 2 years following the first and last islet transplant(s)

- Ryan hypoglycemia severity (HYPO) Score comparing GLASSIA treated versus control CIT-06 subjects at 1 year and 2 years following the first and last islet transplant(s)

- C-peptide: (glucose: creatinine) ratio (CPGCR) comparing GLASSIA treated versus control CIT-06 subjects at 1 year and 2 years following the first and last islet transplant(s)

- Rate of favorable outcome at each center preparing islets (rate of subjects with HbA1c $\leq$ 6.5% and absence of severe hypoglycemic events from Day 28 to Day 730, or reduction in HbA1c of 1 point and absence of severe hypoglycemia from Day 28 to Day 730)

Repeat Transplants

- The proportion of subjects receiving a second islet transplant comparing GLASSIA treated versus control CIT-06 subjects at 1 year and 2 years following the first and last islet transplant(s)

- The proportion of subjects receiving a third islet transplant comparing GLASSIA treated versus control CIT-06 subjects at 1 year and 2 years following the first and last islet transplant(s)

Other Efficacy Endpoints

- Cardiovascular events [death, cerebrovascular accident (CVA), myocardial infarction (MI)] and changes in atherogenic profile for GLASSIA treated versus control subjects at 1 year and 2 years following the first and last islet transplant(s)

- Renal impact measures including renal allograft survival and function measured by serum creatinine (SCr) and urinary albumin creatinine ratio comparing GLASSIA treated versus control subjects at 1 year and 2 years following the first and last islet transplant(s)

Exploratory Mechanistic Endpoints

- Assessment of the effect of AAT on serum cytokines released in the early post transplant period (1 month) comparing GLASSIA versus control subjects from CIT-06
- Assessment of the effect of AAT on the inflammatory gene transcriptional profile of PBL at day 14 and 75 comparing pre-and post transplant samples
- Correlation of reaching target AAT levels with metabolic outcomes
- Histological survival of subcutaneous islets in subcutaneous auxiliary graft and correlation with overall graft survival

Inclusion Criteria

Subjects who meet all of the following criteria are eligible:

1. Male and female subjects age 18 to 70 years.

2. Subjects who are able to provide written informed consent and to comply with the procedures of the study protocol.

3. Subjects must have one of the following payment mechanisms in place:
    a. Medicare,
    b. A third-party insurer who agrees, via pre-authorization, to pay for participation in the study, or
    c. Another mechanism of payment (self-pay, hospital, university, donations, etc.) for participation in the study.

4. Clinical history compatible with T1D with disease onset < 40 years of age and insulin-dependence for ≥ 5 years at the time of enrollment.

5. 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.

6. Subjects who are ≥ 3 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] ± Prednisone ≤ 10 mg/day).

7. Stable renal function as defined by a 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.

8. Subjects who meet one of the options in the following criterion are eligible for transplantation:

    - Reduced awareness of hypoglycemia manifested by a Clarke score of 4 or more measured upon study enrollment and at least one episode of severe hypoglycemia in the 12 months prior to study enrollment.

    OR

    - A subject must have a reduced awareness of hypoglycemia manifested by a Clarke score of 4 or more and at least 1 episode of severe hypoglycemia;

    OR

    - Any subject not meeting the hypoglycemia option must have an HbA1c $\geq$ 7.5%.

Exclusion Criteria

Subjects who meet any of these criteria are not eligible:

1. Weight more than 90 kg or body mass index (BMI) > 30 kg/m^2.

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

3. Other (non-kidney) organ transplants except prior failed pancreatic graft where graft failure is attributed to thrombosis within the first 4 weeks or to other technical reasons that require graft pancreatectomy; with the graft pancreatectomy occurring more than 6 months ago.

4. Untreated or unstable proliferative diabetic retinopathy.

5. Blood Pressure: SBP > 160 mmHg or DBP >100 mmHg despite treatment with antihypertensive agents.

6. Calculated GFR of ≤ 40 mL/min/1.73 m^2 using the subject's measured serum creatinine and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation[1]. Strict vegetarians (vegans) will be excluded only if their estimated GFR is ≤ 35 mL/min/1.73 m^2.

7. Proteinuria (albumin/creatinine ratio or ACr > 300mg/g) of new onset since kidney transplantation.

8. Calculated panel-reactive anti-HLA antibodies > 50%. Subjects with calculated panel reactive anti-HLA antibodies ≤ 50% will be excluded if any of the following are detected:
    - Positive cross-match,
    - Islet donor-directed anti-HLA antibodies detected by Luminex Single Antigen/specificity bead assay including weakly reactive antibodies that would not be detected by a flow cross-match, or
    - Antibodies to the renal donor (i.e. presumed de novo).

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

10. Presence or history of active infection including hepatitis B, hepatitis C, HIV, or tuberculosis (TB). Subjects with laboratory evidence of active infection are excluded even in the absence of clinical evidence of active infection.

11. Negative screen for Epstein-Barr virus (EBV) by IgG determination at time of screening or previous kidney transplant.

12. Invasive aspergillus, histoplasmosis, and coccidoidomycosis infection within the last year.

13. Any history of malignancy except for completely resected squamous or basal cell carcinoma of the skin or other cancers for which the patient is at least 5 years post curative therapy where a very high likelihood (>99%) that cure has been achieved, such as in certain types of low grade thyroid cancer or early stage breast cancer.

14. Known active alcohol or substance abuse.

15. Evidence of Factor V Leiden mutation or other laboratory evidence of hypercoagulable state.

16. Any coagulopathy or medical condition requiring long-term anticoagulant therapy (e.g. warfarin) after islet transplantation (low-dose aspirin treatment [325 mg PO] is allowed) or subjects with international normalized ratio (INR) > 1.5. The use of Plavix is allowed only in conjunction with mini-laparotomy procedure at the time of islet transplant.

17. Severe co-existing cardiac disease, characterized by any one of these conditions:
    - Recent MI (within past 6 months);
    - Evidence of ischemia on functional cardiac exam within the last year;
    - Left ventricular ejection fraction < 30%; or
    - Valvular disease requiring replacement with prosthetic valve.

18. 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. Active pancreatitis.

21. Active peptic ulcer disease, symptomatic gallstones, or portal hypertension.

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

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

24. Any medical condition that, in the opinion of the investigator, will interfere with the safe participation in the trial. (Cancer screenings should be performed per current American Cancer Society guidelines).

25. Positive screen for BK virus by polymerase chain reaction (PCR) performed at time of screening.

26. A kidney transplant patient with type 1 diabetes who has an HbA1c < 7.5 and no history of severe hypoglycemia.

27. Selective or severe IgA deficiency (levels < 5-7 mg/dL)

28. AAT deficiency (defined as plasma level of AAT < 8 μM-50 mg/dL)

Table of Contents

Glossary of Abbreviations

AAT	Alpha-1 antitrypsin
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 Leukocyte Antigen
hOKT3γ1	Anti-CD3 Monoclonal Antibody ala-ala
HSA	Human Serum Albumin
HTK	Histidine-tryptophan-ketoglutarate
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	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
µg	Microgram (10^{-6} gram)
µmole	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
PCP	*Pneumocystis carinii* 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
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-	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

Study Definitions

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

Graft failure: Islet allograft failure will be defined as absence of insulin production by transplanted islets, as evidenced by c-peptide < 0.3 ng/mL. This will be determined by (1) c- peptide <0.3 ng/mL on random testing, followed by (2) c-peptide <0.3 ng/mL at baseline, and at 60 and 90 minutes after MMTT. Participants with graft failure do not need to complete the day 75 metabolic assessments.

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

- One HbA1c level, one fasting serum glucose level, and a Mixed Meal Tolerance Test are documented within the visit window (e.g. 70-80 days at Day 75) and 7 consecutive days of blood sugar and insulin readings are documented within +/- 7 days of the visit window (e.g. 63-87 days at Day 75;
- HbA1c ≤ 6.5% or a ≥ 2.5% decrease from baseline (within 91 days prior to transplant);
- Fasting capillary glucose level should not exceed 140 mg/dL more than three times in 7 consecutive days (fasting is defined as 1st blood sugar reading of the day not noted as post-prandial or bedtime;
- Post-prandial serum glucose ≤ 180 mg/dL at 90 minutes during the MMTT;
- Fasting serum glucose level ≤ 126 mg/dL; if the fasting serum glucose level is > 126 mg/dL, it must be confirmed in an additional one out of two measurements;
- At least one MMTT fasting or stimulated c-peptide ≥ 0.5 ng/mL.

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

Intensive diabetes management: Self-monitoring of glucose values no less than a mean of three times each day averaged over each week and by the administration of three or more insulin injections each day or insulin pump therapy.

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

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

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

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

Treatment failure: Participants with graft failure after their second islet transplant.

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

Wait list (Inactive): Protocol eligible participants will be initially considered 'wait list inactive' while all wait list assessments required to confirm islet transplant eligibility are being completed and results are obtained.

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 are still at risk of a spectrum of microvascular and macrovascular pathologies due to the imperfect glycemic control provided by even the most modern insulin therapy regimens[2]. 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 expenditures[3].

Convincing data exist to document the close association of the degree of glycemic 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 control[4-6]. 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-peptide[5]. Unfortunately, the attempt at rigorous control was associated with a marked increase in hypoglycemic events and related morbidity[7].

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[8,9]. 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 < 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.

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 control[10-12]. 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 neuropathy[13-17].

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[18,19]. 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 Grussner[20] in

response to the report of Venstrom et al[18]. 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.

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 2000[21]. 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 worldwide[22]. 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 achieved[23-28]. The success of islet transplantation in the prior decade provided the foundation for seeking FDA approval for islets as a standard of care cellular therapy for Type I diabetic patients with labile disease manifest by either poor control on optimal therapy or with life threatening hypoglycemia. Phase 3 trials currently being conducted by the Clinical islet transplant consortium CIT are in their final phases, and have the potential to secure FDA approval of islets as a standard therapy in the near future.

In these trials and others, success is highly dependent on securing a sufficient engrafted islet mass to sustain long term normoglycemia. Improvements in donor selection along with better standardization of islet manufacturing now allow a high degree of assurance that a selected donor pancreas will yield a transplantable preparation of islets. The successful infusion rate of islets has been consistently 40-50% in the Edmonton protocol era and about the same in the CIT trial. With this background, our focus has shifted to improving the fate of islets early after transplantation in vivo with the expectation that this will also improve the long term durability of function.

Loss of Islet Mass in the Peritransplant Period

Davalli et al first reported in a quantitative manner that the majority of transplanted islets are lost almost immediately post-transplant[29]. This occurs in both the autograft and allograft setting and in both renal subcapsular and intraportal sites. The mechanisms of early islet loss are not fully understood but inflammatory, coagulation, and complement pathways have been implicated in the process which has been recently termed, an immediate blood islet inflammatory response (IBMIR)[30]. IBMIR has been monitored by detection of thrombin-anti-thrombin complexes (TAT) and C3 levels which rise abruptly during the first hour after the islet infusion. Early islet damage has also been detected with imaging studies and the extent of islet loss is as high as 70-90% in some studies[29, 31-32].

Experimental therapies successful at preventing early islet loss have helped to define the key mediators and pathways involved, even though not all are easily targeted clinically. Embolization of isolated islets into the portal system sets up the unnatural condition of a damaged tissue being placed directly into intravascular space[33]. In mice, islet infusion into the liver leads to occlusion of terminal portal venules and causes areas of hepatic ischemia and infarction[34] and in humans a rise in LFTs signifying hepatocyte injury and may suggest events similar to those in mice[35]. Local stagnation of portal blood further reduces the oxygen tension of an already hypoxic environment, thereby exacerbating islet and surrounding liver ischemia and injury[35]. Islet and liver injury along with inflammatory mediators expressed by islets post isolation injury (tissue factor, monocyte chemoattractant protein-1 (MCP-1) and C5 receptor)[36] and/or surrounding ischemic liver associated danger signals lead to innate immune system activation and to triggering coagulation, activation of platelets and complement[37]. Down regulation of tissue factor improves engraftment[38-39], as does a complement inhibitor specifically targeting C5[40-41], a potent inflammatory stimulus. Toll receptor triggering on islets appears to be a pivotal innate signal as TLR4-TLR2 deficient islets fare better[42] and survival is improved by targeting a natural ligand for TLR2 and TLR4, HMGB1, a key alarmin secreted by injured islets[43].

A variety of anticoagulation approaches, LMW-dextran[44], islet surface heparinization[45], Activated

protein C[46], thrombomodulin[47-48], and islet coating with PEG-urokinase[49] have all been found to increase engrafted mass. Neutralization of potent inflammatory cytokines such as TNFα and IL-1 has also proven beneficial in experimental islet models[50-51]; as a result, blockade of TNFa signaling with a fusion protein of soluble TNFr-Fc (Etanercept) has become standard in clinical islet transplantation[52-54] and targeting IL-1 is under intensive clinical study by a number of groups[55-56]. Some success has also been achieved by targeting more proximal events inciting tissue injury, with free radical scavengers and by attempting to prevent apoptosis of transplanted islets with any of a variety of agents (inhibitors of caspases[57], JNK[58], XIAP[59], and IKK-beta[60], and A20 expression)[61].

