CLINICAL ISLET TRANSPLANTATION (CIT)

PROTOCOL CIT-04

Islet Transplantation in Type 1 Diabetes with LEA29Y (belatacept) Maintenance Therapy

LEA29Y Emory Edmonton Protocol (LEEP)

Version 8.0 (17 January 2013)

BB-IND 9336

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

LEA29Y Manufacturer: Bristol-Myers Squibb (BMS)

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INVESTIGATOR SIGNATURE PAGE	
Protocol Number: CIT-04	**Version / Date:** 8.0/17 January 2013
IND: BB-IND 9336	**CIT Principal Investigators:** **James Shapiro, MD and Nicole Turgeon, MD**
Short Title: *LEA29Y Emory Edmonton Protocol (LEEP)*	
Study Sponsors: The National Institute of Allergy and Infectious Diseases (NIAID) and the National Institute of Diabetes & Digestive & Kidney Diseases (NIDDK)	

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

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

As the Site Principal Investigator, I agree to conduct protocol **CIT-04**, "*LEA29Y Emory Edmonton Protocol (LEEP)*"according to good clinical practices. I agree to carry out the study by the criteria written in the protocol and understand that no changes can be made to this protocol without written permission of the NIAID.

_____

Site Principal Investigator (Print)

_____ _____

Site Principal Investigator (Signature) **Date**

Protocol Synopsis

Title	Islet Transplantation in Type I Diabetes with LEA29Y (belatacept) Maintenance Therapy
Short Title	LEA29Y Emory Edmonton Protocol (LEEP)
Clinical Phase	Phase 2
IND Sponsor	DAIT/NIAID/NIH
IND Number	BB-IND 9336
Activation Date	March 2008
Accrual Objective	10
Accrual Period	24 months
Study Duration	24 months after the final transplant
Study Design	This trial is a prospective, two-center, open-label, pilot study of islet transplantation assessing the safety and efficacy of a steroid-free, calcineurin inhibitor-free based immunosuppressive regimen with belatacept in subjects with long-standing type 1 diabetes (T1D) that is refractory to intensive insulin therapy.
Treatment Description	Subjects will receive up to 3 separate islet transplants to achieve insulin independence. For immunosuppression, the subjects will receive belatacept, basiliximab, and mycophenolate mofetil administered in an open-label fashion.
Primary Endpoint	The proportion of insulin-independent subjects at day 75 (± 5 days) following the first islet transplant.
Secondary Endpoints	The key secondary endpoint is the proportion of subjects with an HbA1c <7.0% AND free of severe hypoglycemic events from Day 28 to Day 365, inclusive, after the first islet transplant. The other secondary endpoint is the proportion of subjects with an HbA1c <7.0% AND free of severe hypoglycemic events from Day 28 to Day 365, inclusive, after the final islet transplant. <u>Efficacy Endpoints</u> At 75 ± 5 days following the <u>first</u> islet transplant and following each <u>subsequent</u> islet transplant(s): • The percent reduction in insulin requirements • HbA1c • Mean amplitude of glycemic excursions (MAGE) • Glycemic lability index (LI) • Ryan hypoglycemia severity (HYPO) score

- Basal (fasting) and 90-min glucose and c-peptide derived from the mixed-meal tolerance test (MMTT)
- β-score
- C-peptide: (glucose·X creatinine) ratio
- Acute insulin response to glucose (AIR_{glu}), insulin sensitivity, and disposition index (DI) derived from the insulin-modified frequently-sampled IV glucose tolerance (FSIGT) test
- Glucose variability and hypoglycemia duration derived from the continuous glucose monitoring system® (CGMS)
- Quality of life (QOL) measures (DQOL, HSQ 2.0, Hypoglycemia Fear Survey-HFS)

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

At 365 ± 14 days following the <u>first</u> and <u>final</u> islet transplant(s):

- The percent reduction in insulin requirements
- HbA1c
- MAGE
- LI
- Clarke score
- HYPO score
- Basal (fasting) and 90-min glucose and c-peptide (MMTT)
- β-score
- C-peptide: (glucose·X creatinine) ratio
- AIR_{glu}, insulin sensitivity, and disposition index (DI) derived from the FSIGT test
- Glucose variability and hypoglycemia duration derived from the continuous glucose monitoring system® (CGMS)
- QOL measures
- The proportion of subjects receiving a second islet transplant
- The proportion of subjects receiving a third islet transplant
- Rate of favorable outcome at each center preparing islets (rate of subjects with an HbA1c < 7.0% and free of severe hypoglycemic events)

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

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

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

- CGMS
- QOL

Secondary Safety Endpoints

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

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

- The incidence and severity of AEs related to the islet transplant procedure including: bleeding (> 2 g/dL decrease in hemoglobin concentration); segmental portal vein thrombosis; biliary puncture; wound complication (infection or subsequent hernia); and increased transaminase levels (> 5 times upper limit of normal [ULN])
- The incidence and severity of AEs related to the immunosuppression including: allergy; reduction in GFR; increase in urinary albumin excretion; addition or intensification of anti-hypertensive therapy; addition or intensification of anti-hyperlipidemic therapy; oral ulcers; lower extremity edema; gastrointestinal toxicity; neutropenia, anemia, or thrombocytopenia; viral, bacterial, or fungal infections; and benign or malignant neoplasms
- The incidence of a change in the immunosuppression drug regimen
- The incidence of immune sensitization defined by presence of anti-HLA antibodies absent prior to transplantation
- The incidence of discontinuation of immunosuppression

At 365 ± 14 days following the first islet transplant:

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

Inclusion Criteria

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

1. Male and female patients age 18 to 65 years of age.
2. Ability to provide written informed consent.

3. Mentally stable and able to comply with the procedures of the study protocol.
4. Clinical history compatible with T1D with onset of disease at < 40 years of age, insulin-dependence for ≥ 5 years at the time of enrollment, and a sum of patient age and insulin dependent diabetes duration of ≥ 28.
5. Absent stimulated c-peptide (<0.3ng/mL) in response to a mixed meal tolerance test (MMTT: Boost® 6 mL/kg body weight to a maximum of 360 mL; another product with equivalent caloric and nutrient content may be substituted for Boost®) measured at 60 and 90 min after the start of consumption.
6. Involvement in intensive diabetes management defined as self monitoring of glucose values no less than a mean of three times each day averaged over each week and by the administration of three or more insulin injections each day or insulin pump therapy. Such management must be under the direction of an endocrinologist, diabetologist, or diabetes specialist with at least 3 clinical evaluations during the 12 months prior to study enrollment.
7. At least one episode of severe hypoglycemia in the 12 months prior to study enrollment.
8. Reduced awareness of hypoglycemia as defined by a Clarke score of 4 or more OR a HYPO score greater than or equal to the 90th percentile (1047) during the screening period and within the last 6 months prior to randomization;

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

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

Exclusion Criteria

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

1. Body mass index (BMI) >30 kg/m^2 or patient weight ≤ 50kg.
2. Insulin requirement of > 1.0 IU/kg/day or < 15 U/day.
3. HbA1c >10%.
4. Untreated proliferative diabetic retinopathy.
5. Blood Pressure: SBP > 160mmHg or DBP > 100mmHg.
6. Measured glomerular filtration rate (using iohexol) of <80 $mL/min/1.73m^2$ (or for subjects with an iodine allergy, calculated using the subject's measured serum creatinine and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation[1]). Strict

vegetarians (vegans) with a calculated GFR < 70 mL/min/1.73m^2 are excluded. The absolute (raw) GFR value will be used for subjects with body surface areas > 1.73 m^2.

7. Presence or history of macroalbuminuria (>300mg/g creatinine).
8. Presence or history of panel-reactive anti-HLA antibodies above background by flow cytometry.
9. For female subjects: a) Positive serum pregnancy test (minimum sensitivity 25 IU/L or equivalent units of human chorionic gonadotropin [HCG]) within 72 hours prior to the start of study medication; b) presently breast-feeding; c) unwillingness to use effective contraceptive measures to avoid pregnancy in such a manner that the risk of pregnancy is minimized 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. Subjects must use two acceptable methods of contraception while taking mycophenolate mofetil (MMF). For females of child bearing potential, the two methods should be started 4 weeks prior to the first dose of MMF. Oral contraceptives, Norplant®, Depo-Provera®, and barrier devices with spermicide are acceptable contraceptive methods; condoms used alone are not acceptable.
10. All women ≥ 35 years and women of any age who have first degree relatives with a history of breast carcinoma, or who have other risk factors of breast carcinoma, must have a screening mammogram, or provide results of a screening mammogram performed within 6 months of enrollment. Subjects with a mammogram that is suspicious for malignancy and in whom the possibility of malignancy cannot be reasonably excluded following additional clinical, laboratory, or other diagnostic evaluations will be excluded.
11. Active infection including hepatitis B, hepatitis C, or HIV.
12. Presence or history of active tuberculosis (TB). Subjects with laboratory evidence of active infection are excluded even in the absence of clinical evidence of active infection..
13. Negative screen for Epstein-Barr Virus (EBV) by IgG determination.
14. Invasive aspergillus, histoplasmosis, or coccidioidomycosis infection within one year prior to study enrollment.
15. Any history of malignancy except for completely resected squamous or basal cell carcinoma of the skin.
16. Known active alcohol or substance abuse.
17. Baseline Hb below the lower limits of normal at the local laboratory; lymphopenia (<1,000/μL), neutropenia (<1,500/μL), or thrombocytopenia (platelets <100,000/ μL). Participants with lymphopenia are allowed if the investigator determines there is no additional risk and obtains clearance from an independent hematologist.