Alpha-1 antitrypsin (AAT), a 52 Kd glycoprotein acute phase reactant and serum serine protease inhibitor that is released during injury and inflammation[62]. It is the most abundant proteinase in the serum with normal levels of 1.5-3.5 ng/ml that increase ~twofold with inflammation. A number of human serum derived AAT preparations are approved for use in patients with functional AAT deficiency[63]. AAT exhibits diverse anti-inflammatory properties by blocking inflammatory mediators such as IL-1, IL-6, IL-8 and TNFa and leads to increases in natural anti-inflammatory proteins IL-10 and IL-1rA and may block complement activation and DC maturation. In vitro, AAT has demonstrated cytoprotective effects on islets[64]. Interest in applying AAT to promote islet graft survival was spurred by studies by Dinarello showing that AAT treatment prolonged mouse islet allograft survival[65-66]. The benefit of AAT was limited by rapid development of mouse anti-human AAT (hAAT) antibodies that defeated the activity of AAT. In subsequent studies, antibody development was avoided using mice tolerant to hAAT by low level hAAT transgene expression. When the immune response to the xeno-protein was eliminated, the beneficial effect of AAT on islet allograft survival was pronounced; in fact, with AAT treatment initiated on d-1 and extending to 14 to 30 days in the absence of any other therapy, not only did allogeneic islet grafts survive long term, but also, donor strain tolerance was achieved[66]. Analysis of tolerant mice found islets to be rich in Tregs and to contain high levels of TGF-β, IL-10, and IL-1 but low expression of inflammatory mediators by RT-PCR. There was also evidence for preserved IL-2 activity in the presence of suppressed inflammation, a setting speculated to be conducive to spontaneous tolerance.

Koulmanda et al have recently investigated the molecular basis of the effect of AAT on islet engraftment using a syngeneic islet transplant model[67]. Transplanting a marginal islet mass that reverses diabetes in only 10-25% of untreated mice, the authors found that short-term AAT treatment resulted in normoglycemia in 100% of hosts. Transcriptional profiling of grafts at day 3 and gene autology and the ingenuity pathway analysis found AAT treated grafts to have down-regulated innate immune inflammatory pathways including IL-6, IL-8, IL-10, chemokine signaling, DC maturation and cross-talk between DC and NK cells; key molecular hubs of action included inflammatory molecules TLR, IFN, TNF-α and NF-κB and chemokines/interleukins.

Also relevant to the application of AAT in clinical islet transplantation is the immunomodulatory effects observed in the context of experimental autoimmune diabetes in NOD mice. When a brief course is administered to recent onset diabetic NOD mice, diabetes is reversed and euglycemia restored in 21 of 24 mice for the duration of the experiment[68-69]. Moreover, the authors demonstrated that autoimmunity to islets had been eliminated; after rendering treated mice diabetic again (chemically) newly transplanted islet isografts survived without recurrent autoimmunity, providing provocative evidence that AAT treatment allowed self-tolerance to islet autoantigens to be reset.

Also relevant to our proposal is the recent report by Koulmanda et al that AAT administration in an NHP islet auto transplant model can dramatically alter the outcome of the graft. In this work, cynomolgus monkeys underwent a 70-80% partial pancreatectomy and the residual islets were ablated with streptozotocin. Islets recovered from the removed segment provided a marginal mass for transplantation of only 1600-4100 IEq/Kg. In untreated controls, islet function was gradually lost at 3-6 months post transplant by non-immunological causes (perhaps exhaustion) with glycemic instability and falling c-peptide levels. In contrast, recipients treated similarly but who received AAT 60 mg/kg on d-1,3,7 and 14 manifest normoglycemia for >700-1200 days with none reverting to hyperglycemia. The transcriptional signature of treated versus controls at the time function was being lost in the controls

was also informative. Using pathway and gene network analysis, critical hubs of interest included NFkB, AKT and TGFb.[70]

With these data as a foundation, a number of trials have been initiated treating new-onset diabetics with AAT in an attempt to ameliorate the accompanying inflammatory lesion and potentially reestablish self-tolerance. The first is a pilot (RETAIN Part I) being conducted by our collaborator (co-investigator) on the study proposed herein (Dr. Mario Ehlers with the ITN) who treated 8 pediatric and 8 adult new-onset type I diabetic patients. They used a treatment regimen beginning with a dose of 45 mg/kg dosed weekly for 6 weeks, followed by a 3-week washout period and then increased the dose to 90 mg/kg/week for 6 weeks (for a total of 12 weekly infusions). Potentially important in the design of the trial is the analysis by the ITN mechanistic core, which revealed that inhibition of inflammatory cytokines and modulation of NFκB and apoptotic pathway genes occurred at blood levels that were attained with the 90 mg/kg dose but not the 45 mg/kg dose; moreover, the 90 mg/kg dose was safely administered without any serious AEs. Metabolic data at 12 months (stimulated C-peptide secretion, insulin use, and HbA1c) are stable and show preservation of C-peptide responses at 1 year relative to baseline. Based on further analysis of mechanistic and PK data, they concluded that a dose in the range of 90-180 mg/kg/week will be optimal in terms of the plasma concentrations attained and the duration of drug levels above the threshold for 50% inhibition of cytokine and NFκB responses.

Gottlieb et al studied AAT treatment in a phase I open label trial in 12 recent onset Type I diabetic patients treated with 8 consecutive weekly infusions of 80 mg/kg. That 5 of the 12 patients manifest sustained c-peptide responses at 12 ad 18 months was viewed as encouraging data. I addition, mechanistic analyses revealed reduced levels of TLR induced IL-1b expression in monocytes and DCs at 12 months post AAT treatment[71].

Also noteworthy is that there are two corporate-sponsored trials of AAT in new-onset T1D that have recently started recruiting including: one study sponsored by Grifols Therapeutics Inc., which the investigators will explore doses up to 180 mg/kg/week (NCT02093221) and the second by Kamada Ltd., which will explore doses up to 120 mg/kg/week (NCT02005848).

A more recent analysis studied AAT treated bone marrow transplant recipients with steroid refractory GVHD, Marcondes et al utilized a modified every other day regimen of AAT (GLASSIA) that achieved stable serum AAT levels in the desired target range[72]. Their regimen consisted of a 90 mg/kg load followed by 60 mg/kg every other day for 8 doses. We have adopted a modified version of this regimen for our trial that contains a total weekly dose in the Grifols trial of 120 mg/kg/wk.

Based on the above findings, we contend that the diverse inflammation targeting properties of AAT may simultaneously impact critical impediments to islet transplantation – alloimmunity, autoimmunity, and innate/inflammation induced early post-transplant islet loss. Given that in addition to the study patients noted above, AAT has been administered safely long term to large numbers of patients as replacement therapy in the setting of AAT deficiency[73-74], and has proven highly safe even in immunosuppressed post-lung transplant patients, in conjunction with the fact that it is readily commercially available, makes the agent an exciting therapy to improve islet transplant success.

In the current trial, we will capitalize on data from the CIT-06 trial (with permission of the CIT steering committee) that has completed enrollment of 24 IAK patients and has now passed the 1 year primary endpoint. Since the proposed cohort to be treated with AAT is otherwise similar in terms of recipient selection, islet manufacturing, medical management and post transplant follow-up, we will have a large carefully studies control cohort that will maximize trial efficiency and power to detect meaningful increases in islet mass manifest by single donor insulin independence rates at day 75. Since the day 75 single donor insulin independence rate in CIT-06 was relatively low (and nearly identical to the IAK experience in the CITR registry) at 17%, meaningful increases in engrafted mass gained by AAT therapy should be readily detectable in our study.

Use of an auxiliary islet graft in the subcutaneous tissue to monitor islet histopathology
To date, intraportal infusion has proven to be the only consistently effective site for clinical islet transplantation. There are a number of theoretical and practical disadvantages of this site including bleeding and clotting risks. Equally problematic is that biopsy of liver engrafted islets is difficult; only a fraction of cores capture islets and when islets are seen they are generally few in number. In addition, a percutaneous liver biopsy carries a small but significant risk (bleeding, AV fistula, organ injury). However, for all other organ transplants, histopathology is the definitive diagnostic modality.

To develop a more clinically feasible approach to islet biopsy we will take advantage of recent remarkable findings by Naji et al that islets readily engraft and function in the subcutaneous tissue in rodents and NHP's when co-transplanted with collagen. We thus plan to transplant a small fraction of the total islet mass (~2-3%) into the subcutaneous tissue at the time of portal infusion into at least 4 discrete locations marked by a suture or surgical clip. Since we plan to transplant islets via mini-laparotomy, this will not add significant morbidity or risk to the procedure. By marking the location of sites with radio-opaque clips, we will be able to biopsy the site under local anesthesia in the OR at any time post transplant. If 8-10,000 islets of a 400,000-500,000 islet preparation are placed in this location (2000-2500 islets per pocket in 0.5 ml of collagen emulsion (Cosmoderm)); the auxiliary graft will readily allow us to obtain a sizeable biopsy of hundreds to thousands of islets for microscopic evaluation rather than an occasional one or two islets seen with liver core biopsy.

Biopsy of the auxiliary graft will be performed at 1 year post initial islet infusion or at the time of any second islet infusion at laparotomy and at the time of onset of graft dysfunction.

The pilot use of the subcutaneous site may not only prove invaluable in discerning immune vs. non-immune injury, but also will provide preliminary data to substantiate the clinical relevance of this site and potentially leading to justification for later evaluation of transplantation of the entire islet mass to this site (not within the scope of the current protocol). One potential pitfall of the auxiliary site is the theoretical possibility of discordant events between subcutaneous position and the islets in the liver. To evaluate this, at the time of open procedures for second doses, we will perform core liver biopsies (if liver exposure is easy/safe) to allow correlation of the pathology present in the two sites.

A second theoretical concern is the possibility that the subcutaneous site is more immunogenic than the portal site, potentially increasing the risk of rejection of islets at both sites. This risk is assumed to be low but impossible to calculate precisely. The patients will be informed of this risk and will be given the option to not participate in this portion of the trial.

1.2 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 obligated to chronic life-long immunosuppression. Thus, the nominal risk of the islet transplant procedure itself is minimal compared to the risks for non-uremic patients with diabetes receiving new immunosuppression in addition to the transplant procedure[75-77]. 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[78-80].

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 clinician. Such management should consist of a target HbA1c levels of < 6.5% as suggested by the ACE Consensus Statement on Guidelines for Glycemic Control[8]. Following a period of ≥ 4 months on this therapy, only patients who evidence continued inadequate glucose control characterized by either HbA1c ≥ 7.5% or HbA1c < 7.5% but with severe hypoglycemic episodes (≥ 1 severe episode) as defined by the DCCT trial (and Clarke hypoglycemia score > 4) will be considered for transplant. This approach will target the group of post-renal transplant diabetics most likely to benefit from islet transplantation.

1.3 Rationale for Selection of Study Treatment Regimen

1.3.1 Investigational Product: Alpha 1 Antitrypsin (AAT) (GLASSIA)

AAT has been found to have potent anti-inflammatory and immunomodulatory properties in small and large animal studies and has been found to promote islet engraftment and survival in small and large animal experiments. It is approved for use in treating patients with congenital AAT deficiency. GLASSIA will be administered 90 mg/kg IV prior to transplant and the 30mg/kg dose repeated Monday/Wednesday/Friday through day 21 post-transplant.

1.3.2 Investigational Product: Allogeneic Islets

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 profile[23-28]. 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 evaluation. Donor suitability must conform to the standards established in the U.S. guidance's (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 perfluorocarbon[46].

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 component[47] and is identified by the CIT (Clinical Islet Transplant Consortium).Islets will be cultured for ≤ 72 hours prior to transplantation. The final product formulation is presented in Table 2.

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).

1.3.3 Immunosuppressive Medications for Initial Islet Transplant

1.3.3.1 Rabbit Anti-Thymocyte Globulin (Thymoglobulin®)

Rabbit ATG is a polyclonal antibody preparation that depletes T and B cells following IV administration[28, 81-85]. 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 on day -2 relative to the planned islet transplant by assessment of T cell depletion by CD3 (total T lymphocyte) counts measured after administration of the first ATG dose. If lack of efficacy is detected, basiliximab (Simulect®) can be substituted for rabbit ATG using the

regimen detailed below for subsequent transplants. Methylprednisolone, acetaminophen, pentoxifylline, and diphenhydramine will be administered as premedications 1 hour prior to the first dose of rabbit ATG.

1.3.3.2 Methylprednisolone (Solumedrol®)

Methylprednisolone is a synthetic glucocorticoid with potent anti-inflammatory 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 as needed through the first ATG infusion only (i.e. on day -2). Patients will not receive any additional doses of this drug.

1.3.3.3 Basiliximab (Simulect®)

Basiliximab is a chimeric (murine/human) monoclonal antibody (IgG1k) approved by the Food and Drug Administration (FDA) for prophylaxis against acute organ rejection in adult recipients of renal allografts. With subsequent islet transplants and second attempts at initial transplants, as a substitute for rabbit ATG, basiliximab may be administered (20 mg IV) on Days 0 and 4.