18. A history of Factor V deficiency.
19. Any coagulopathy or medical condition requiring long-term anticoagulant therapy (e.g., warfarin) after islet transplantation (low-dose aspirin treatment is allowed) or patients with an international normalized ration (INR) >1.5. The use of Plavix is allowed only when portal vein access is obtained using a mini-laparotomy procedure at the time of islet transplant.
20. Severe co-existing cardiac disease, characterized by any one of these conditions:

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

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

    c) Left ventricular ejection fraction <30%.
21. Persistent elevation of liver function tests at the time of study entry. Persistent serum glutamic-oxaloacetic transaminase (SGOT [AST]), serum glutamate pyruvate transaminase (SGPT [ALT]), Alk Phos or total bilirubin, with values >1.5 times normal upper limits will exclude a patient. Known cirrhosis of the liver or portal hypertension.
22. Symptomatic cholecystolithiasis.
23. Acute or chronic pancreatitis.
24. Symptomatic peptic ulcer disease.
25. Severe unremitting diarrhea, vomiting or other gastrointestinal disorders potentially interfering with the ability to absorb oral medications.
26. Hyperlipidemia despite medical therapy (fasting low-density lipoprotein[LDL] cholesterol > 130 mg/dL, treated or untreated; and/or fasting triglycerides > 200 mg/dL).
27. Receiving treatment for a medical condition requiring chronic use of systemic steroids, except for the use of ≤ 5 mg prednisone daily, or an equivalent dose of hydrocortisone, for physiological replacement only.
28. Treatment with any anti-diabetic medication other than insulin within 4 weeks of enrollment.
29. Use of any other investigational agents within 4 weeks of enrollment.
30. Subjects previously treated with belatacept.
31. Administration of live attenuated vaccine(s) within 2 months of enrollment.
32. Any medical condition that, in the opinion of the investigator, will interfere with the safe participation in the trial.
33. Prisoners or subjects who are compulsorily detained (involuntarily incarcerated) for treatment of either a psychiatric or physical (e.g., infectious disease) illness.

34. Treatment with any immunosuppressive regimen at the time of enrollment, or subjects with comorbidities for which treatment with such agents are likely during the trial.
35. A previous islet transplant.
36. A previous pancreas transplant, unless the graft failed within the first week due to thrombosis, followed by pancreatectomy and the transplant occurred more than 6 months prior to enrollment.
37. Known hypersensitivity to mycophenolate mofetil or any of the drug's components.
38. Rare hereditary deficiency of hypoxanthine-guanine phosphoribosyltransferase (HGPRT) such as Lesch-Nyhan and Kelly-Seegmiller syndrome.
39. Dietary restriction of phenylalanine.

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Glossary of Abbreviations

ACR	American College of Rheumatology
AE	Adverse Event
AIDS	Acquired Immunodeficiency Syndrome
AIR_{glu}	Acute Insulin Response to Glucose
APC	Antigen Presenting Cell
AUC	Area under the curve
BG	Blood Glucose
BPAR	Biopsy-proven acute rejection
BW	Body Weight
CAN	Chronic allograft nephropathy
CBC	Complete Blood Count
CFR	Code of Federal Regulations
cGMP	Current Good Manufacturing Practices
CGMS	Continuous Glucose Monitoring System®
CI	Confidence intervals
CTCAE	Common Terminology Criteria for Adverse Events
CIT	Clinical Islet Transplantation Consortium
CITR	Collaborative Islet Transplant Registry
CMV	Cytomegalovirus
CNI	Calcineurin Inhibitor
CPGCR	C-peptide (glucose X creatinine) Ratio
CRF	Case Report Form
CRO	Clinical Research Organization
CsA	Cyclosporine A
CSBPAR	Clinically suspected and biopsy-proven acute rejection
CTCAE	Common Terminology Criteria for Adverse Events
DAIT	Division of Allergy, Immunology, and Transplantation
DCCT	Diabetes Control and Complications Trial
DGF	Delayed graft function
DI	Disposition Index
DIC	Disseminated Intravascular Coagulation
DSMB	Data Safety Monitoring Board
EBV	Epstein Barr Virus

EC	Ethics Committee
EDTA	Ethylenediaminetetraacetic Acid
ELISA	Enzyme-linked Immunosorbent Assay
FDA	Food and Drug Administration
FSIGT	Frequently Sampled Intravenous Glucose Tolerance
GCP	Good Clinical Practice
G-CSF	Granulocyte Colony Stimulating Factor
GFR	Glomerular Filtration Rate
HbA1c	Glycosylated Hemoglobin
HDL	High-density lipoprotein
HFS	Hypoglycemic Fear Survey
HGPRT	Hypoxanthine-guanine phosphoribosyltransferase
HIV	Human Immunodeficiency Virus
HLA	Histocompatibility Antigen
HSV	Herpes Simplex Virus
ICH	International Conference on Harmonization
IDDM	Insulin-dependent diabetes mellitus
IEQ	Islet Equivalents
IITR	International Islet Transplant Registry
IND	Investigational New Drug
INR	International Normalized Ratio
IRB	Institutional Review Board
ITN	Immune Tolerance Network
ITT	Intent to treat
IV	Intravenous
LDL	Low-density Lipoprotein
LI	Less intensive
LI	Lability Index
MAGE	Mean Amplitude of Glycemic Excursions
MedDRA	Medical Dictionary for Drug Regulatory Activities
MHC	Major Histocompatibility Complex
MI	More intensive
MMF	Maintenance mycophenolate mofetil
MMTT	Mixed Meal Tolerance Test
MPA	Mycophenolic acid

NCI	National Cancer Institute
NIAID	National Institute of Allergy and Infectious Disease
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NIH	National Institutes of Health
NOD	Non-obese Diabetic
PAID	Problem Areas in Diabetes
PI	Principal Investigator
pit-hGH	Pituitary Growth Hormone
PNF	Primary Non-function
PRA	Panel Reactive Antibodies
PTDM	Post-transplant Diabetes Mellitus
PTLD	Post-transplant Lymphoproliferative Disorder
PTT	Partial Thromboplastin Time
QOL	Quality of Life
RNA	Ribonucleic Acid
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SC	Subcutaneous
SCr	Serum Creatinine
SGOT	Serum glutamic-oxaloacetic transaminase
SGPT	Serum glutamate pyruvate transaminase
SOC	System Organ Class
SSL	Secure Socket Layer
T1D	Type 1 Diabetes
TAT	Thrombin-Antithrombin
TB	Tuberculosis
TCAE	Terminology Criteria for Adverse Events
TCR	T-cell receptor
TGs	Triglycerides
TMRE	Tetramethyl Rhodamine
ULN	Upper Limit of Normal
UNOS	United Network for Organ Sharing Disorders
WHO	World Health Organization
WOCBP	Women of Child Bearing Potential

Study Definitions

Full Graft Function

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

Graft Failure

Islet allograft failure will be defined as absence of insulin production by transplanted islets, as evidenced by c-peptide < 0.3 ng/mL. This will be determined by (1) c-peptide <0.3 ng.mL on random testing, followed by (2) c-peptide <0.3 ng/mL at baseline, and at 60 and 90 minutes after MMTT. C-peptide levels obtained in the course of the MMTT will be run at the core lab in Seattle, WA; allow 72 hours for results. Participants with graft failure do not need to complete the day 75 metabolic assessments.

Insulin Independent

Subjects will be considered insulin independent if they are able to titrate off insulin therapy for at least 1 week and all of the following criteria are met:

- One HbA1c level, one fasting serum glucose level, and a Mixed Meal Tolerance Test are documented within the visit window (e.g. 70-80 days at Day 75) and 7 consecutive days of blood sugar and insulin readings are documented within +/- 7 days of the visit window (e.g. 63 – 87 days at Day 75);
- HbA1c < 7.0% or ≥ 2.5% decrease from baseline;
- Fasting capillary glucose level should not exceed 140 mg/dL (7.8 mmol/L) 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 (10.0 mmol/L) at 90 minutes during the MMTT;
- Fasting serum glucose level ≤ 126 mg/dL (7.0 mmol/L); if the fasting serum glucose level is > 126 mg/dL (7.0 mmol/L), it must be confirmed in an additional one out of two measurements;
- At least one MMTT fasting or stimulated c-peptide ≥ 0.5 ng/ml.