1.3.3.4 Etanercept (Enbrel®)

Etanercept is an engineered soluble tumor necrosis factor (TNF) receptor-Fc that blocks TNF binding and reduces inflammation[86-91]. 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.

1.4 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 transplantation. Long-term insulin independence, however, is inferior, and a high proportion of islet-alone patients have required reinstitution of small doses of insulin over time[23]. Recently reported and unpublished preliminary data from US centers performing IAK transplants suggest that under Edmonton-like immunosuppression, islets transplanted even to chronically immunosuppressed individuals may experience a similar fate[36].

The etiology of the inexorable loss of islet mass using the Edmonton regimen has yet be explained but allo and autoimmunity have been variable implicated as have immunosuppression medication toxicity and islet exhaustion. Informative in this regard are experiments in humans and animals using syngeneic islets, a case in which immune destruction is excluded. In human islet autotransplantation in the setting of chronic pancreatitis, insulin independence is often lost over time and is associated with transplantation of a marginal islet mass. This has led to the conclusion that a marginal islet mass may deteriorate over time due to metabolic stress induced exhaustion. Similar conclusions have been reported in experimental small and large animal systems (as detailed above). A relevant example is the recent study by Koulmanda et al in which a marginal mass of islets recovered by partial pancreatectomy failed over time despite the absence of allo and autoimmunity. A brief course of AAT effectively stabilized insulin independence presumably by decreasing early islet loss thereby increasing the functional mass of engrafted islets [70].

Given that AAT is FDA approved (for use in humans congenitally deficient in AAT) and that it has a proven safety record over 20 year period, we found AAT as an ideal candidate for application in human islet transplantation with the goal of increasing the engrafted mass and stabilizing long term function by

avoiding marginal mass induced exhaustion.

We have paired GLASSIA with what has become the standard for immunosuppression in islet transplantation based on the recent work of the CIT and the seminal studies of Hering et al who reported what are arguably the most successful series of islet-alone transplants using potent induction immunosuppression and high quality islets. In a series of 10 consecutive patients treated with rabbit ATG induction therapy (6 mg/kg total dose), 9 became insulin independent with a single infusion of islets[92]. 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.

Based on these data, the CIT adopted the regimen based on Thymoglobulin induction combined with a brief course of Etanercept 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. It is well recognized that TNF-α and TNF-β play multiple roles in the development and function of the immune system and have pleomorphic regulatory effects on the development and expression of autoimmunity[93]. Blockade of TNF in the neonatal period results in a dramatic increase in the levels of CD4+CD25+ regulatory T cells in NOD mice[94, 95].

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 cells[95]. In murine models, selective inhibition of TNF-α in the peritransplant period has promoted reversal of diabetes after marginal-mass islet isografts[96]. Temporary etanercept administration has previously been studied in globally immunosuppressed kidney[89] and bone marrow transplant recipients[87, 88]. In renal transplant recipients, etanercept was combined with depleting T cell antibodies (hOKT3γ1 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 hOKT3γ1 ala-ala and ATG administration.

Compared with the hOKT3γ1 ala-ala trial[26], 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 trial[92] had a significantly higher acute c-peptide response to arginine (ACRArg) on days ≥ 180 post-transplant: 1.07 ± 0.15 ng/mL (vs. 0.74 ± 0.21 ng/mL in the hOKT3γ1 ala-ala trial[26]; p=0.028). This improvement occurred despite transplantation of fewer islets: 7,271 ± 1,035 IEq/kg (vs. 10,302 ± 2,594 IEq/kg in the hOKT3γ1 ala-ala trial; p=0.01). To facilitate comparison of the proportion of engrafted islets between studies, the ACRArg was corrected for implanted IEq/kg, and expressed as the engraftment index.

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[82-85, 97]. In many kidney series, 1-year rejection rates as low as 5% have been achieved using a brief course of rabbit ATG induction in low immunological risk patients. 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 organs[83, 98].

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 Europe[85, 99]. 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%)[100].

The current protocol plans to rely on the CIT backbone regimen of rabbit ATG and Etanercept to allow us to assess whether the addition of GLASSIA improves initial engraftment and long-term survival.

1.5 Known and Potential Risks and Benefits to Human Participants

1.5.1 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 in a separate section.

1.5.1.1 Transmission of Disease from Donor to Recipient

Selection of potential donors for islet isolation must follow stringent guidelines. The aim of this process is to avoid use of any potential donor that might harbor transmissible 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, 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 who 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. 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. EBV by PCR monitoring will be carried out routinely after transplantation at defined intervals throughout the trial. EBV disease and the risk of PTLD have not

been reported in the recent era of clinical islet transplantation, suggesting that the risk of this complication may be less than 2%.

1.5.1.2 Risk of Microbial Contamination of Islet Preparations

Because isolated islets go through an extensive processing technique, the potential risk of bacterial contamination of the cellular product exists[101, 102]. 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 level is determined (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 so that the absence of any adventitious microbial and fungal contaminants can be confirmed. Broad-spectrum antibiotics are added to the released final product prior to transplant to further diminish the subjects' risk of infection. With this approach, intrahepatic sepsis post-transplant of islet allografts has not been reported in the recent era of islet transplantation.

This contrasts with the human islet autotransplant experience in which bacterial contamination of the preparation is relatively common but generally well-tolerated by the recipient. In one series, positive cultures from islet tissue preparations were identified in 11 of 29 subjects (38%) receiving autologous islets. The occurrence of serious morbidity due to infection (as defined by 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 found 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.

Importantly, since 2000, pancreatectomy specimens for clinical islet allotransplantation have been processed exclusively 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.

1.5.1.3 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 leukocyte antigens (HLA) in islet allotransplant recipients with failing grafts have been communicated from several islet transplant centers[103-107]. 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. 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[108]. 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 future.

1.5.1.4 Acceleration of Retinopathy with Acute Correction in Glycemic Control

In the DCCT study[7], about 10% of subjects with pre-existing retinopathy receiving intensive insulin 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 baseline[109]. Long-term follow-up of both groups suggested that successful pancreas transplantation may have a late beneficial effect that becomes evident only after 36 months.

1.5.1.5 Risk of Triggering Renal Graft Failure

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.

1.5.1.6 Psychological Impact of Successful of Failed Islet Transplant

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.

1.5.2 Risks of Use of Investigational Agent: GLASSIA

GLASSIA is human alpha1 proteinase inhibitor indicated in patients with lung disease secondary to severe hereditary deficiency of alpha1-antitrypsin (AAT). For this purpose the recommended dose for chronic therapy is 60 mg/kg once weekly at a rate of 0.2 ml/kg-bw/min via iv infusion. For this study we will be dosing at 30 mg/kg per dose.Its use is contraindicated in patients with IgA deficiency with anti-IgA antibodies as this may result in severe hypersensitivity and or anaphylaxis. GLASSIA is pregnancy category C.

Since GLASSIA is product purified from human plasma, there is a theoretical risk of transmitting infectious agents such as viruses, including CJ virus. However, no seroconversions for hepatitis (HBV and HCV), HIV or other known infectious agents have been reported in clinical studies with GLASSIA.

Common adverse reactions $\geq$ 5% include: headache, musculoskeletal discomfort, vessel puncture site bruise, Headache, reaction at the injection site such as redness, rash, swelling, itching, nausea, and rhinorrhea.

Adverse reactions occurring at >0.5% noted in the package insert include: pharyngitis (1.2%), headache (0.8%) and cough (0.5%).

Rare but serious reactions include: increased clotting in the blood and viral infection.

For the current trial we will apply a dose of 90 mg/kg load with 30 mg/kg intravenously 3x/week based on studies of GLASSIA in bone marrow patients. In part I of an ITN-sponsored trial of AAT in new-onset type I diabetic patients (the RETAIN trial, NCT01183468) encouraging preliminary results were obtained that are relevant to the current trial (see letter from Dr. Ehlers attached and abstracted below). In brief, 8 pediatric and 8 adult new-onset type I diabetic patients were treated beginning with a dose of 45 mg/kg dosed weekly for 6 weeks, followed by a 3-week washout period and then increased the dose to 90 mg/kg/week for 6 weeks (for a total of 12 weekly infusions). Mechanistic analysis by the ITN mechanistic core revealed that inhibition of inflammatory cytokines and modulation of NFκB and apoptotic pathway genes occurred at blood levels that were attained with the 90 mg/kg dose but not the 45 mg/kg dose; moreover, the 90 mg/kg dose was safely administered without any serious AEs. Metabolic data at 12 months (stimulated C-peptide secretion, insulin use, and HbA1c) are stable and show preservation of C-peptide responses at 1 year relative to baseline. Based on further analysis of mechanistic and PK data, it was concluded that a dose in the range of 90-180 mg/kg/week will be optimal in terms of the plasma concentrations attained and the duration of drug levels above the threshold for 50% inhibition of cytokine and NFκB responses. These studies are consistent with the bone marrow patient dosing at least 60 mg/kg 3x/week but the more frequent dosing will help to maintain the serum AAT level within the desired target range during the critical period of islet engraftment. We will measure a AAT trough between the 2nd and 3rd dose of AAT. If the trough falls below 2.5-3.5 mg/ml, we will increase 60mg/kg/dose. If further tested troughs still remain below specified range, we will not increase the dose further.

Providing support for our plan to administer AAT in the islet transplant setting is the observation from the RETAIN pilot trial that C-peptide levels were preserved for both adult and pediatric patients for the 12 month study period despite what we regard as under-dosing of AAT in the critical early months of treatment. Admittedly, the data must be interpreted with caution as we do not have a control population, but based on comparison to historical data on C-peptide decline in new-onset patients the data are encouraging, and the ITN is enthusiastic about supporting the next stages of this work. Similarly, the anti-inflammatory properties seen in GVH patients and the early evidence of some benefit are encouraging.

1.5.3 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 immunosuppressive regimen for their kidney transplant. Subjects who receive islet transplantation as part of this study will have the additional risks from administration of a) basiliximab, b) rabbit ATG, and c) anti-inflammatory therapy (i.e., etanercept).

1.5.3.1 Basiliximab (Simulect®)

Basiliximab is a chimeric (murine/human) monoclonal antibody (IgG1k) approved by the Food and Drug Administration (FDA) for prophylaxis against acute organ rejection in adult recipients of renal allografts. It is usually given at a dose of 20 mg IV on Days 0 and 4.

Basiliximab is associated with constipation, nausea, abdominal pain, vomiting, diarrhea, dyspepsia, peripheral edema, fever, viral infections, hyperkalemia, hypokalemia, hyperglycemia,

hypercholesterolemia, hypophosphatemia, hyperuricemia, urinary tract infections, upper respiratory infections, surgical wound complications, acne, hypertension, headache, tremor, insomnia, and anemia. In the four placebo-controlled studies, the pattern of adverse events in 590 patients treated with the recommended dose of basiliximab was similar to that in 594 patients treated with placebo (see product monograph for details). Basiliximab did not increase the incidence of serious adverse events observed compared with placebo. As with any protein product, anaphylaxis can occur, particularly with repeated administration, but this has been reported only rarely.

1.5.3.2 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, leukopenia (47%), and thrombocytopenia (30%). CMV infection occurred in 13% and PTLD 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®[29]. Hering et al described a beneficial role of Thymoglobulin® induction (6 mg/kg) in 8 subjects with T1D receiving single donor islet grafts, all of whom achieved insulin independence and were protected against recurrence of hypoglycemia[92]. 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 utilized[92].

1.5.4 Risks of Immunosuppressive / Anti-inflammatory Therapy

1.5.4.1 Etanercept (Enbrel®)

Etanercept is a dimeric soluble form of the p75 TNFR receptor that blocks TNF binding and reduces inflammation[86-89, 96]. 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 patients[110-112], and based on post-marketing studies a warnings have been issued about the following conditions, which have been reported with the use of Enbrel®: serious infections and sepsis, including fatalities; an increased risk of lymphoma and other malignancies in children and adolescents; and leukemia.

Many of the serious infections occurred in patients on concomitant immunosuppressive therapy.

Experience with anti-TNF alpha therapies in clinical and experimental islet transplantation has been limited. Farney et al described a beneficial role of etanercept in promoting engraftment of marginal mass islet grafts in mice[96]. 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 transplantation[92].

1.5.4.2 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 as needed through the first ATG infusion only (i.e. on day -2). 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.

1.5.5 Risks of Study Procedures

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

1.5.5.1 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.

1.5.5.2 Metabolic Stimulation Testing

The risks associated with metabolic testing are generally regarded as minor. Placement of IV cannulae may be associated with pain and discomfort at the puncture site, bruising, bleeding, 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.

1.5.5.3 The Procedural Risks of Islet Transplantation

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

- *Open Surgical Approach*

This procedure is usually carried out under general anesthesia, but can be performed occasionally under local anesthesia if required. The potential risk of acute bleeding is anticipated to be less with a controlled operative approach as opposed to a percutaneous approach, especially where a transplant site does not have access to local expertise in advanced interventional radiological procedures. Access to a tributary of the portal vein using the open technique requires a surgical

incision for exposure, and direct cannulation of a branch of the middle colic vein, the inferior mesenteric vein, a tributary of the superior mesenteric vein, or direct cannulation of a small omental vein. Potential acute surgical risks include bleeding at the surgical site, portal 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.

- *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.

1.5.5.4 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 liver[113, 114]. 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[115]. 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 Registry[115]. Reports on intra-abdominal (n=1)[25] and intrathoracic bleeding (n=1)[34] have been published. The risk of significant hemorrhage after percutaneous islet transplantation as defined by a drop in Hb of more than 2.5 g/L or the need for transfusion or surgery was 9% in the Edmonton series[116]. Subsequently, a further increase in risk of bleeding has been observed by the Edmonton program and has been attributed in part to concomitant aspirin therapy[116]. The risk has since been ameliorated by avoidance of pre-transplant aspirin and more effective measures to seal the catheter tract in the liver[114]. When effective methods are used to ablate the transhepatic portal catheter tract, bleeding can be avoided completely; at the University of Miami D-Stat thrombostatic agent has been used to seal the catheter tract and has avoided risk of bleeding[121]. At the University of Minnesota, no bleed-related complications occurred in 20 consecutive subjects when the catheter tract was sealed with coils and Gelfoam.

1.5.5.5 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 CITR[115]. 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.

1.5.5.6 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 post transplant 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 surgery[123].

1.5.5.7 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 procedure[117-120].

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.

1.5.5.8 Hepatic Dysfunction and Steatosis

Transient abnormalities in liver enzyme tests have been observed immediately following intraportal islet transplantation[120]. Three of the 86 islet transplant recipients reported to the CITR have experienced transient elevations of liver enzymes requiring prolongation of posttransplant hospitalization or admission[115]. Persistence of laboratory abnormalities indicative of liver dysfunction and likely or definitely induced by intraportal islet transplantation is a rare event; abnormalities in liver function tests usually resolved within 4 weeks[28]. 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[121,122] 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[123], 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.

1.5.5.9 Portal Hypertension

Portal hypertension following intraportal infusion of unpurified allogeneic islet tissue resulted in a tear of the splenic capsule requiring splenectomy in one case[123]. 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 later[124]. 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)[10]. The baseline pressures were normal in all cases, suggesting absence of chronic portal hypertension[10].

1.5.5.10 Portal Vein Thrombosis

Transplanted islets release tissue factor and exhibit prothrombotic properties when infused to an intravascular site such as the portal vein[125]. A partial portal vein thrombosis has been reported in one of six subjects transplanted at the intramural National Institutes of Health (NIH) program[25]. In the Edmonton single-center experience, the risk of partial portal vein thrombosis was 3% in more than 100 intraportal islet transplants[113]. The management of partial vein thrombosis includes anticoagulation therapy which may lead to intra-abdominal hemorrhage requiring transfusion and/or surgical intervention[125, 126]. 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 liver[126, 127]. 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 post transplant. Early diagnosis and prompt management of branch vein portal occlusion with systemic heparinization may prevent clot propagation. Repeated intraportal islet transplants are generally contraindicated in subjects who have experienced prior portal thrombosis.

1.5.5.11 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 Registry[108]. 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[115]. Gall bladder hematoma (n=1) and gall bladder opacification (n=2) have been observed as well.

1.5.5.12 Risk of Auxiliary Graft Biopsy

This biopsy is a minor procedure that will be done under direct visualization under local anesthesia. The main risks associated with this are superficial infection and bleeding.

1.5.6 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 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 < 100 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 ≥ 100 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.

1.5.7 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 diabetes management by an experienced diabetologist. Transplantation should also benefit subjects who are well- controlled but have multiple episodes of hypoglycemia.

Of note, 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[128-130]. In addition, experimental evidence suggests that even with partial graft function, providing endogenously produced c-peptide may contribute to restoration of physiological function.

2. OBJECTIVES

2.1 Primary Objective

The primary objective is to test the hypothesis that AAT treatment during the period of islet engraftment will increase the functional engrafted islet mass and that this will increase insulin independence rates at day 75, and secondarily would improve the long-term durability of islet function.

2.2 Secondary Objectives

Secondary objectives of this study will assess whether AAT treatment compared to control therapy at the time of islet transplantation, improves metabolic control and reduces the risk of cardiovascular and renal complications from diabetes. Mechanistic studies will probe the mechanism of action of AAT on recipient PBL and their correlation with success at the primary end-point and engrafted mass.

3. SELECTION AND WITHDRAWAL OF SUBJECTS

3.1 Inclusion Criteria

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

1. Male and female subjects age 18 to 70 years.

2. Subjects who are able to provide written informed consent and to comply with the procedures of the study protocol.

3. Subjects in the United States must have one of the following payment mechanisms in place:

    a. Medicare,
    b. A third-party insurer who agrees, via pre-authorization, to pay for participation in the study, or
    c. Another mechanism of payment (self-pay, hospital, university, donations, etc.) for participation in the study.

4. Clinical history compatible with T1D with disease onset < 40 years of age and insulin-dependence for ≥ 5 years at the time of enrollment.

5. 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.

6. Subjects who are ≥ 3 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] ± Prednisone ≤ 10 mg/day).

7. Stable renal function as defined by a 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.

8. Subjects who meet one of the options in the following criterion are eligible for transplantation:

    - Reduced awareness of hypoglycemia manifested by a Clarke score of 4 or more measured upon study enrollment and at least one episode of severe hypoglycemia in the 12 months prior to study enrollment.

    OR

    - a subject must have a reduced awareness of hypoglycemia manifested by a Clarke score of 4 or more and at least 1 episode of severe hypoglycemia;

    OR

    - Any subject not meeting the hypoglycemia option must have an HbA1c $\geq$ 7.5%.

3.2 Exclusion Criteria

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

1. Weight more than 90 kg or body mass index (BMI) > 30 kg/m2.

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

3. Other (non-kidney) organ transplants except prior failed pancreatic graft where graft failure is attributed to thrombosis within the first 4 weeks or to other technical reasons that require graft pancreatectomy; with the graft pancreatectomy occurring more than 6 months ago.

4. Untreated or unstable proliferative diabetic retinopathy.

5. Blood Pressure: SBP > 160 mmHg or DBP >100 mmHg despite treatment with antihypertensive agents.

6. Calculated GFR of ≤ 40 mL/min/1.73 m2 using the subject's measured serum creatinine and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [1]. Strict vegetarians (vegans) will be excluded only if their estimated GFR is ≤35 mL/min/1.73 m2.

7. Proteinuria (albumin/creatinine ratio or ACr > 300mg/g) of new onset since kidney transplantation.

8. Calculated panel-reactive anti-HLA antibodies > 50%. Subjects with calculated panel reactive anti-HLA antibodies ≤ 50% will be excluded if any of the following are detected:
    - Positive cross-match,
    - Islet donor-directed anti-HLA antibodies detected by Luminex Single Antigen/specificity bead assay including weakly reactive antibodies that would not be detected by a flow cross-match, or
    - Antibodies to the renal donor (i.e. presumed *de novo*).

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

10. Presence or history of active infection including hepatitis B, hepatitis C, HIV, or tuberculosis (TB). Subjects with laboratory evidence of active infection are excluded even in the absence of clinical evidence of active infection.

11. Negative screen for Epstein-Barr virus (EBV) by IgG determination at time of screening or previous kidney transplant.

12. Invasive aspergillus, histoplasmosis, and coccidoidomycosis infection within the last year.

13. Any history of maliganancy except for completeley resected squamous or basal cell carcinoma of the skin or other cancers for which the patient is at least 5 years post curative therapy where a very high likelihood (>99%) that cure has been achieved, such as in certain types of low grade thyroid cancer or early stage breast cancer.

14. Known active alcohol or substance abuse.

15. Evidence of Factor V Leiden mutation or other laboratory evidence of hypercoagulable state.

16. Any coagulopathy or medical condition requiring long-term anticoagulant therapy (e.g. warfarin) after islet transplantation (low-dose aspirin treatment [325 mg PO] is allowed) or subjects with international normalized ratio (INR) > 1.5. The use of Plavix is allowed only in conjunction with mini- laparotomy procedure at the time of islet transplant.

17. Severe co-existing cardiac disease, characterized by any one of these conditions:
    - Recent MI (within past 6 months);
    - Evidence of ischemia on functional cardiac exam within the last year;
    - Left ventricular ejection fraction < 30%; or
    - Valvular disease requiring replacement with prosthetic valve.

18. 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. Active pancreatitis.

21. Active peptic ulcer disease, symptomatic gallstones, or portal hypertension.

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

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

24. Any medical condition that, in the opinion of the investigator, will interfere with the safe participation in the trial. (Cancer screenings should be performed per current American Cancer Society guidelines).

25. Positive screen for BK virus by polymerase chain reaction (PCR) performed at time of screening.

26. A kidney transplant patient with type 1 diabetes who has an HbA1c < 7.5 and no history of severe hypoglycemia.

27. Selective or severe IgA deficiency (levels < 5-7 mg/dL)

28. AAT deficiency (defined as plasma level of AAT < 8 μM-50 mg/dL)

4. STUDY DESIGN

The study will be a prospective, multi-center clinical trial in kidney transplant recipients with T1D, assessing the effect of AAT on islet transplant engraftment and durability of function. Subjects considered for enrollment will be: 1) at least 3 months post-renal transplant, 2) have stable renal graft function and calculated GFR by measured SCr and the CKD-EPI equation, 3) be free of renal rejection episodes for ≥ 3 months, and 4) receive standard of care diabetes management either prior to enrollment or during the study.

If subjects have not received appropriate diabetes management in the 12 months prior to enrollment, they must undergo a period of standardized diabetes care by an experienced clinician at the transplant center using the current ADA's standards of medical care in diabetes. After at least 4 months of structured care by an experienced diabetologist, subjects with a Clarke score of 4 or more and at least one episode of severe hypoglycemia will be consented and listed for islet transplantation. If after 12 months subjects do not achieve acceptable glycemic control (HbA1c ≥ 7.5%), they will be consented and listed for islet transplantation. If after 12 months a subject has either a severe hypoglycemic event and Clarke score of 4 or more, or an HbA1c≥ 7.5%, the subject will be eligible for islet transplantation.

Subjects may undergo a period of standardized diabetes care by an experienced diabetologist. These subjects will be treated by diabetes management following current ADA guidelines for glycemic control. Similar guidelines for insulin therapy will also be applied to subjects with partial function following islet transplantation. This will include a target HbA1c of < 7.5%, 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.

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.

Hypoglycemic events that occur within 28 days 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 who is crossmatch negative will be selected from the active wait list.

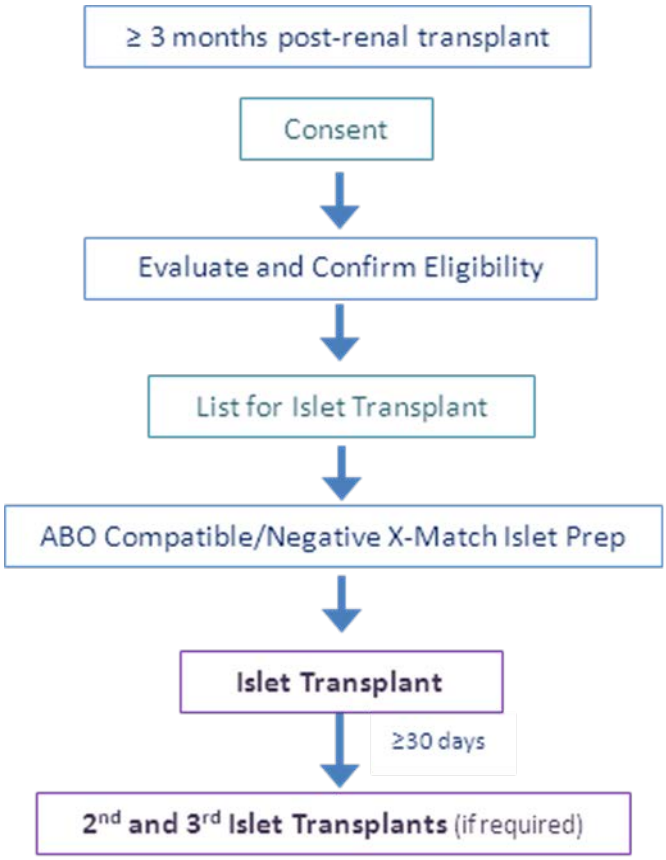
After preliminary testing, a subject will be invited to the hospital for transplantation upon determination of preliminary islet suitability based on count, quality, ABO and negative serum crossmatch. The subject will begin the planned induction therapy and Solumedrol premedication. These drugs can be administered via central vein catheter, peripherally inserted central catheter (PICC) line, or peripheral IV[98].

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.