Insulin Dependent

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

Intensive Diabetes Management

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

Partial Graft Function

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

Protocol Eligible

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

Primary Nonfunction (PNF)	Graft failure that occurs between 3 and 7 days post-transplant.
Severe Hypoglycemic Event Definition	A severe hypoglycemic event is defined as an event with one of the following symptoms: memory loss; confusion; uncontrollable behavior; irrational behavior; unusual difficulty in awakening; suspected seizure; seizure; loss of consciousness; or visual symptoms, in which the subject was unable to treat him/herself and which was associated with either a blood glucose level < 54 mg/dL (3.0 mmol/L) or prompt recovery after oral carbohydrate, IV glucose, or glucagon administration).
Wait List	Protocol eligible participants who have been listed for islet transplant with UNOS or an equivalent transplant network.
Women of Child Bearing Potential (WOCBP)	WOCBP includes any female who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) or is not postmenopausal (defined as amenorrhea ≥ 12 consecutive months; or women on hormone replacement therapy with documented serum follicle stimulating hormone level > 35 mIU/mL). Even women who are using oral, implanted, or injectable contraceptive hormones or mechanical products such as an intrauterine device or barrier methods (diaphragm, condoms, spermicides) to prevent pregnancy or practicing abstinence or where the partner is sterile (e.g., vasectomy), should be considered to be of child bearing potential.

1. BACKGROUND AND RATIONALE

1.1 Background

More than 1 million North Americans are afflicted with Type I Diabetes (T1D). Each year, an estimated 30,000 new cases of insulin-dependent diabetes mellitus are diagnosed in the United States. Despite steady improvements in the management of this disease, its victims remain at greatly increased risk for stroke, myocardial infarction, amputation, and premature death. Diabetes-related health care costs are staggering, and the life expectancy of a teenager is reduced by thirty years from the onset of insulin-dependent diabetes mellitus (IDDM) [2]. For many patients with T1D exogenous insulin therapy is not adequate to prevent the complications of the disease. The Diabetes Control and Complications Trial (DCCT) found that intensive insulin therapy delayed the onset and slowed the progression of retinopathy, nephropathy, and neuropathy in patients with IDDM [3, 4]. Unfortunately, intensive insulin therapy is not attainable for many patients with T1D. Even with careful monitoring, patients receiving intensive insulin therapy in the DCCT had significantly more episodes of severe hypoglycemia compared to conventionally treated patients. Thus, results of the DCCT would support the rationale for transplantation of insulin producing cells if this can be achieved with minimal morbidity. Approximately 10% of the T1D population develops severe and uncontrollable recurrent hypoglycemia or glycemic lability despite optimized insulin therapy. For these individuals, alternative therapies to injectable insulin are more urgently required.

Transplantation of isolated pancreatic islets is an appealing approach to the treatment of insulin-dependent diabetes mellitus. However, the perennial hope that such an approach would result in long-term freedom from the need for exogenous insulin, with stabilization of the secondary complications of diabetes, has been slow to materialize in practice. Of the 447 patients transplanted from 1990 to 1999, less than 10 percent remained free of insulin for longer than one year [5, 6]. In the majority of these procedures, the regimen of immunosuppression consisted of antibody induction with an anti-lymphocyte globulin combined with cyclosporine, azathioprine, and glucocorticoids.

1.2 Preclinical and Clinical Experience

1.2.1 Preclinical Studies

1. NULOJIX® (belatacept) is a higher avidity CTLA4Ig (abatacept) molecule. The identification of CD28 as a critical costimulatory molecule for T cell activation led to considerable enthusiasm that the fusion protein CTLA4-Ig would prove to be as effective in primate and clinical transplantation studies as it was in initial rodent studies. Unfortunately, the potency of this fusion protein was considerably less effective in non-human primate renal transplantation models as compared to rodent models. Recently, it was shown that the binding affinity of CTLA4-Ig was insufficient to completely block CD28/CD86 interactions in in vivo studies. This may be related to the faster dissociation rate of CTLA4-Ig from CD86 as compared to CD80. High avidity CTLA4-Ig molecules were developed at Bristol Myers Squibb using a mutagenesis and screening strategy of over 2,300 fusion proteins. Belatacept was identified as the most potent candidate, as it binds to human CD86 with 4-fold, and to CD80 with 2-fold

increased avidity. Belatacept was ten times more potent than CTLA4-Ig at inhibiting T cell proliferative responses in vitro.

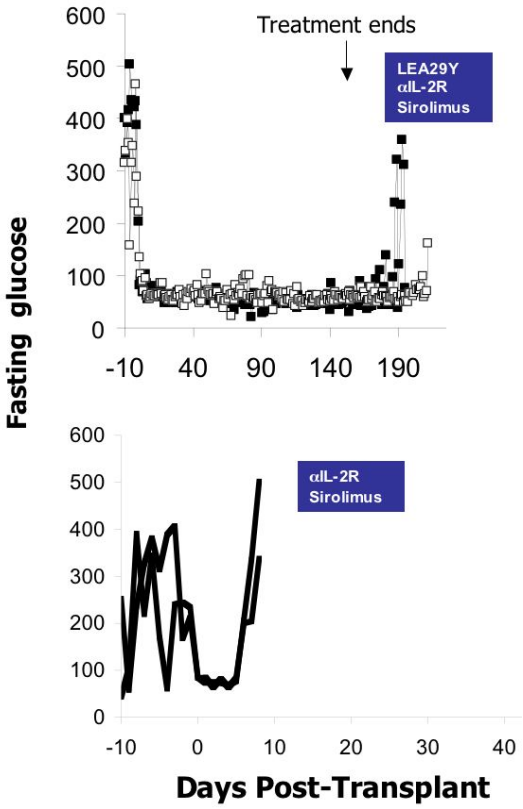
2. *NULOJIX® (belatacept) prolongs renal allograft survival and synergizes with conventional immunosuppression in non human primates.* Given the promising in vitro and in vivo immunosuppressive activity of belatacept, we tested the ability of the molecule to prevent renal allograft rejection in non-human primates. Our initial studies with belatacept monotherapy (> 20 μg/mL for 90 days post transplant) demonstrated superior efficacy to CTLA4-Ig (median survival time for the belatacept group 45d vs. 8d for CTLA4-Ig, p=0.008). These data indicate that the enhanced binding activity of belatacept results in enhanced immunosuppressive activity when compared to the parent molecule CTLA4-Ig.

We found that the combination of belatacept with the chimeric anti-human IL-2R mAb (basiliximab) led to potentiation of efficacy, with 5 of 6 recipients demonstrating stable serum Cr and survival for > 100d. However, after cessation of belatacept treatment (day 70), all recipients ultimately rejected their allografts. Importantly, no adverse effects related to the administration of belatacept (e.g., thrombosis, hypertension, hyperglycemia, or hypercholesterolemia) were observed in any of the experimental animals by clinical assessment, by laboratory analysis, or at necropsy[7].

3. *NULOJIX® (belatacept) inhibits the development of anti-donor antibody responses.* Despite progressive improvements in acute rejection rates in recent years the rate of allograft loss per year has remained essentially unchanged. The development of antibodies specific for donor MHC molecules is thought to be an important factor contributing to the process of chronic rejection. In addition to its possible role in the process of chronic rejection the development of antibodies to donor MHC antigens following transplantation has a major impact on the prospect of re-transplantation. To evaluate the potential impact of belatacept treatment on this process we examined the development of anti-donor antibody responses in the various experimental groups. Animals treated with basiliximab alone failed to develop detectable anti-donor antibodies, presumably because the allograft failed before an effective antibody response could be mounted. Animals treated with MMF and Cs generated strong anti-donor antibody responses at the time of rejection (between days 25 and 36). In contrast, none of the animals treated with belatacept developed anti-donor antibodies during therapy, even when rejection occurred during belatacept treatment. Following withdrawal of belatacept, however, animals from each of the experimental groups developed anti-donor antibodies at the time of rejection.

These data provide evidence that blockade of T-cell costimulatory pathways is a promising strategy for the development of potentially less toxic immunosuppressive medications for islet transplantation.

4. *A calcineurin inhibitor-free, belatacept based protocol protects allogeneic islets in non-human primates.*



Building on our primate renal data, we further optimized the belatacept-based regimen for use in primate islet transplantation by eliminating both steroids and calcineurin inhibitors for a less diabetogenic, 'islet-friendly' maintenance strategy. Belatacept was evaluated for its potential to replace tacrolimus and protect allogeneic islets in a preclinical primate model, when compared to the Edmonton Protocol-equivalent immunosuppression [8]. As detailed in our Diabetes paper, animals receiving the belatacept/sirolimus/anti–IL-2R regimen (n=5) had significantly prolonged islet allograft survival (204, 190, 216, 56, and >220 days). Importantly, the belatacept-based regimen prevented the priming of anti-donor T- and B-cell responses, as detected by interferon-gamma enzyme-linked immunospot and allo-antibody production, respectively. The results of this promising study suggest that NULOJIX® (belatacept) is a potent immunosuppressant that can effectively prevent rejection in a steroid-free immunosuppressive protocol and produce marked prolongation of islet allograft survival in a preclinical model, and forms the foundation of our initial clinical trial.

In summary, we have identified a novel calcineurin inhibitor/steroid-free immunosuppressive medication that provides significant protection from rejection and prolongs the survival of islet allografts in non-human primates. Together, the encouraging results of clinical trials using the lower affinity parent molecule, CTLA4-Ig, in rheumatoid arthritis, the results using NULOJIX® (belatacept) in human renal transplantation and the results described here provide a strong rationale for clinical trials to test these strategies in human islet transplantation.

1.2.2 NULOJIX® (belatacept) in Clinical Studies

NULOJIX® (belatacept) has been studied in four BMS-sponsored clinical trials protocols: IM103-001 (completed), IM103 002 (completed), IM103-100 (completed), and a Phase III kidney transplant study.[9] Protocol IM103-001 was a Phase I, randomized, double blind, placebo-controlled study to assess the safety, pharmacokinetics and immunogenicity of escalating doses of intravenously administered belatacept in healthy volunteers. Protocol IM103-002 was a Phase II, randomized, double blind, placebo-controlled study to evaluate the safety, preliminary clinical activity, immunogenicity and pharmacokinetics of multiple doses of belatacept and CTLA4Ig administered intravenously to subjects with active rheumatoid arthritis. IM103-100 was a Phase II, randomized, open-label, controlled study to directly compare the safety and preliminary clinical activity of belatacept with cyclosporin (Neoral®) in kidney transplant recipients. BENEFIT was a Phase III, randomized, open-label, partially blinded study to

compare the safety and efficacy of belatacept-basedimmunosuppression regimens versus cyclosporine in renal transplant recipients.

Results of Phase I studies pharmacokinetics and safety of a single dose, dose escalation study of Belatacept in Healthy Volunteers (IM103-001):

A single-dose Phase I study with belatacept was performed in 40 healthy volunteers. Subjects received single IV infusions of 0.1, 1, 5, 10, or 20 mg/kg belatacept. At each dose level, 6 subjects received active drug and 2 received placebo. Pharmacokinetic sampling and analysis indicated that Cmax values increased in a dose-proportional manner and were in a range similar to that observed with the parent molecule. Both Cmax and AUC (area under the curve) appeared to increase in ratio comparable to the dose increment ratio. The half-life ranged between 176 - 210 hr (~7-9 days) between the 5 and 20 mg/kg dose levels. Overall, the pharmacokinetics of belatacept appears to be linear following intravenous (IV) administration to humans.

Review of the safety data from this trial indicates that single, IV doses of belatacept of 0.1 to 20.0 mg/kg were well tolerated. No deaths or serious adverse events were reported. No histamine-like peri-infusional AEs were reported. No clinically significant changes in vital signs or laboratory parameters were observed. There was no evidence for the development of anti-belatacept antibodies.

Results of a Phase II Study of Belatacept in Rheumatoid Arthritis (IM103-002)

Study IM103002 was a Phase 2 pilot study that assessed the efficacy, safety, and immunogenicity of multiple IV doses of belatacept, CTLA4Ig, and placebo in 214 subjects with RA. Eligible subjects had a diagnosis of RA for ≤ 7 years, had failed at least 1 disease-modifying anti-rheumatic drug therapy, including etanercept, and had active disease (≥ 10 swollen joints, ≥ 12 tender joints, an erythrocyte sedimentation rate ≥ 28 mm/h, and morning stiffness ≥ 45 minutes). Overall, belatacept demonstrated dose-dependent efficacy in this subject population, as evidenced by American College of Rheumatology scores. With respect to safety, no deaths were reported during the treatment or follow-up period (through Day 169), and 12 subjects reported SAEs, although no SAEs were considered drug related by the investigators.

Results of a Phase II Study of Belatacept in Solid Organ Transplantation (IM103-100)

Study IM103100 was a 1-year, partially-blinded, randomized, active-controlled, multiple-dose, multicenter non-inferiority study in de novo renal transplant recipients. All subjects received basiliximab induction and background maintenance immunosuppression with MMF and corticosteroids. Subjects were randomized in a 1:1:1 ratio to treatment with belatacept (more intensive [MI] or less intensive [LI] regimens) or CsA (open-label dosed twice daily to achieve a specified trough serum concentration range). Belatacept was administered in a double-blind fashion, with the investigator and subject blinded to the identity of the belatacept dose regimen.

Belatacept subjects were dosed with 10 mg/kg on Days 1, 5, 15, 29, 43, 57, 71, 85, 113, 141, and 169 (MI regimen) or 10 mg/kg on Days 1, 15, 29, 57, and 85 (LI regimen). Subjects were reallocated on Days 85 (LI regimen) and 169 (MI regimen) to a 5 mg/kg dose of the drug every 4 or 8 weeks through Day 365.

The primary efficacy variable was the incidence of clinically-suspected and biopsy-proven acute rejection (CSBPAR) at 6 months post-transplantation. CSBPAR was defined as an increase in

serum creatinine (SCr) of at least 0.5 mg/dL compared to the baseline value in the absence of other factors known to adversely affect renal function that led the investigator to suspect acute rejection, which was then confirmed by centrally-assessed biopsy. Secondary efficacy variables were the incidence of all biopsy-proven acute rejections (BPARs), including those without an increase in SCr of at least 0.5 mg/dL, as well as the composite endpoints of CSBPAR or presumed acute rejection and BPAR or presumed acute rejection at 6 months and 1 year, and death and/or graft loss at 1 year. 'Presumed acute rejection' was defined as an elevation in SCr (at least 0.5 mg/dL compared to the baseline value in the absence of other factors known to adversely affect renal function) that led the investigator to suspect and treat the subject for acute rejection without a biopsy to confirm the diagnosis, or despite a biopsy that did not confirm acute rejection. All biopsies were assessed in a blinded fashion by a central pathologist. The primary cause of graft loss and death was also adjudicated.

The safety evaluation included AEs (including infections), vital signs, physical examinations, electrocardiograms, and laboratory parameters (hematology, biochemistry, and urinalysis). Topics of special interest were renal function (GFR, as determined by iohexol clearance, SCr, and calculated creatinine clearance or GFR at 1, 6, and 12 months), BP parameters (systolic diastolic pressure [SBP] and diastolic blood pressure [DBP], presence of hypertension), fasting serum cholesterol and triglycerides (TGs), and the presence of post-transplant diabetes mellitus (PTDM).

The efficacy and safety results for Study IM103100 study are presented in the following sections. For more detailed information on the efficacy and safety of belatacept, see the Investigator Brochure. Overall, the mean duration of exposure was comparable across all 3 treatment groups. Specifically, mean duration of exposure was 300, 308, and 294 days in the belatacept MI and LI groups and the CsA group, respectively.

Acute Rejection

The primary endpoint, CSBPAR at 6 months, occurred infrequently in all treatment groups. The incidence rate was slightly lower in the belatacept groups than in the CsA group. The criteria for non-inferiority to CsA were easily satisfied for both belatacept groups; however, the number of events was too small to support any further conclusions regarding the relative efficacy of the 3 regimens. The distribution of events by severity (as indicated by histological grade) was similar across the 3 treatment groups. Identical results were observed at 12 months.

The secondary endpoint of BPAR occurred 2 to 4 times more frequently than the primary endpoint of CSBPAR, indicating that most BPAR were subclinical (i.e., not associated with an increase in SCr ≥ 0.5 mg/dL). These episodes of subclinical rejection were observed on biopsies taken to satisfy the protocol requirements, according to local practice, or for other reasons than an increase in SCr ≥ 0.5 mg/dL.

BPAR occurred most frequently in the belatacept LI group. As the rate of CSBPAR was comparable across treatment groups, the difference in the rate of BPAR was due to an increase in the number of subclinical rejection episodes in the belatacept LI arm. In addition, reallocation of subjects to an 8-week infusion schedule rather than a 4-week infusion schedule in the maintenance phase was associated with an increased frequency of subclinical rejection.

Overall, the histological severity grade of acute rejection episodes appeared to be similar across the 3 treatment groups. While Grade IIB rejection, as assessed by Banff 97 criteria, occurred more frequently in the belatacept groups, the number of such events was small, and the confidence intervals (CIs) around the incidence rates broadly overlapped (belatacept MI: 1.0.%-12.5%; belatacept LI: 1.1%-13%; and CsA: 0%-6.5%).

Recurrent Acute Rejection

Overall, the average number of rejection episodes per subject (~1.2) was similar among the 3 treatment groups.

Chronic Allograft Nephropathy (CAN)

Biopsy specimens were also examined for CAN by an independent blinded central histopathologist using Banff 97 working classification of kidney transplant pathology.[10] By Month 12, CAN was approximately 30%-50%, in relative terms, less common with belatacept than with CsA.

Subject and Graft Survival

Death and/or graft loss occurred infrequently in all treatment groups, and was least frequently reported in the belatacept LI group. Most graft losses occurred for technical, rather than immunological, reasons.

Five deaths (4 in the CsA group and 1 in the belatacept MI group) occurred and were analyzed according to the intent-to-treat (ITT) principle. Two of these deaths – both in the CsA group – occurred on therapy or within 56 days of the last dose of study therapy. Accordingly, these deaths also are counted under the prespecified safety conventions.

Three other deaths qualify under the ITT principle, but not under the safety conventions because they either never received study drug or the death was an event subsequent to the discontinuation of study drug + 56 days. One death in the CsA group and 1 in the belatacept MI group, occurred > 56 days after the last dose of study therapy. One death in the CsA group occurred in a subject who was randomized, but never treated.

Adverse Events

Overall Adverse Events

The overall incidence of AEs is summarized in Table 1.3.4.4A.