At the end of the culture period after the islets have been inspected, the intended recipient will receive

GLASSIA, administered preoperatively at a dose of 90 mg/kg at a rate of 0.14 ml/minute and if tolerated without evidence of hypersensitivity, the rate will be increased to 0.2ml/min. Subsequent doses (30 mg/kg) will be administered during the engraftment period, three times a week over the first three weeks.

Figure 1. *Timeline for Enrollment and Transplantation*



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.

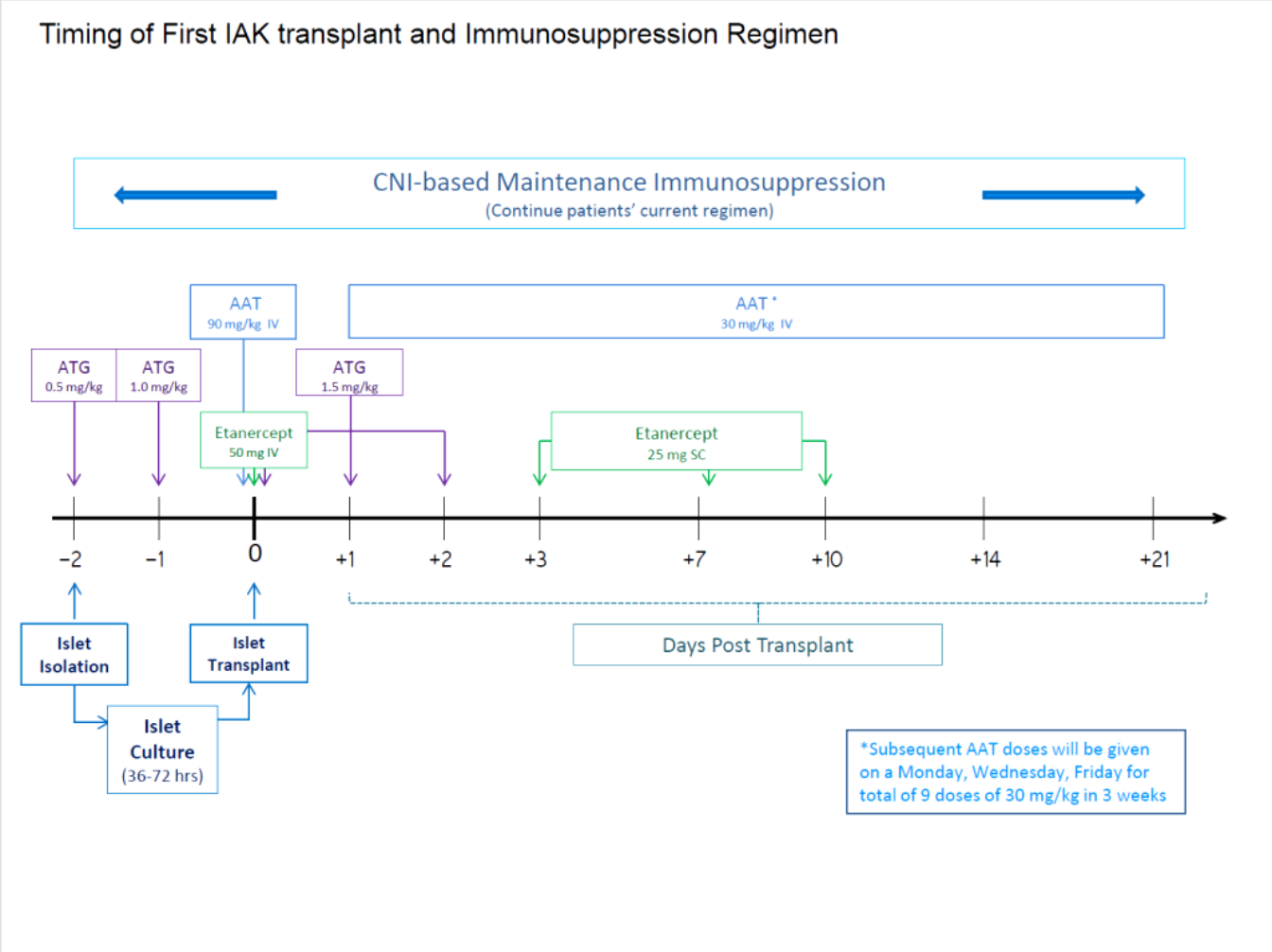
If possible, islet transplant procedure will be performed by open mini-laparotomy approach to facilitate implantation of an auxiliary graft in the subcutaneous space. If the open approach is not safe, the percutaneous approach is acceptable and the auxiliary graft will be transplanted by a small incision under local anesthesia. Each approach is capable of gaining access to the portal system and there is no evidence to suggest that the choice of approach will impact the function of the transplanted islets. Islets may be infused together with heparin according to the section entitled “Heparin”. Following completion of heparin, subjects will be treated with enoxaparin/dalteparin, aspirin, and pentoxifylline according to section entitled “Anticoagulation Prophylaxis / Hematological Agents”.

Following transplant, subjects will receive prophylactic antibiotics according to section entitled

"Antibacterial, Antifungal, and Antiviral Prophylaxis". Optimal glycemic control will be achieved according to section entitled "Updated Vaccinations". Subjects will remain up to date on CDC-recommended adult vaccinations; please refer to the MOP for guidance.

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 for the first transplant, and ≥ 4,000 IEq/kg recipient BW for subsequent transplants. It is expected that achieving insulin independence will potentially 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 after their initial transplant will no longer be eligible for additional islet transplants under this protocol. It is recognized that availability of suitable donor pancreata may affect the interval between transplants.

Figure 2. *Timing of First IAK Transplant and Immunosuppression Regimen*



Centers are encouraged to re-infuse subjects with partial graft function 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 can receive a maximum of 3 allogeneic donor islet infusions for the duration of the study.

4.1 Study Endpoints

Since our primary thesis is that GLASSIA will increase islet engraftment in the early perioperative period our primary endpoint for which the study is powered is a comparison of insulin independence rates of GLASSIA treated versus control patients at day 75 post transplant. We are fortunate to have permission to utilize data from the CIT-06 trial which will allow a large number of control patients. Since our proposed trial was directly derived from CIT-06 in terms of entry criteria, treatment regimen and post transplant monitoring except for treatment with GLASSIA and related mechanistic studies, these cohorts provide us a highly relevant comparator and allow us to enroll more GLASSIA treated patients than if a randomized trial. This increases the power of the trial making it likely that we will be able to detect differences between treated and control groups should GLASSIA exert the hypothesized benefit to engraftment.

For our primary mechanistic assessment to measure engrafted functional islet mass we will rely on the frequently sampled intravenous glucose tolerance test (FSIGT) from which an AUC for insulin secretion correlates with islet mass. Each of the 24 CIT-06 patients had this test performed at day 75. Of note, we had previously considered use of a glucose potentiated arginine stimulation test for this purpose. However, no CIT-06 patients had this test performed[131-132].

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 metabolic impact 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 secondary endpoints, and additional stimulatory tests of islet graft function utilizing MMTT, glucose, 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®), cardiovascular impact, and renal impact will be assessed as additional secondary endpoints[132-136]. Each of these were collected in CIT-06 patients and will add to the robustness of our comparison of the metabolic outcome between GLASSIA treated and control CIT-06 subjects.

Study endpoints are listed relative to the islet transplant occurring on Day = 0. The timing of all assessments is provided in the Schedule of Events (Appendix 1). For logistical purposes, the day 75 time point is equivalent to the 3 month evaluation.

4.1.1 Primary Endpoints

Primary Clinical Endpoint

The proportion of GLASSIA versus control CIT-06 subjects achieving insulin independence at day 75 after the first infusion of single donor islets.

4.1.2 Secondary Endpoints

Key Secondary Endpoint

Insulin independence

- The proportion of GLASSIA treated versus control CIT-06 subjects who are insulin independent after 1 or more islet infusions at:
    - 1 year after the first islet infusion
    - 1 year after the last islet infusion
    - 2 years after the first islet infusion
    - 2 years after the last islet infusion

Other Secondary Endpoints
Insulin Utilization

- The proportion of GLASSIA treated versus control CIT-06 subjects who are insulin independent after 1 or more islet infusions at:
    - 1 year after the first islet infusion
    - 1 year after the last islet infusion
    - 2 years after the first islet infusion
    - 2 years after the last islet infusion

- Percent change from baseline insulin requirement comparing GLASSIA treated versus control CIT-06 subjects at Day 75, 1 year and 2 years following the first and last islet transplant(s)

Islet Mass

The relative functional engrafted islet mass comparing GLASSIA treated versus control CIT-06 subjects using FSIGT testing at:

- Day 75 after the first islet transplant
- 1 year after the first islet transplant
- 2 years after the first islet transplant

- Correlation of markers of early islet loss as assessed by the rapid release of thrombin-anti thrombin complexes (TAT), C3 and c-peptide, and insulin specific DNA with FSIGT and clinical markers of islet function (HbA1c, insulin independence rates, duration of islet function and insulin independence)

- The proportion of GLASSIA treated versus CIT-06 control subjects with both an HbA1c ≤ 6.5% AND an absence of severe hypoglycemic events:
    - From Day 28 to Day 365 after the first islet transplant
    - From Day 28 to Day 730 after the first islet transplant.

- The proportion of GLASSIA treated versus control subjects with both an HbA1c < 7.0% AND free of severe hypoglycemic events:
    - From Day 28 to Day 365 after the first islet transplant.
    - From Day 28 to Day 730 after the first islet transplant

- The proportion of GLASSIA treated versus control CIT-06 subjects A reduction in HbA1c of 1 point AND an absence of severe hypoglycemia from:
    - From Day 28 to Day 365 after the first islet transplant
    - From Day 28 to Day 730 after the first islet transplant

- Number of severe hypoglycemic events comparing GLASSIA treated versus control CIT-06

subjects at 1 year and 2 years following the *first* and *last* islet transplant(s)

- HbA1c comparing GLASSIA treated versus control CIT-06 subjects at 1 year and 2 years following the *first* and *last* islet transplant(s)

Glycemic Control

- The change in Clarke score from baseline in GLASSIA treated versus control CIT-06 subjects at:
    - 1 year after the first islet transplant.
    - 2 Years after the first islet transplant

- β-score comparing GLASSIA treated versus control CIT-06 subjects at 1 year and 2 years following the *first* and *last* islet transplant(s)

- Glucose variability and hypoglycemia duration derived from the CGMS comparing GLASSIA treated versus control CIT-06 subjects at 1 year and 2 years following the first and last islet transplant(s)

- Basal (fasting) and 90-min glucose and c-peptide derived from the mixed-meal tolerance test (MMTT) comparing GLASSIA treated versus control CIT-06 subjects at 1 year and 2 years following the first and last islet transplant(s)

- MAGE comparing GLASSIA treated versus control CIT-06 subjects at 1 year and 2 years following the first and last islet transplant(s)

- Glycemic lability index (LI) comparing GLASSIA treated versus CIT-06 control subjects at 1 year and 2 years following the first and last islet transplant(s)

- Ryan hypoglycemia severity (HYPO) Score comparing GLASSIA treated versus control CIT-06 subjects at 1 year and 2 years following the first and last islet transplant(s)

- C-peptide: (glucose· creatinine) ratio (CPGCR) comparing GLASSIA treated versus control CIT-06 subjects at 1 year and 2 years following the first and last islet transplant(s)

- Rate of favorable outcome at each center preparing islets (rate of subjects with HbA1c $\leq$ 6.5% and absence of severe hypoglycemic events from Day 28 to Day 730, or reduction in HbA1c of 1 point and absence of severe hypoglycemia from Day 28 to Day 730).

Repeat Transplants

- The proportion of subjects receiving a second islet transplant comparing GLASSIA treated versus control CIT-06 subjects at 1 year and 2 years following the first and last islet transplant(s)

- The proportion of subjects receiving a third islet transplant comparing GLASSIA treated versus control CIT-06 subjects at 1 year and 2 years following the first and last islet transplant(s)

Other Efficacy Endpoints

- Cardiovascular events [death, cerebrovascular accident (CVA), myocardial infarction (MI)] and changes in atherogenic profile for GLASSIA treated versus control subjects at 1 year and 2 years following the first and last islet transplant(s)

- Renal impact measures including renal allograft survival and function measured by glomerular filtration rate (GFR), serum creatinine (SCr) and urinary albumin creatinine ratio comparing

GLASSIA treated versus control subjects at 1 year and 2 years following the first and last islet transplant(s)

Exploratory Mechanistic Endpoints

- Assessment of the effect of AAT on serum cytokines released in the early post transplant period (1 month) comparing GLASSIA versus control subjects from CIT-06
- Assessment of the effect of AAT on the inflammatory gene transcriptional profile of PBL at day 14 and 75 comparing pre-and post transplant samples
- Correlation of reaching target AAT levels with metabolic outcomes
- Histological survival of subcutaneous islets in subcutaneous auxiliary graft and correlation with overall graft survival

5. STUDY TREATMENT REGIMEN

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

Table 1. *Islet Transplant and Immunosuppression Regimen*

	Days Relative to Transplant												
	-2	-1	0	1	2	3	4	5	6	7	10	14	21
Islet Transplant			X										
ATG (Initial Transplant Only)	X	X	X	X	X								
Basiliximab (Subsequent Transplants Only)			X				X						
AAT			X	AAT will be given on Monday, Wednesday, Friday schedule for total of 9 doses in 3 weeks									
Etanercept			X			X				X	X		

5.1 Investigational Agent: GLASSIA

GLASSIA is human alpha1 proteinase inhibitor indicated in patients with lung disease secondary to severe hereditary deficiency of alpha1-antitrypsin (AAT). For this purpose the recommended dose for chronic therapy is 30 mg/kg once weekly at a rate of 0.2 ml/kg/min via iv infusion. In the current trial, patients will receive a 90 mg/kg load followed by 30mg/kg 3x/week for 3 weeks. It should take approximately 30 minutes to infuse. Its use is contraindicated in patients with IgA deficiency with anti-IgA antibodies as this may result in severe hypersensitivity and or anaphylaxis. GLASSIA is pregnancy category C.