Table 1.3.4.4A: Overall Incidence of Adverse Events Through Day 56 After Double-blind Period (Randomized, Transplanted and Treated Population) - Study IM103100

	No. (%) of Subjects		
	Belatacept MI (N=74)	**Belatacept LI (N=71)**	**CsA (N=71)**
Adverse Events	73 (98.6)	69 (97.2)	68 (95.8)
Discontinued Due to Adverse Events	13 (17.6)	15 (21.1)	14 (19.7)
Related Adverse Events	43 (58.1)	40 (56.3)	50 (70.4)
Serious Adverse Events	50 (67.6)	52 (73.2)	41 (57.7)
Related Serious Adverse Events	20 (27.0)	23 (32.4)	21 (29.6)
Deaths[a]	0	0	2 (2.8)

- [a] Includes all deaths up to 56 days after last dose of study therapy, by therapy received.

CsA = cyclosporine, LI = less intensive, and MI = more intensive.

The rate of AEs, including AEs resulting in discontinuation, was similar across the 3 treatment groups. The rate of SAEs was somewhat higher for both belatacept treatment groups than for the CsA treatment group. As described below, this difference is due to an increased number of reports of AEs of transplant rejection, not subsequently confirmed as transplant rejection, in the belatacept treatment groups.

The incidence of AEs, by Medical Dictionary for Drug Regulatory Activities (MedDRA) system organ class (SOC) and preferred term, is summarized in Table 1.3.4.4B.

Table 1.3.4.4B: Most Frequent Adverse Events (At Least 10% in Any Group) Through Day 56 After Double-blind Period (Randomized, Transplanted and Treated Population) - Study IM103100

	No. (%) of Subjects		
MedDRA System Organ Class Preferred Term	**Belatacept MI (N=74)**	**Belatacept LI (N=71)**	**CsA (N=71)**
Subjects with Any Adverse Events	73 (98.6)	69 (97.2)	68 (95.8)
Blood & Lymphatic System Disorders	29 (39.2)	28 (39.4)	40 (56.3)
Leukopenia	14 (18.9)	12 (16.9)	21 (29.6)
Anemia	13 (17.6)	12 (16.9)	21 (29.6)
Cardiac Disorders	10 (13.5)	10 (14.1)	10 (14.1)
Endocrine Disorders	4 (5.4)	8 (11.3)	9 (12.7)
Gastrointestinal Disorders	45 (60.8)	45 (63.4)	42 (59.2)
Nausea	19 (25.7)	18 (25.4)	16 (22.5)
Diarrhea	17 (23.0)	18 (25.4)	17 (23.9)
Constipation	16 (21.6)	22 (31.0)	20 (28.2)
Vomiting	11 (14.9)	14 (19.7)	11 (15.5)
General Disorders & Administration Site Conds.	43 (58.1)	40 (56.3)	42 (59.2)
Edema Peripheral	23 (31.1)	20 (28.2)	21 (29.6)
Pyrexia	15 (20.3)	19 (26.8)	15 (21.1)
Pain	7 (9.5)	6 (8.5)	9 (12.7)
Fatigue	6 (8.1)	6 (8.5)	9 (12.7)
Edema	6 (8.1)	7 (9.9)	11 (15.5)
Immune System Disorders	22 (28.7)	29 (40.8)	16 (22.5)
Transplant Rejection	19 (25.7)	23 (32.4)	11 (15.5)
Infections & Infestations	54 (73.0)	52 (73.2)	53 (74.6)
Urinary Tract Infection	17 (23.0)	17 (23.9)	22 (31.0)
Cytomegalovirus Infection	11 (14.9)	10 (14.1)	13 (18.3)
Nasopharyngitis	9 (12.2)	10 (14.1)	11 (15.5)
Injury, Poisoning & Procedural Complications	44 (59.5)	45 (63.4)	45 (63.4)
Incision Site Complication	17 (23.0)	16 (22.5)	13 (18.3)
Post Procedural Pain	14 (18.9)	17 (23.9)	15 (21.1)
Graft Dysfunction	9 (12.2)	10 (14.1)	10 (14.1)

Table 1.3.4.4B: Most Frequent Adverse Events (At Least 10% in Any Group) Through Day 56 After Double-blind Period (Randomized, Transplanted and Treated Population) - Study IM103100

	No. (%) of Subjects		
MedDRA System Organ Class Preferred Term	**Belatacept MI (N=74)**	**Belatacept LI (N=71)**	**CsA (N=71)**
Investigations	26 (35.1)	22 (31.0)	29 (40.8)
Blood Creatinine Increased	13 (17.6)	10 (14.1)	13 (18.3)
Metabolism & Nutrition Disorders	36 (48.6)	35 (49.3)	42 (59.2)
Hypophosphatemia	14 (18.9)	24 (33.8)	15 (21.1)
Hyperlipidemia	9 (12.2)	8 (11.3)	6 (8.5)
Hypercholesterolemia	6 (8.1)	4 (5.6)	9 (12.7)
Hypokalemia	5 (6.8)	5 (7.0)	9 (12.7)
Musculoskeletal & Connective Tissue Disorders	26 (35.1)	20 (28.2)	20 (28.2)
Arthralgia	8 (10.8)	6 (8.5)	4 (5.6)
Back Pain	8 (10.8)	3 (4.2)	6 (8.5)
Nervous System Disorders	26 (35.1)	20 (28.2)	26 (36.6)
Headache	13 (17.6)	10 (14.1)	8 (11.3)
Tremor	8 (10.8)	10 (14.1)	14 (19.7)
Psychiatric Disorders	18 (24.3)	27 (38.0)	20 (28.2)
Insomnia	12 (16.2)	19 (26.8)	17 (23.9)
Renal & Urinary Disorders	28 (37.8)	27 (38.0)	25 (35.2)
Reproductive System & Breast Disorders	7 (9.5)	12 (16.9)	7 (9.9)
Respiratory, Thoracic & Mediastinal Disorders	23 (31.1)	24 (33.8)	29 (40.8)
Cough	7 (9.5)	8 (11.3)	11 (15.5)
Dyspnea	5 (6.8)	6 (8.5)	9 (12.7)
Skin & Subcutaneous Tissue Disorders	26 (35.1)	18 (25.4)	18 (25.4)
Vascular Disorders	27 (36.5)	29 (40.8)	29 (40.8)
Hypertension	16 (21.6)	17 (23.9)	22 (31.0)

Note: The number of adverse events for transplant rejections includes investigator-reported transplant rejections, often obtained at the time of biopsy, irrespective of central blinded histological evaluation and/or local evaluation. All cases of centrally-confirmed clinically-suspected and biopsy-proven acute rejection and biopsy-proven acute rejection are reported in Table 1.3.4.3.A.

CsA = cyclosporine, LI = less intensive, MedDRA = Medical Dictionary of Drug Regulatory Activities, and MI = more intensive.

Transplant rejection was reported more commonly with both doses of belatacept than with CsA. Subsequent evaluation revealed that these reports reflected episodes of suspected acute

rejection later disproved by central biopsy, as well as episodes that resolved spontaneously without treatment. All reported AEs of transplant rejection were subsequently confirmed by biopsy. AEs commonly observed during CsA treatment, such as anemia, leukopenia, hirsutism, tremor, hypomagnesemia, and hypertension, were reported less frequently with belatacept than with CsA in this study. Infectious complications occurred with comparable frequency. Pulmonary edema and proteinuria were reported more frequently with belatacept than with CsA. The significance of these events requires further evaluation.

Serious Adverse Events

SAEs were reported somewhat more frequently in the belatacept treatment groups than in the CsA group (see Table 1.3.4.4C). This difference is accounted for by an increased frequency of reporting acute rejection as an AE in the belatacept groups. Subsequent evaluation revealed that these reports reflected episodes of suspected rejection later disproved by central biopsy, as well as episodes that resolved spontaneously without treatment.

Three subjects treated with the belatacept MI regimen developed post-transplant lymphoproliferative disorder (PTLD). One case occurred on treatment and the others occurred 2 months and > 1 year after discontinuation of the study drug. The subject that developed PTLD on treatment was Epstein-Barr virus (EBV) negative and received an EBV positive allograft. This subject was diagnosed with PTLD 9 months after transplantation from a biopsy of a lesion near the basal ganglia, and belatacept was discontinued. The subject died 5 months later from *Pneumocystis carinii* pneumonia and recurrent *Cytomegalovirus* (CMV) infection while receiving dexamethasone and sirolimus. A second subject was diagnosed with PTLD 4 months after transplantation and 2 months after discontinuation of belatacept with initiation of tacrolimus. The diagnosis was based upon a renal allograft biopsy performed for suspected acute rejection. The tumor tissue and urine tested positive for EBV, and retrospective analysis of stored sera from the recipient tested negative for EBV. This subject underwent a transplant nephrectomy. A final subject received 4 doses of belatacept before discontinuation for a Grade IIB rejection, which was treated with a 10-day course of OKT3®. PTLD was diagnosed from an excisional biopsy of an anterior cervical lymph node 12 months after discontinuation of study drug. Additional information on these cases is provided in the Investigator Brochure.