5.1.1 Formulation, Dosing, Administration and Emergency Precautions

GLASSIA (Kamada) is available commercially as a ready to use, liquid preparation of purified human-alpha$_1$-protease inhibitor (Alpha$_1$-PI). It is prepared from human plasma though cold ethanol fractionation. GLASSIA is available in with UPS Water for Injection (to 1 gm/50ml).

The first dose of GLASSIA will be calculated with the recipients body weight recorded during the admission for transplant. Subsequent doses will be calculated using the weight recorded on the admission for that dosing. The dose will be rounded to the nearest whole vial dose but must be within

10% of calculated dose. Patients will receive a loading dose of 90 mg/kg followed by maintenece dose of 30mg/kg/dose. A trough level will be measured between 2nd and 3rd dose. If the AAT level is not between 2.5-3.5 mg/ml we will increase dose to 60mg/kg/dose for the remaining doses.
GLASSIA will be used within three hours per package insert guidelines. The infusion will be administered by a qualified individual, using a 5 micron in-line filter during the infusion. Vital signs will be recorded within 30 minutes before initiating the infusion and every 30 minutes during the infusion and within 15 minutes after completing the infusion.

With each infusion, medical personnel will 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.

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 Investigational Agent: Allogeneic Islets

5.2.1 Formulation, Dosage, and Administration

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

Table 2. *Composition of Final Drug Product [Product Code PHPI-A-01]*

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

Administration:

The islet mixture is delivered slowly via gravity drainage from a bag attached to the catheter in the portal vein or portal vein tributary. Access to the portal vein is achieved by mini-laparotomy to gain percutaneous transhepatic access under fluoroscopic, ultrasonographic, or real-time CT (computed tomography) guidance. If this approach is deemed unsafe, the portal vein can be accessed by a mesenteric or omental venous tributary of the portal vein under general anesthesia.

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.

At the end of the each islet infusion, we plan to transplant a small fraction of the total islet mass (~2-3%) into the abdominal wall subcutaneous tissue or preperitoneal space at the time of portal infusion into at least 4 discrete locations marked by a suture or surgical clip. We estimate that 8,000-10,000 islets of a 400,000-500,000 islet preparation are placed in this location (2000-2500 islets per pocket in 0.5 ml of collagen emulsion (Cosmoderm)), the auxiliary graft will readily allow us to obtain a sizeable biopsy of hundreds to thousands of islets for microscopic evaluation rather than an occasional one or two islets seen with liver core biopsy. Since we plan to transplant islets via mini-laparotomy, this will not add surgical morbidity or risk to the procedure. By marking the location of sites with radio-opaque clips, we will be able to biopsy the site under local anesthesia in the OR at any time post transplant. It is conceivable that subcutaneous islets could be more immunogenic than those in the liver and could even trigger rejection of the islet mass in the liver. Although it is difficult to estimate this risk, we assume it is very low and will include this risk in the patient's consent. In addition, the consent will be written in such a way to allow study candidates to opt in or out for this proportion of the procedure.

5.2.2 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.

5.3 Immunosuppression Medications

5.3.1 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.

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 1.5 mg/kg on day –2, 1.0 mg/kg on day –1, and 1.5 mg/kg on days 0, +1, and +2. The doses will be administered as directed on the package insert and in the CIT-06 Manual of Procedures. 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 day – 2 relative to the islet transplant by assessment of T cell depletion by CD3 counts measured after administration of the first ATG dose. If lack of efficacy is detected, basiliximab

(Simulect®) 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 as needed 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.

5.3.2 Subsequent Allogeneic Islet Transplants and Second Attempts at Initial Transplants

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

BASILIXIMAB (SIMULECT®)

Two IV doses of basiliximab, a monoclonal antibody IL-2 receptor blocker, may be given with subsequent islet transplants and second attempts at initial transplant. If basiliximab is administered, the first dose will be 20 mg and will be given within two hours prior to islet transplant on the day of islet transplantation. The second dose will be given on Day 4 after the transplant.

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

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

5.4 Concomitant Medications

5.4.1 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).

5.4.2 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.

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 6 months after islet transplantation for prevention of Pneumocystis carinii pneumonia (PCP). 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. 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.

CLOTRIMAZOLE (MYCELEX TROCHE®)

Clotrimazole will be administered as 1 troche PO QID starting on day –2 relative to initial transplant, day -1 for subsequent transplants, to be continued for 3 months after transplantation. Patients may receive antifungal prophylaxis according to local standard of care.

VALGANCICLOVIR (VALCYTE®)

Valganciclovir is an antiviral drug which will be given to prevent CMV infection starting on Day -2 for initial transplants, Day -1 for subsequent transplants, at a dose of 450 mg PO QD, increasing to 900 mg QD by Day 12 and continuing for 14 weeks posttransplant. If the CMV status of the donor and recipient are both negative, then valganciclovir administration may be adjusted or eliminated. 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.

5.4.3 Anticoagulation Prophylaxis / Hematological Agents

HEPARIN

Heparin may 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.

ENOXAPARIN (LOVENOX®)

Enoxaparin will be administered at a dose of 30 mg sc BID through day 7 post-islet transplant, with the first dose given 48 hours after the transplant procedure (when heparin is discontinued). The dose can be modified or extended at the discretion of the investigator.
Dalteparin can be substituted for enoxaparin at the PI's discretion.

ASPIRIN

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

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). *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 load[137].

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.

5.4.4 Updated Vaccinations

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

5.4.5 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.

5.4.6 Other Standard Therapies

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

5.5 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. If graft dysfunction is accompanied by biopsy proven rejection, the option for treatment will be discussed with the patient.

5.6 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 than Zithromax
- Other investigational products
- Immunomodulatory agents
- Other anti-diabetic agents
- Dapsone
- > 10 mg Prednisone (nasal steroids are allowed)

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 Sugar Records, 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, basiliximab, and rabbit ATG will be allowed in the event of complications.

5.8.2 Modification of Standard Immunosuppression

ISLETS ARE UNSUITABLE

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

GRAFT FAILURE

Subjects who experience islet graft failure will be maintained on their current immunosuppressive regimen for their renal graft as long as a subsequent islet transplant is possible. If/when it is determined that a subject will not receive a subsequent islet transplant, the subject will move to the reduced follow-up schedule.

ALLERGIC REACTION TO ATG

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

INTOLERANCE OF PROTOCOL MEDICATIONS

In the event that protocol-regulated concomitant medications are not tolerated, the subject will continue taking the immunosuppressive therapy in order to protect the islet 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.

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.

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.

NEUTROPENIA

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

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

- Reduce 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/μL 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/µL and the subject is afebrile, then the following will be done:

- Hold rabbit ATG.
- Hold administration of trimethoprim/sulfamethoxazole and/or valganciclovir.
- Review and obtain current sirolimus trough levels and hold dose if trough 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/µL 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.

THROMBOCYTOPENIA

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

NEPHROTOXICITY

A sustained 33% increase in SCr 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 3.

Table 3. *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.
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 "Reduced Follow- up" Treatment)

Study treatment will begin at the time of the first dose of induction antibody therapy for an islet transplant. Study treatment may be prematurely discontinued for any subject for any of the following reasons:

- The subject is unwilling or unable to comply with the protocol.
- The investigator believes that the study treatment is no longer in the best interest of the subject.
- The renal allograft is lost and the subject elects to terminate chronic immunosuppression.
- Graft Failure: See Study Definitions and Section 5.8.2.
- An unexpected related SAE. The agent(s) to which the event is attributed will be discontinued.

Subjects who prematurely discontinue study treatment will remain in the study until normal termination, for the purpose of monitoring safety and efficacy parameters and will enter the reduced follow-up schedule outlined in Appendix 2. Data from these subjects will be used in the intent-to-treat analysis.

6. CRITERIA FOR PREMATURE TERMINATION OF THE STUDY

6.1 Participant Withdrawal Criteria

In general, subjects may be prematurely terminated from the 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 meeting the definition for intent-to-treat who prematurely terminate from study treatment 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), and the Data Safety Monitoring Board (DSMB), if any one of the following occurs:

1. The Protocol Chair finds any unexpected fatal or life-threatening AE possibly related to the use of the test therapy.
2. Primary non-function (PNF) occurs in 3 or more consecutive subjects at 2 or more participating clinical sites.
3. There are 4consecutive 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 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 later in this study protocol.

After the protocol is placed on hold, no additional transplants within the trial will be performed at any participating clinical site until the 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, and the DSMB, if any one of the following occurs:

1. Any possibly study-related grade 5 AE; or
2. Two SAEs related to the islet transplant procedure (e.g., bleeding, thrombosis, gall bladder injury); or
3. Two consecutive primary non-functioning transplants. See Study Definitions.

After any site is placed on hold, no additional transplants will be performed at that site until the 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.

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

At the screening visit, the screening procedures and diabetes management details 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 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 and retinologist. 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 4. *Timeframes for local screening assessments*

Local Screening Assessments	Allowable timeframe prior to the date of consent
EBV IgG	No limit. Positive result required for eligibility.
Retinopathy evaluation; Physical exam; Abdominal US; electrocardiogram (ECG); Cardiac Stress Test; TB Test; TSH; Serology; Coagulation; CMV IgG/IgM (if neg), AAT level, IgA antibody level	Within one year
CBC; Chemistry	Within 6 months
CXR	Within 1 month

The screening pregnancy test, first morning spot urine, and blood draws for all laboratory assessments must be done at the study site after informed consent has been signed. Pregnancy and blood transfusion history will be collected and provided to the local lab for Alloantibody analysis. In addition to the protocol required screening assessments, subjects should meet site-specific requirements for transplant.

Once all eligibility criteria (inclusion and exclusion) are met, subjects will be referred to a study

diabetologist for assessment and additional diabetes care if needed, which will be administered per the guidelines in Appendix 3. All subjects will return to the clinic at least quarterly from the time of enrollment.

7.2 Standard Diabetes Management

After completion of the screening assessments confirming eligibility for the study, a subject will undergo 12 months of standard diabetes management by an experienced clinician (see Appendix 3). If a subject received at least 12 months of diabetes management prior to enrollment and experienced at least on episode of severe hypoglycemia during that time, the subject would not be required to receive standard diabetes care while on study as long as they have a Clarke score of 4 or more. A subject is required to return to the study site for clinic visits every 3 months while undergoing routine care and assessment and while on the waitlist. Monthly HbA1c sampling between the required 3 month interval visits may be drawn and assessed locally. Eligibility will be reconfirmed after 4 months and after 12 months of standard diabetes care. All eligibility assessments must be within the windows required for initial study enrollment. Once eligibility has been reconfirmed, a subject will be placed on the wait list. The subject will continue with standard diabetes management while on the wait list.

7.3 Waitlist/Baseline

Waitlist assessments will be repeated at pre-defined intervals as detailed in Appendix 1. Results from repeat assessments done closest to transplantation will be used as the subject's baseline values. During this period when subjects are awaiting their first transplant, CGMS should be completed as time allows. All one-time baseline assessments should be completed on Day -2, whenever possible, but always prior to the start of induction immunosuppression.

As in any other transplant situation, medical conditions that arise (*e.g.*, new serious infections, 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.

7.4 Islet Transplant and Study Treatment Visits

Blood group compatible and crossmatch negative subjects selected for 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[98]. 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.

Once islets have been examined at the end of the culture period but prior to final release testing, the pharmacy will compile the needed dose to allow the infusion to be completed within three hours of reconstitution. GLASSIA will be infused within three hours before the intended start of the islet infusion but after the final release criteria confirm islet suitability.

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.

7.5 Follow-up Visits

Islet transplant recipients will undergo a minimum 36-month follow-up period following the last islet transplant to include time points 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

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

7.6.1 Second Islet Transplant Criteria

Islet transplant recipients with partial islet graft function (see Study Definitions) will be considered for a second islet transplant in the interim between 30 ± 3 days and 8 months post-initial infusion.

Islet transplant recipients with graft failure will be considered for a second islet transplant before 8 months post-initial infusion. Please refer to the MOP for details on this process, which includes review of the potency testing from the first transplant product and post- transplant clinical data.

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

1. Subject received ≤ 5,000 IEq/kg with the first transplant, but failed to achieve or maintain insulin independence.
2. Subject has been compliant with study monitoring and prescribed immunosuppressive therapy.
3. 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.
4. Subject has no unresolved SAEs.
5. No evidence of PTLD, requiring complete withdrawal from immunosuppressive therapy.
6. No evidence of hypersensitization, allergic responses, or other potentially serious drug reactions to medications required by the protocol.
7. 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.
8. Absence of any medical condition that, in the opinion of the investigator, will interfere with a safe and successful second islet transplant.
9. Absence of anti-HLA antibodies directed to the kidney transplant or to the current islet preparation detected by Luminex Single Antigen/specificity bead assay (including weakly reactive antibodies that would not be detected by a flow cross-match). All kidney and currently-proposed islet preparation donor specificities must be avoided in 2nd and 3rd islet transplants.

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

7.6.2 Third Islet Transplant Criteria

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

1. The subject received greater than 4,000 IEq/kg following the second transplant, but remains dependent on insulin for longer than one month after the second transplant.
2. There is evidence of partial islet graft function at one month.
3. The Protocol Chair, and the Site Principal Investigators (PIs), determined that there were no relevant protocol deviations at the site.
4. The subject has been compliant with study monitoring and prescribed immunosuppressive therapy.
5. No evidence of a serious and life-threatening infection, AE, or other condition that precludes attempting an intraportal injection or continuation of the post-transplant treatment regimen.
6. Subject has no unresolved SAEs.
7. No evidence of PTLD, requiring complete withdrawal from immunosuppressive therapy.
8. No evidence of hypersensitization, allergic responses, or other potentially serious drug reactions to medications required by the protocol.
9. 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.
10. Absence of anti-HLA antibodies directed to the kidney transplant or to the current islet preparation detected by Luminex Single Antigen/specificity bead assay (including weakly reactive antibodies that would not be detected by a flow cross-match). All kidney and currently-proposed islet preparation donor specificities must be avoided in 2nd and 3rd islet transplants.

Subjects who have completed 8 months follow-up post-initial transplant will no longer be eligible for additional islet transplants funded under this protocol.

7.7 Visit Windows

The post-transplant 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 the 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, non-randomized study. Therefore, no blinded treatment codes are required for the assignment of study treatment.

8. SAFETY MONITORING

AEs that are classified as serious according to the definition set forth by the health authorities must be reported promptly to University of Iowa 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 4.1 (July 16, 2008),* 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

Only AEs that are associated with a protocol mandated procedure, which is not part of the normal standard of care for the subject, and severe hypoglycemic events (see study definitions), will be collected after the screening consent has been obtained and until the subject initiates standard diabetes care or is placed on the wait list (whichever comes first). All AEs will be collected from at the time the subject initiates study-directed diabetes care or is placed on the protocol waitlist (whichever comes first) 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

GRADING CRITERIA

The study site will grade the severity of AEs experienced by 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 5. *General Severity Definition of Adverse Event*

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

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

DEFINITION OF ATTRIBUTION

Attribution will only be determined and collected for serious adverse events.
The relatedness, or attribution, of an AE to islet transplantation, which includes the transplant 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 standard diabetes care will be defined by using the descriptors provided below.

Table 6. *Attribution of Adverse Events*

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

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.

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 University Of Iowa DCC medical reviewer and the PI.

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.

REPORTING TIMELINE – FROM THE SITE TO THE UNIVERSITY OF IOWA DCC

When an investigator identifies an SAE, he or she must notify the University Of Iowa 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 other than SAEs will be reported to the DCC by the sites on at least a monthly basis.

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.

NOTIFYING THE DATA AND SAFETY MONITORING BOARD

The DCC will provide the DSMB with listings of all SAEs on an ongoing basis, and at least yearly.

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.

REPORTING PREGNANCY AS A SERIOUS ADVERSE EVENT

Any pregnancy that occurs during a clinical study that is using an investigational drug must be reported to the DCC utilizing the SAE report form. This report is *for tracking purposes only*. All pregnancies that are identified during the study must be followed to conclusion and the outcome of each must be reported. The investigator should report all pregnancies within 24 hours using the SAE report form. The investigator should counsel the subject and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. A woman who becomes pregnant or wishes to while on the study will be counseled as to her choices and will be encouraged to discuss those choices with her obstetrician. Monitoring of the subject should continue until the conclusion of the pregnancy, and a follow-up SAE report form detailing the outcome of the pregnancy should be submitted 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

Time points 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 time point is equivalent to the 3 month evaluation.

9.1 Metabolic Testing

The timing of all assessments is provided in the Schedule of Events (Appendix 1).

9.1.1 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 at a minimum of 4 out of 7 days per week; 2-hour post-prandial capillary glucose levels should not exceed 180 mg/dL more than 3 times per week. Average daily insulin requirements will be obtained. It will also be determined if the subject achieved the targeted level of metabolic control (HbA1c ≤ 6.5%).

9.1.2 Glycemic Control

Glycemic control will be assessed by HbA1c (%) which will be analyzed by local labs at each center.

9.1.3 Glycemic Lability

Glycemic lability will be assessed by both the MAGE[134] and the LI[129].

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 mg/dL glucose. A MAGE > 200 mg/dL is indicative of marked glycemic lability.

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

9.1.4 Hypoglycemia

Episodes of severe hypoglycemia will be documented 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 or prompt recovery after oral carbohydrate, IV glucose, or glucagon administration[7].

In addition, composite indices of hypoglycemia frequency, severity, and symptom recognition will be assessed by both the Clarke survey[132] and the HYPO score[133].

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 <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.

9.1.5 Mixed-Meal Tolerance Test

Basal (fasting) and stimulated glucose and c-peptide levels will be determined using the MMTT. Subjects will be instructed not to eat or inject short-acting (or bolus) insulin after 8 PM the night before the test. Evening or bedtime administration of long-acting insulin will be permitted, as will consumption of water. Subjects receiving CSII 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 or ≥ 180 mg/dL, 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 = 60 and 90 minutes, stimulated glucose and c-peptide levels will again be drawn.

Each blood sample collected for c-peptide and glucose determination will be drawn and processed according to the local site's guidelines.

9.1.6 B-Score: A Composite Index of Post-transplant Graft Function

The β-score will be determined from the HbA1c, insulin requirements, fasting (basal) serum glucose, and basal or stimulated c-peptide as developed by Ryan et al[129]. 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 ≤ 180 mg/dL, indicative of excellent graft function.

9.1.7 The C-peptide to Glucose, Creatinine Ratio

The c-peptide to glucose, creatinine ratio (CPGCR) will be determined 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[138,139]. The CPGCR is calculated as [c-peptide (ng/mL) * 100]/[glucose (mg/dL) * creatinine (mg/dL)]. An index of islet graft function, this measure correlates well with both the 90-minute serum glucose levels during a MMTT and with the β- score.

9.1.8 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 100 mg/dL), number and duration of hyper- (>180 mg/dL) and hypo- (< 54 mg/dL) glycemic episodes, and total duration of hypoglycemia[135,140].

9.1.9 Measures of Cardiovascular Outcomes

CARDIOVASCULAR CHANGES

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

- Subject mortality (all cause and cardiac related). Standardized follow-up history and physical examinations for cardiovascular disease will be conducted.
- Cardiovascular events (MI, CVA) will be recorded at this time. See Appendix 4 for definitions of cardiovascular events.
- Atherogenic profile consisting of a fasting lipid panel [triglycerides, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL, calculated) and non-HDL cholesterol (calculated), c-reactive protein, apolipoprotein A1 and apolipoprotein B will be assessed. Each blood sample collected for the atherogenic profile will be analyzed at each site.
- The ratio of apolipoprotein A1 and apolipoprotein B.

9.1.10 Renal Impact

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

- Loss of renal allograft survival defined as a permanent return to dialysis, retransplant, or death;
- Renal allograft function measured by SCr, eGFR, spot urine albumin creatinine ratios, and protein excretion; and
- The investigators at each site have agreed that renal biopsies may be done for clinical purposes.

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 anti-donor reactivity.

9.2.1 Immune Assays

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.

ALLOANTIBODY

Development of alloantibody is generally associated with long 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. Site will perform Luminex (single antigen bead) testing via their local laboratories.

AUTOANTIBODY

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

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. Samples will be sent to Quest Laboratories for analysis.

ARCHIVED SERUM AND DNA

In order to ensure that we will ultimately gain as much information as possible from this trial and due to the ongoing development of assays, serum and DNA will be archived and batch shipped to Kevan Herold at Yale, for analysis.

10. STATISTICAL CONSIDERATIONS AND ANALYTICAL PLAN

10.1 Study Endpoint Assessment

10.1.1 Primary Endpoint

Since our primary thesis is that GLASSIA will increase islet engraftment in the early perioperative period our primary endpoint for which the study is powered is a mechanistic assessment of the functional islet mass early (day 75) post transplant. We will rely on the frequently sampled intravenous glucose tolerance test (FSIGT) from which an AUC for insulin secretion correlates with islet mass for our primary mechanistic assessment of functional islet mass. Data from the CIT06 trial, which performed this test on all patients at day 75, will serve as the control. Such controls increase the power of the trial making it likely that we will be able to detect differences between treated and control groups should GLASSIA exert the hypothesized benefit to engraftment.

10.1.2 Secondary Endpoints

Secondary objectives of this study will assess whether AAT treatment compared to control therapy at the time of islet transplantation, improves the rate of insulin independence over time (1 and 2 years post op), metabolic control and reduces the risk of cardiovascular and renal complications from diabetes.

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 endpoint and other secondary endpoints. The key secondary endpoint is separated from the other secondary endpoints because it provides direct evidence of the durability of the primary outcome at one year after the final infusion. By identifying the other important secondary endpoints in the protocol we expect favorable changes 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 other 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 multiplicity comparisons even for the other secondary endpoints.

Most of the secondary endpoints are measured before transplant, at day 75 (A1c, MMT, FSIGT, MAGE, c-peptide and Beta score) and at one year post-initial transplant (HbA1c, fasting and 90 minute MMTT, c-peptide, glucose, and c- peptide glucose creatinine ratio, MAGE, HYPO, Clarke survey, and Beta score). We will compare these markers with those in the CIT-06 controls using student's t-test, nonparametric test (e.g., Mann-Whitney U test, Wilcoxon signed rank test), or chi-square test when appropriate. A pre-post analysis will be used for these variables. For continuous variables that are normally distributed or that can be transformed to a normal distribution, we will compute an appropriate estimate and 95% confidence interval for the mean change and a paired t-test for testing that the mean change is zero. If an appropriate normalizing transform cannot be identified then a signed-rank test will be used to test for a significant change. Similarly, a McNemar test will be used to test for a significant change for dichotomous outcome variables.

For those continuous secondary endpoints that are only observable after transplantation or for whom

change from baseline are not meaningful we will compute estimates of the mean and 95% confidence intervals. Similarly, for binary endpoints we will compute the observed rates and exact 95% confidence intervals Levels of these markers will be compared with those in the CIT-06 controls using student's t-test or nonparametric test (e.g., Mann-Whitney U test) when appropriate.

Regression models for continuous longitudinal data (mixed models) will be used to describe the profiles of change over time for each of the response variables where measurements are repeated over 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.

10.1.3 Exploratory Endpoints

Exploring novel approaches to monitoring islet graft injury will serve as the basis for hypotheses generation in future trials. We will calculate descriptive statistics for the safety and efficacy of the new approaches. Adverse events rates will be tabulated and 95% confidence intervals (CI) will be calculated. We will calculate the mean, median, minimum, maximum, inter-quartile ranges, and 95% CI of loss of engrafted islets estimated by early post infusion release of insulin specific DNA, TAT and C3a. Success rate and 95% CI will be estimated.

Other secondary and exploratory endpoints include relative functional engrafted islet mass, the proportion achieving insulin independence, the proportion achieving HbA1c ≤ 6.5% and an absence of severe hypoglycemic events, the proportion achieving HbA1c < 7.0%, reduction in HbA1c of at least 1 point, the change in Clarke score, c-peptide, Glycemic labiality index (LI), glucose and glucose variability. These endpoints will also be analyzed using descriptive statistics.

10.2 Subject and Demographic Data

10.2.1 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, range, and 95% confidence interval.
b. Categorical data (*i.e.*, sex and race) will be presented as enumerations and percentages.

10.2.2 Medical History

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

10.2.3 Use of Medications

All medications used will be coded using the MedDRA 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.

10.2.4 Changes in Renal Function

Statistical presentation of changes in renal function over time will be further defined in the SAP.

10.2.5 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.

10.3 Sample Size and Power Calculations

10.3.1 Power for Primary Endpoint

The primary end-point is the rate of insulin independence 75 days after the first single donor islet infusion. Our hypothesis is that the greater engrafted mass achieved with AAT will result in a significant increase in the proportion of subjects achieving insulin independence after the first dose. In the Edmonton era, single donor insulin independence occurred in less than 10% of patients. It appears that this is increased in patients treated with T cell depletion and Etanercept to 18%. In the CIT06 study patient who received islet transplantation after kidney grafting showed an insulin independence rate of 16.7%. A similar rate of insulin independence in IAK patients is seen in the CITR registry.