One subject treated with the belatacept MI regimen developed breast cancer after 12 months of treatment. In retrospect, the baseline mammogram for this subject was abnormal. No subjects treated with the belatacept LI regimen developed malignancies. Two subjects treated with CsA developed malignancies – squamous cell carcinoma of the skin and thyroid cancer – while a third subject developed a parathyroid nodule not yet confirmed to be malignant.

Table 1.3.4.4C: Most Frequent (At Least 5% in Any Group) Serious Adverse Events Through Day 56 After Double-blind Period (Randomized, Transplanted and Treated Population) - Study IM103100

MedDRA System Organ Class Preferred Term	No. (%) of Subjects Belatacept MI (N=74)	Belatacept LI (N=71)	CsA (N=71)
Subjects with Any Serious Adverse Events	50 (67.6)	52 (73.2)	41 (57.7)
Blood & Lymphatic System Disorders	2 (2.7)	3 (4.2)	4 (5.6)
Gastrointestinal Disorders	7 (9.5)	7 (9.9)	5 (7.0)
General Disorders & Administration Site Conds.	5 (6.8)	8 (11.3)	7 (9.9)
Pyrexia	4 (5.4)	8 (11.3)	6 (8.5)
Immune System Disorders	20 (27.0)	23 (32.4)	13 (18.3)
Transplant Rejection	18 (24.3)	20 (28.2)	9 (12.7)
Infections & Infestations	17 (23.0)	12 (16.9)	18 (25.4)
Cytomegalovirus	5 (6.8)	4 (5.6)	7 (9.9)
Pyelonephritis	4 (5.4)	1 (1.4)	2 (2.8)
Urinary Tract Infection	2 (2.7)	0	4 (5.6)
Injury, Poisoning & Procedural Complications	8 (10.8)	6 (8.5)	9 (12.7)
Investigations	8 (10.8)	2 (2.8)	4 (5.6)
Blood Creatinine Increased	8 (10.8)	2 (2.8)	4 (5.6)
Metabolism & Nutrition Disorders	1 (1.4)	2 (2.8)	4 (5.6)
Renal & Urinary Disorders	9 (12.2)	11 (15.5)	9 (12.7)
Respiratory, Thoracic & Mediastinal Disorders	6 (8.1)	3 (4.2)	4 (5.6)
Vascular Disorders	3 (4.1)	5 (7.0)	8 (11.3)

- CsA = cyclosporine, LI = less intensive, MedDRA = Medical Dictionary of Drug Regulatory Activities, and MI = more intensive.

IM103-100 Study Follow-Up

The most recent unpublished analysis of the IM103-100 trial of Belatacept + cellcept and low dose steroid (5-10 mg prednisone per day) in clinical renal transplantation shows that there are 75 subjects on belatacept and Cellcept®-based regimens in long-term extension trials from the original IM103-100 study with no reports of subsequent post transplant lymphoproliferative disorder in this group.

Nine of these long-term study subjects are being followed at Emory University. Out of the 9, one was non-compliant with therapy and had a rejection episode that was successfully reversed. Eight of the 9 Emory subjects have excellent, stable renal function (mean Cr = 0.80) with up to 6 years of follow-up.

Results of a Phase III Study of Belatacept in Renal Transplantation (BENEFIT)[9]

In the phase III BENEFIT trial, one of the largest studies in kidney allograft recipients, two NULOJIX® (belatacept) regimens were compared to cyclosporine as the cornerstone of maintenance therapy for standard risk recipients from standard risk donors. All subjects received basiliximab induction and maintenance therapy consisting of mycophenolate mofetil (MMF) and prednisone. At 12 months, both belatacept regimens demonstrated similar patient and graft survival as a composite endpoint compared with cyclosporine (95% MI; 97% LI; 93% cyclosporine). Belatacept was associated with superior renal function compared with cyclosporine as measured by a composite renal impairment endpoint (defined as CrCl < 60 ml/min/1.73 m2 or a decrease in CrCl of > 10% between months 3 and 12; 55% MI; 54% LI; 78% cyclosporine; P≤ 0.001 MI or LI vs. cyclosporine), and measured glomerular filtration rate at Month 12 (65, 63, and 50 mL/min for MI, LI and cyclosporine, respectively; P≤ 0.001 MI or LI vs. cyclosporine).

However, belatacept-treated patients experienced a higher incidence (22% MI; 17% LI; 7% CsA) and grade (more frequent grade ≥2) of acute rejection episodes. Despite this ostensibly more aggressive rejection profile, belatacept-treated subjects had very low rates of developing donor specific antibodies that trended toward being lower than the rate observed in the cyclosporine comparison group. Importantly, belatacept-treated patients with acute rejection had better renal function at 12 months than cyclosporine-treated patients without acute rejection. In addition, belatacept-treated subjects showed a trend toward less chronic allograft nephropathy and improved cardiovascular and metabolic profiles (superior blood pressure control and lipid profiles) compared with cyclosporine-treated subjects one year post-transplant, despite the increase in early acute rejection.

1.3 Rationale for Selection of Study Population

Iatrogenic hypoglycemia is a major unresolved problem for many patients with T1D. It is the limiting factor in the management of T1D, causing some deaths as well as recurrent physical, and recurrent (or even persistent) psychosocial, morbidity [11]. Iatrogenic hypoglycemia is a consequence of 3 compromised defense mechanisms, whose pathophysiology was thoroughly reviewed by Cryer [11-14].

First and perhaps most important, glucose-regulated insulin levels are not present in c-peptide-negative T1D patients. The second defense mechanism, glucagon secretion in response to developing hypoglycemia, is lost in virtually all patients with T1D within 5 to 10 years after its onset [15]. Third, epinephrine response to falling glucose levels is compromised, in terms of the magnitude of the response and the threshold for the response [16], in a subgroup of patients with T1D. Epinephrine is not normally critical, but becomes so when the insulin and glucagon responses are deficient or absent. Those T1D patients with an absent insulin response and combined deficiencies of their glucagon and epinephrine responses to falling plasma glucose levels have the clinical syndrome of defective glucose counterregulation; their risk of severe hypoglycemia is 25-fold or more higher than that of those with absent glucagon but intact epinephrine responses [17, 18]. T1D patients with impaired epinephrine responses also have the clinical syndrome of hypoglycemia unawareness, which refers to the absence of adequate autonomic warning symptoms of developing hypoglycemia.

Hypoglycemia unawareness and the associated inability to respond adequately to falling glucose levels explain the frequent episodes of neuroglycopenia in such patients. Moderate

hypoglycemia refers to a hypoglycemic episode complicated by neuroglycopenia in which the patient is still able to overcome the situation without assistance. Severe hypoglycemia refers to a situation in which neurologic impairment is severe enough to prevent self-treatment, placing patients at risk for injury to themselves or others. Accordingly, the DCCT Research Group defined severe hypoglycemia as an event with symptoms consistent with hypoglycemia in which the patient requires the assistance of another person; it is associated with a blood glucose level below 50 mg/dL and with prompt recovery after oral carbohydrate, intravenous glucose, or glucagon administration [19]. The DCCT Research Group definition replaced the more stringent 1980s definition of severe hypoglycemia based on loss of consciousness [20-22].

Cryer suggested viewing the 3 clinical syndromes (defective glucose counterregulation, hypoglycemia unawareness, and elevated glycemic thresholds) during effective intensive insulin therapy as manifestations of hypoglycemia-associated autonomic failure. All 3 syndromes segregate together and are associated with a high frequency of iatrogenic hypoglycemia. Parenthetically, they do not segregate with classical diabetic autonomic neuropathy [17, 23, 24]. Hypoglycemia-associated autonomic failure is triggered by recurrent episodes of hypoglycemia, which reduce the magnitude of hormonal counterregulation and reduce symptomatic responses to a given degree of subsequent hypoglycemia [13, 25], thereby initiating and perpetuating a vicious cycle.

Hypoglycemia-associated autonomic failure is an important risk factor for severe hypoglycemia, which is associated with significant morbidity and mortality. Patients with hypoglycemia unawareness have a nearly 7-fold increased risk of severe hypoglycemia [26]. Those with combined deficiencies of their glucagon and epinephrine responses to falling plasma glucose levels have a 25-fold or more greater risk of subsequent severe hypoglycemia, as compared with those with absent glucagon but intact epinephrine responses [17, 18]. The patient characteristic that most strongly predicted severe hypoglycemia in the DCCT was a history of prior severe hypoglycemic events [27].

In addition to the increased morbidity and mortality associated with severe hypoglycemia, it also has detrimental psychosocial consequences. For example, hypoglycemia is said to be a major concern of prospective employers [28]. Neuroglycopenia can cause social embarrassment, and even lead to ostracism or be mistaken for disorderly or unlawful behavior [11]. The more distressing the severe hypoglycemic episode, the greater the psychological fear of hypoglycemia[29]. The threat and fear of severe hypoglycemia can significantly discourage patients and health care providers from pursuing intensive insulin therapy and can therefore can be a major but unrecognized impediment to achieving euglycemia [11, 30]. Pramming et al. found that their patients were as concerned about the development of severe hypoglycemia as they were about the development of blindness or renal failure [31].

In patients with long-term (i.e., >15 years) T1D, scrupulous avoidance of hypoglycemia fails to restore normal glycemic thresholds or magnitudes of hormonal counterregulation to hypoglycemia. Avoidance of iatrogenic hypoglycemia sufficient to reverse the clinical syndrome of hypoglycemia unawareness does not normalize the key components of the clinical syndrome of defective glucose counterregulation (i.e., deficient glucagon and epinephrine responses to hypoglycemia)[32-36]. In one recent report on patients with autonomic neuropathy and longstanding diabetes, Fanelli et al. demonstrated that, after meticulous prevention of hypoglycemia, only the threshold – not the magnitude – of responses of autonomic symptoms was normalized. In addition, the recovery of epinephrine responses to hypoglycemia was

barely appreciable [37]. Thus, it appears that, while hypoglycemia unawareness is reversible by meticulous prevention of hypoglycemia, defective glucose counterregulation may not be reversible [38].

A successful pancreas transplant restores epinephrine responses and symptom recognition during hypoglycemia in patients with longstanding T1D and autonomic neuropathy [39]. In T1D islet transplant recipients with documented pretransplant hypoglycemia unawareness and defective hormonal counterregulatory responses during hypoglycemia, Myer et al. demonstrated, at 1 month post-transplant, improved glycemic thresholds and/or peak incremental responses of epinephrine, norepinephrine, and cortisol, as well as restoration of autonomic warning symptoms during hypoglycemia [40]. In a more recent study by Paty et al., intrahepatic islet transplantation did not restore hypoglycemic hormonal counterregulation or symptom recognition in T1D recipients[41]. Ryan et al. documented the absence of episodes of severe hypoglycemia in 12 successful islet transplant recipients (median follow-up, 10.2 months) [42] whose diabetes was complicated by recurrent episodes of severe hypoglycemia pretransplant. This would suggest that hypoglycemia associated autonomic failure in conjunction with defective counterregulation and impaired sympathoadrenal responses are not just due to recurrent hypoglycemia. After a sustained period without any hypoglycemia most patients post islet transplant still had defective responses to hypoglycemia. The absence of clinically significant hypoglycemia post islet transplant despite the persistent defect in counterregulation in most subjects demonstrates the dominance of the absence of glucose regulated insulin secretion in the pathogenesis of severe hypoglycemia. Correction of this can only currently be attained with transplantation of beta cell tissue.

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

1.3.1 Glycemic Lability

Defining labile diabetes is a challenge but a working definition of labile diabetes may be: “Very variable glucose control associated with unpredictable responses to insulin”. Labile diabetes is akin to the more extreme brittle diabetes which has been defined as describing the patient “whose life is constantly being disrupted by episodes of hypo- or hyperglycemia whatever their cause“ [43, 44]. Brittle diabetes in addition to lability has the added connotation that there may be associated frequent admissions to hospital [45, 46]. Given the rationing of health care over the last decade use of such parameters such as admission to hospital has become problematic. Early publications postulated that brittle diabetes was related to SC insulin degradation [47] but now the most severe cases are recognized to usually have a factitious origin [48]. While the most extreme cases of labile diabetes, whether associated with recurrent hypoglycemia or diabetic ketoacidosis, may fall into the traditional brittle definitions, there are many patients with T1D who have very labile glucose control that is a source of frustration for them and their caregivers.

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

The HbA1c is the standard measure of glucose control and is used in all major studies as an endpoint of glycemic control. It has been valuable as a risk predictor of diabetes complications. Yet the HbA1c may be misleading. Patients with erratic glucose control, especially if having hypoglycemic unawareness, can have an HbA1c below 7%, yet the most chaotic and difficult glycemic control. Mean glucose values suffer the same problem in that swings in glucose values between 2 and 20 and back to 2 mmol/L may give a mean glucose of 8 mmol/L, a poor reflection of the real state of affairs.

Previous efforts at measuring glucose lability have ranged from qualitative to quantitative. Earlier definitions of brittle diabetes have incorporated visits to the hospital [45, 46] but these are subject to the vagaries of local practice. More quantitative measures have been the mean amplitude of glycemic excursions (MAGE) [49] and the M value of Schlichtkrull [50]. The MAGE relies on capillary glucose readings over two days (a minimum of seven readings a day) and an amplitude is an excursion of glucose in excess of the standard deviation of the mean values for the day. If the mean of these amplitudes is ≥ 11.1 mmol/L the subject is considered to have labile diabetes. Where the MAGE fails as a measure of lability is with the subject who has a gradual decline in glucose over the day from 22 to 2 mmol/L. Such a profile will give a MAGE of 20 but such a gradual decline need not be considered truly labile.

Also used in the past has been the M value of Schlichtkrull [50] but this logarithmic expression of the glucose deviation from a standard glucose level has not been validated. More recently [51] the advent of continuous glucose monitoring system (CGMS®) has allowed insight into the patterns of glucose. The CGMS® profiles give exquisite details that have been quantified in terms of mean and standard deviation. Determining lability with this process has been more difficult and the suggested method has been the determination of the absolute value of measured glucose minus 5.5 mmol/L. This has the drawback that sustained high glucose levels will result in a high value but the profile may not necessarily be labile. In addition, the technique is currently limited to three days of monitoring and may be less accurate at low glucose levels [52].

Any measure needs to be robust enough to handle a variety of glucose monitoring patterns used in day-to-day diabetes practice, intuitive in that it measured glucose swings, mathematically rigorous and finally easy to use. A newer measure of lability based on the change in glucose over time has been the Lability Index (LI) [53]. A typical range for a diabetes population was calculated in 100 subjects with T1D who were not selected because of any particular problems. Most subjects have scores under 300 mmol/L^2/h·wk^{-1} with a median of 223 (25 – 75th percentiles 130 – 329 mmol/L^2/h·wk^{-1}). An LI ≥ 433 mmol/L^2/h·wk^{-1} (90th percentile) indicated serious problems with glycaemic lability. The LI correlated well with a

clinical scoring of lability by diabetologists and showed improvement after successful islet transplantation and rose when graft function was lost.

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

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

1.4 Rationale for Selection of Study Regimen

1.4.1 Investigational Products:

1.4.1.1 ALLOGENEIC ISLETS

T1D is an autoimmune disease where destruction of the insulin producing pancreatic β cells occurs, leading to severely dysregulated glucose homeostasis. It afflicts nearly 2 million people in the United States, most of them children or young adults. Despite the effectiveness of insulin therapy in allowing these patients to survive, the imperfect control of BG excursions common with insulin injections eventually results in vascular complications in many. In fact, in adults diabetic retinopathy is the most common cause of blindness and diabetic nephropathy is the most common indication for kidney transplantation. The DCCT established that these microvascular complications of diabetes can be prevented by maintaining near-normal glucose control using multiple daily injections of insulin or insulin "pump" therapy in patients with T1D. [54] However, this degree of control can be impossible to achieve in many patients despite modern insulin analogs and delivery systems, [55] and also leads to life threatening episodes of insulin-induced hypoglycemia. [56]

The hope of achieving near-normal glucose control without hypoglycemia in T1D patients has provided the strong impetus for developing effective strategies for β-cell replacement via pancreas or isolated islet transplantation. When successful, pancreas transplantation can normalize BG in diabetic recipients, with resultant stabilization and even reversal of microvascular complications. [57] However, despite the ability of whole organ pancreas

transplantation to correct glucose homeostasis in T1D, the procedure requires major surgery and is not without risk. According to United Network for Organ Sharing (UNOS) pancreas registry data, almost 10% of whole organ pancreas grafts fail early due to technical complications and require an additional laparotomy for graft removal. Other morbid complications such as anastomotic leak, bleeding, and infection are even more common. As a result of the magnitude of the operation and its potential complications (including death – usually from a perioperative myocardial infarction), this procedure is generally reserved in most centers for diabetics who are less than 50 years of age, have minimal if any coronary artery disease, and because of the risks of chronic immunosuppression, already require a kidney transplant for the treatment of end-stage diabetic nephropathy. While whole pancreas transplantation has been performed in non-uremic T1D patients experiencing severe problems with metabolic control, long-term pancreatic graft function and survival is inferior when compared to simultaneous pancreas-kidney transplantation, primarily due to immunologic graft loss. Thus, T1D patients in need of β-cell replacement to stabilize their metabolic control are often excluded from whole pancreas transplantation unless they also require a kidney graft.

Transplantation of isolated pancreatic islets offers the distinct advantage over whole organ pancreas transplantation that it can be accomplished with less procedural related morbidity. Consequently, isolated islet transplantation is a much safer treatment, and so may be considered as an option for patients before the development of irreversible diabetic complications. But until recently, <10% of islet transplant recipients experienced insulin-independence after one year, in contrast to the ~ 80% of whole pancreas transplant recipients. The lower rate of insulin-independence following islet transplantation was attributed to a low engrafted islet mass combined with a high metabolic demand imposed by the glucocorticoids used as part of the immunosuppression. Accordingly, the islet transplant group from Edmonton initiated a protocol where islets isolated from two or more donor pancreata were transplanted under a glucocorticoid-free immunotherapy regimen.