Table 7 gives the statistical power to reject the null hypothesis that the insulin independence rate is ≤16.7%, assuming a sample size of 20, using one-sided binomial test with 2.5% type 1 error. Sample size calculations assume asymptotic normality. We propose that AAT treatment will achieve single donor insulin independence in 50% of patients. With 20 patients we will have approximately an 87% power to reject the null hypothesis that the insulin independence rate is ≤16.7% using a one-sided binomial test with 2.5% type 1 error. We believe the insulin independence rate after the first single donor islet infusion is at least as high as the control (reported in the CIT-06 study) using a one-sided test. If the true insulin independence rate is 55%, we would have 94%power to reject the null hypothesis. This suggests that we have a good chance of finding meaningful trends and potentially statistically significant differences between groups using highly relevant clinical end points in this pilot trial.

Table 7 Statistical Power to Reject the Null Hypothesis

True insulin independence rate in the study	Statistical power
45%	75%
50%	87%
55%	94%
60%	98%

We have chosen to transplant 20 subjects. This will provide approximately 87% power to detect a difference of 50% insulin independence rate in islet transplanted subjects versus the estimated 16.7% insulin independence rate. Table 8 displays minimal detectable differences with 70%, 80% and 90% power. The smallest difference that can be detected with 90% power by a one-sided 2.5% level binomial test is 52% compared to 16.7%. The minimal detectable difference is 47% compared to 16.7% with 80% power. Using a one-sided test with 10% type 1 error rate, we could detect a medium effect of slightly more than 2 fold difference (38% vs. 16.7%) with 70% power.

Table 8 Minimum Detectable Difference for Insulin Independence Rate

Power	Minimum detectable difference
70%	43% vs. 16.7%
80%	47% vs. 16.7%

90%	52% vs. 16.7%

10.3.2 Precision of Estimates of Secondary Endpoints

We calculate the half width of the 95% confidence interval of the mean for each secondary endpoint based on their estimated standard deviation and the sample size. That is, we can say with 95% confidence that the true value of the mean will be no further from the observed estimate than the value reported. Table 9 provides the half width of the confidence interval for HbA1c, c-Peptide, MMTT, Hypo score, MAGE and LI.

Table 9. Precision of Estimates of Secondary Endpoints

Variable	Mean	Standard Deviation	Half Width of 95% CI
HbA1c	6	0.7	0.31
c-Peptide	2	0.7	0.31
MMTT	133	55	24
Hypo Score	850	750	329
MAGE	8	4	1.8
LI	223	200	88

10.4 Interim Analyses to Ensure Patient Safety

We will use the method for interim analyses described by Emerson and Fleming to provide cut-points for an interim analysis for futility.

We use the following notions to describe the interim analyses plan.
Let p represent the true insulin independence rate 75 days after the first single donor islet infusion.
The efficacy hypothesis is

$$H_{01}: p \leq 0.167 \text{ versus } H_{a1}: p > 0.167.$$

Rejecting this null hypothesis would lead to concluding that 75 day insulin independence rate is better than 16.7% in patients who receive AAT. In order to ensure that the study provides
the maximum safety information, this study will not be stopped for efficacy.

The futility hypothesis is

$$H_{02}: p \geq 0.60 \text{ versus } H_{a2}: p < 0.60.$$

Rejecting this hypothesis would lead to concluding that 75 day insulin independence rate in patients who receive AAT is not greater than 0.60. The type II error (accepting the null hypothesis when it is false) is no greater than 0.025.

Table 10 assumes two equally spaced analyses; an interim analysis is planned when 10 subjects have completed the study and a final analysis will happen when all 20 subjects have completed the study. The table provides cut-points for a recommendation for futility (reject H_{02}). Column 3 in this table (labeled “Number of insulin independence at 75 days required”) provides the largest number of observed 75 day insulin independence that would lead to recommending futility.
At the first interim analysis (when 10 subjects have completed the study) the monitoring plan would recommend for futility if no more than 2 of the first 10 transplant subjects demonstrate a insulin

independence at 75 days after their transplant.

Table 10: Sequential Monitoring Plan

Patients Accrued Fixed Sample		Number of insulin independence at 75 days required*
10	Reject H_{02}	≤1
20	Accept H_{01}	≤7

* Based on exact binomial probabilities

10.5 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. 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. 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 database training materials.

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 an encrypted database supported 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 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.

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*, 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 DCC, 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 DCC 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 DCC.

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 unique identification number and these numbers rather than names will be used to collect, store, and report subject information.

14. PUBLICATION POLICY

Any publications of study results will adhere to the NIH Public Access Policy.

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APPENDIX 1: Sequence of Events

Time points (days relative to transplant)	SCREEN	WL[1]	BL[2]	D 0[3]	D 3	W 1 D 7	W 2 D 14	W 3 D 21	W 4 D 28	M 2 D 56	M 2½ D 75	M 6 D 180	M 9 D 270	M 12 D 365	365 post-initial Tx	Year 2				730 post-initial Tx
																M 15	M 18	M 21	M 24	
Visit #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Y1	15	16	17	18	Y2
Visit Windows (specified in days)	N/A	N/A	≤ 2	N/A	N/A	± 3				± 10	± 5	± 7	±14							± 90
Informed Consent	X																			
Medical and Diabetes History	X																			
Inclusion / Exclusion	X		X																	
Retinopathy Exam	X	X-yrly																		
Radiologic Exams																				
ECG	X	X-yrly	X											X					X	
Chest X-Ray[7]	X		X																	
Abdominal US (or MRI if clinically indicated)	X				X[5]															
Cardiac Persantine thallium or Stress echo or equivalent	X																			
General Assessments																				
Physical Exam[6]	X	X-yrly	X[6]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Review Medications[8]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
AE/Hypo.Events		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Clarke Score	X	X-q6Mo									X			X	X				X	X

Time points (days relative to transplant)	SCREEN	WL[1]	BL[2]	D 0[3]	D 3	W 1 D 7	W 2 D 14	W 3 D 21	W 4 D 28	M 2 D 56	M 2½ D 75	M 6 D 180	M 9 D 270	M 12 D 365	365 post-initial Tx	Year 2				730 post-initial Tx
																M 15	M 18	M 21	M 24	
Visit #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Y1	15	16	17	18	Y2
Visit Windows (specified in days)	N/A	N/A	≤ 2	N/A	N/A	± 3				± 10	± 5	± 7	±14							± 90
Local Laboratory Assessments																				
CBC w/ Diff	X	X-q6mo	X	X[10]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Comprehensive Metabolic Panel[11]	X	X-q6mo	X	X[10]	X	X	X	X	X-q1mo[12]						X	X	X	X	X	X
Calcineurin levels	X	X-q6mo	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
PRA by Luminex[4]	X	X-qMo												X	X				X	X
Coagulation (PT, PTT, INR)	X		X																	
Pregnancy test (females)[13]	X		X																	
Serology (HBV, HCV, HIV)[9]	X	X-yrly												X	X				X	
Fasting Lipids (TG, TC, HDL, LDL)	X	X-q6mo												X	X				X	
Apolipoprotien A1 & B, C-Reactive Protein		X												X	X				X	
T-Spot TB Test (or equivalent)	X	X-yrly												X					X	
AAT Levels [26] (Send out to Quest Diagnositcs)							X													
Immunoglobulin A (IgA)	X																			
Urinalysis	X																			
Blood type/HLA	X[21]		X																	

Time points (days relative to transplant)	SCREEN	WL[1]	BL[2]	D 0[3]	D 3	W 1 D 7	W 2 D 14	W 3 D 21	W 4 D 28	M 2 D 56	M 2½ D 75	M 6 D 180	M 9 D 270	M 12 D 365	365 post-initial Tx	Year 2				730 post-initial Tx
																M 15	M 18	M 21	M 24	
Visit #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Y1	15	16	17	18	Y2
Visit Windows (specified in days)	N/A	N/A	≤ 2	N/A	N/A	± 3				± 10	± 5	± 7	±14							± 90
(repeat test at each islet transplant)																				
Crossmatch (repeat test at each transplant)[14]			X																	
Thyroid Function (TSH)	X	X-yrly																		
Fasting C-Pep	X		X				X	X	X-q1mo [12]						X	X	X	X	X	X
First morning spot urine (albumin & creatinine)	X	X-q3mo							X		X			X	X				X	X
HbA1c	X	X-q3mo							X	X	X	X	X	X	X	X	X	X	X	X
Fasting & post-prandial c-peptide[15]					X	X														
Glucose immediately post-transplant[16]				X																
EBV IgG	X																			
CMV IgG, CMV IgM		X-yrly													X					X
CMV, EBV by PCR		X-yrly	X[17]								X	X								
BKV by PCR (blood)	X	X-yrly	X[17]																	
GFR (CKD-EPI calculated)	X																			
MMTT - 60 & 90 min	X										X	X	X	X	X	X	X	X	X	X

Time points (days relative to transplant)	SCREEN	WL[1]	BL[2]	D 0[3]	D 3	W 1 D 7	W 2 D 14	W 3 D 21	W 4 D 28	M 2 D 56	M 2½ D 75	M 6 D 180	M 9 D 270	M 12 D 365	365 post-initial Tx	Year 2				730 post-initial Tx
																M 15	M 18	M 21	M 24	
Visit #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Y1	15	16	17	18	Y2
Visit Windows (specified in days)	N/A	N/A	≤ 2	N/A	N/A	± 3				± 10	± 5	± 7	±14							± 90
c-peptide & glucose[18]																				
FSIGT Test	X[27]										X			X					X	
CGMS		X-yrly									X				X	X	X	X	X	X
Local Pathology Testing																				
Biopsy of Auxiliary Graft				X[24]											X[25]					
Mechanistic Assays																				
TAT, C3a, C-Pep[22] Quest (San Jan Cap)			X[20]	X[20]																
Autoantibody[23] Barbara Davis		X										X	X	X	X	X	X	X	X	X
Serum AAT level (ITN)	X					X[19]	X[19]	X[19]	X[19]											
Beta Cell DNA[23] (Yale)		X						X	X	X	X	X	X	X	X		X		X	

1: WL = Waiting List. Repeat assessments as indicated (i.e. yrly, q3mo), while the subject is on the WL.
2: BL = Baseline. Baseline window for patients is -2 days relative to transplant.
3: Day 0 is the day of islet transplant. This SOE applies to the 1st, 2nd, and 3rd islet transplant; restarting at Day 0 for each subsequent transplant.
4: After listing, draw sample monthly for crossmatch. Run PRA by Luminex every 3 months.
5: Abdominal ultrasound to be performed within 3 days post-transplant.
6: Cardiovascular history and physical to be conducted at BL, Y1, Y2, Y3, in addition to patient physical.
7: 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.
8: At screening, review medications. Review changes at subsequent visits.
9: Serology includes: HBc Ab, HBs Ab, HBs Ag, HCV Ab, and HIV. Do not repeat Hep B tests if HBs Ab was previously positive.
10: Test repeated Days -1, 0, +1, +2.

11: Comprehensive Metabolic Panel includes: Na^+, albumin, Mg^{2+}, Cl^- , K^+, alk phosphatase, total bilirubin, CO_2, creatinine, ALT(SGPT), BUN, gamma GT, glucose, ST (SGOT), Ca^{2+}, phosphorus.
12: Visit window for monthly labs is +/- 10 days.
13: Complete urine or serum pregnancy at screening. Complete serum pregnancy test within 72 hours prior to transplant. If urine tests positive at anytime, confirm by serum pregnancy.
14: 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.
15: C-peptide should be drawn fasting, and twice between 1-3 hrs post-prandial on Day 3 and Day 7 post-transplant.
16: Finger stick glucose should be drawn every hour for the first 6 hours immediately post-transplant.
17: CMV, EBV, and BKV by PCR immediately prior to the transplant. Post-transplant testing may be more frequent per standard of care.
18: MMTT can be done as necessary when determining islet graft failure.
19: Drawn daily during hospitalization and prior to each AAT dose.
20: TAT, C3a, & c-peptide: pre-induction AND pre transplant, 15, 30, 60, 120 min post-tx.
21: At screening, may use results from kidney transplant.
22: Serum frozen and batch shipped after each transplant.
23: Serum frozen and batch shipped at end of study.
24: Biopsy of auxiliary graft with subsequent transplants only
25: To be done in the event of graft failure for patients who do not receive subsequent transplants26: Drawn and shipped in real time to Quest Diagnositcs between 2^{nd} and 3^{rd} dose of AAT
27: A baseline FSIGT Test will be optional

APPENDIX 2: Reduced Follow-up Schedule of Events

Subjects prematurely discontinued from study treatment according to the criteria previously stated will remain in the study until normal termination. For the purpose of monitoring safety and efficacy parameters, the subjects 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**	**2 years post- final**	**3 years post- final**
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
Alloantibody (central)				X			X	X	X
HbA1c (central)							X	X	X
SCr (central)							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: Study Contacts

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APPENDIX 4: 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 an 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 ≥ 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.