In the year 2000, the initial report of success with the "Edmonton protocol" represented a major advance in the field of clinical islet transplantation, where insulin-independent amelioration of hyper- and hypoglycemia occurred in seven consecutive T1D recipients with a median follow-up of 12 months.[58] The immunosuppression regimen consisted of a combination of novel T lymphocyte directed induction therapy with the interleukin-2 receptor monoclonal antibody daclizumab, and maintenance therapy with the potent calcineurin-inhibitor (CNI) tacrolimus and the more recently developed agent rapamycin. The efficacy of the Edmonton approach has now been confirmed by several other centers, including reports where single donor transplant recipients enjoyed a high rate of initial insulin-independence. [59] Unfortunately, loss of graft function occurs over time, and insulin-independence rates at Edmonton have declined from 72% at one year to 28% by three years[60]. Similarly insulin-independence rates at Miami have declined from 79% at one year to 20% by three years[61]. Recent data demonstrate a functionally low engrafted β-cell mass in insulin-independent transplant recipients under Edmonton immunosuppression that likely declines over time[62], suggesting that the eventual recurrence of diabetes and return to insulin therapy may result from both early (engraftment) and late (survival) immunologic graft loss. Novel strategies aimed at promoting the engraftment or survival of transplanted islets may lead to improved long-term graft function and more sustained insulin-independence for T1D patients.

1.4.1.2 NUJOLIX® (BELATACEPT)

NUJOLIX® (belatacept) is approved in the U.S. for the prophylaxis of organ rejection in adult patients receiving a kidney transplant, in combination with basiliximab induction, mycophenolate mofetil (MMF), and corticosteroids.

Belatacept is a soluble chimeric protein designed to selectively inhibit costimulation of T-cells. T-cells require 2 signals for activation. The first signal, which is antigen specific, is delivered by engagement of the T-cell receptor (TCR) with antigen presented in context with major histocompatibility complex molecules on the APC. The second, or co-stimulatory signal, is delivered by engagement of co-stimulatory ligand on the APC with a receptor on the T-cell. A key co-stimulatory signal is provided by the interaction of B7-1 (CD80) and B7-2 (CD86) on APCs with CD28 expressed on T-cells. In the absence of this second signal, the T-cell becomes anergic (unresponsive) or undergoes apoptosis.

Conversely, if the T-cell becomes fully activated, CTLA4 (CD152) becomes expressed on the cell surface. CTLA4 has a substantially higher avidity than CD28 for CD80 and CD86 (approximately 500- to 2,500-fold). The increased avidity of endogenous CTLA4, in comparison with CD28, affords a homeostatic mechanism to down-regulate T-cell activity.

Belatacept was derived from CTLA4Ig (abatacept, BMS-188667), a novel fusion protein consisting of the extracellular domain of human CTLA4 fused to fragment of the Fc domain of a human immunoglobulin (Ig) G1 antibody. By binding avidly to CD80/86, CTLA4Ig blocks the interaction of the T-cell's CD28 with the APCs CD80/CD86, thus preventing T-cells from receiving the required second costimulatory signal. CTLA4Ig has been shown to be efficacious in a wide variety of preclinical models and in subjects with psoriasis and rheumatoid arthritis. With respect to transplantation, CTLA4Ig demonstrated efficacy in rodent models of transplantation, but did not demonstrate substantial efficacy in non-human primate models (cynomolgus monkeys). Therefore belatacept, a 2 amino acid variant of CTLA4Ig was developed. This alteration resulted in markedly increased binding avidity for B7 molecules. Belatacept was subsequently shown to have efficacy in non-human primate renal transplant model in which CTLA4Ig was not efficacious[7]. Belatacept was also shown to be efficacious in Phase 2 and 3 clinical trials in de novo renal transplant recipients.

1.4.2 Immunosuppressive Medications

In this trial, we hypothesize that avoidance of the diabetogenic drug, tacrolimus and complete avoidance of the anti-angiogenic drug, sirolimus will create a more favorable environment for islet engraftment and function, and will thus substantially improve the rate of success with single donor transplants and minimize or avoid non-immune toxicities such as hypertension, hyperlipidemia, and nephrotoxicity. The trial is a two-center, prospective pilot study of islet transplantation using a steroid-free, calcineurin-inhibitor-free belatacept- based immunosuppressive medication in 20 subjects with long-standing T1D that is refractory to intensive insulin therapy that meet all additional entry criteria and has given informed consent.

The study medication, belatacept, will be administered using the modified less intensive regimen that was derived from the phase 2 human renal transplant trial and is consistent with the less intensive dosing regimen used in the phase III program. Based on results from the

completed phase 2 study, both the less intensive and more intensive regimens appear acceptable. Both regimens met the primary endpoint of non-inferiority to CsA in acute rejection. Both exhibited subject/graft survival similar to CsA, improved GFR, and a lower incidence of CAN. The less intensive dosing regimen will be augmented with a dose at Day 4 to ensure adequate immunosuppression in the early, immunologically-critical, period. Based on this information we will employ the modified lower intensity regimen when testing belatacept. This should result in achieving target levels consistently without excessive immunosuppression.

Since tacrolimus target trough ranges in the Edmonton protocol are 3-6 ng/mL, we have set our dose for mycophenolate mofetil in the proposed belatacept trial at 1g PO BID. The long term follow-up data from the IM103-100 study suggest synergy and tolerability and safety with the combination of mycophenolate mofetil and belatacept. This suggests a similar protection from rejection for the proposed trial. We further believe that the side effect profile associated with mycophenolate mofetil will be far superior to that associated with high dose sirolimus, and that there will be a far more favorable toxicity profile compared to Edmonton Protocol treated patients. The dominant side effects of mycophenolate mofetil (for example, gastrointestinal toxicity) will be more tolerable than the primary side effects of sirolimus. A monoclonal antibody IL-2 receptor blocker - basiliximab – will be administered with each transplant. As new information becomes available from the belatacept kidney trial our plan will be reevaluated.

1.5 Known and Potential Risks and Benefits to Human Subjects

1.5.1 Risks of Primary Investigational Agent: Allogeneic Islets

Transplantation of islets is associated with several potential risks. These risks may be categorized in terms of: a) transmission of disease from donor to recipient, b) risk of microbial contamination of islet preparations, c) sensitization of the recipient to donor antigens, d) acceleration of retinopathy with acute correction in glycemic control, and e) psychological impact of successful or failed islet transplantation. Other risks including portal thrombosis, portal hypertension, bleeding or hepatic steatosis are discussed separately in Section 1.5.4.

1.5.1.1 TRANSMISSION OF DISEASE FROM DONOR TO RECIPIENT

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

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

Donors are excluded if: a) there is known pre-existing metabolic disease including T1 or Type 2 diabetes, or if the HbA1c is elevated above 6.1% in the absence of transfusions in the week prior

to death, b) if there is malignancy other than primary brain tumors, c) septicemia is present or suspected at the time of death, d) there is evidence of clinical or active viral hepatitis (A, B or C), acquired immunodeficiency syndrome (AIDS), syphilis, active viral encephalitis of unknown origin, Creutzfeldt-Jacob disease, rabies, treated or active tuberculosis, septicemia, dementia, individuals that have received pituitary growth hormone (pit-hGH), or serious illness of unknown etiology.

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

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

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

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

1.5.1.2 RISK OF MICROBIAL CONTAMINATION OF ISLET PREPARATIONS

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

In 152 islet preparations transplanted consecutively at the University of Alberta since 1999, there have been no cases of transmission of bacterial or fungal disease through islet

transplantation, when islets are prepared under current Good Manufacturing Practices (cGMP) conditions. One recipient of an islet autograft received an infected islet preparation as the autograft pancreas contained a chronic embedded pancreatic stent that likely led to bacterial colonization and contamination. This recipient developed transient complete thrombosis of the portal vein with subsequent recanalization.

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

There have been previous reports of two cases of islet transplantation-related septicemia (Enterobacter cloacae) due to transplantation of contaminated cryopreserved pancreatic islets [63]. Additionally, the University of Minnesota investigators have previously reported on the incidence and significance of contaminated islet preparations in clinical islet auto- and allotransplantation [64]. Positive cultures from islet tissue preparations were identified in 11 of 29 patients (38%) receiving autologous islets. The occurrence of serious infection morbidity (as defined as positive blood cultures, abscesses, or intra-abdominal infections) did not differ significantly between the positive and negative culture groups (p=0.99). In the allogeneic islet transplant group, 7 of 33 patients (21%) received tissue that retrospectively was determined to be contaminated. None of these patients developed serious infectious complications (despite broad-spectrum immunosuppression). Despite the occurrence of contaminated grafts, there was no serious increase in infectious morbidity. Presumably the inocula were kept low by the multiple washing steps allowing the recipients to clear the organisms without serious sequelae.

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

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

1.5.1.3 SENSITIZATION OF THE RECIPIENT TO DONOR ANTIGENS

As with any allogeneic transplant, islet transplant recipients may become sensitized to islet-donor histocompatibility antigens (HLA), leading to development of panel reactive alloantibodies (PRA). These alloantibodies may develop while the recipients demonstrate full or partial islet function on maintenance immunosuppression. Furthermore, donor specific alloantibodies may develop after loss of the islet transplant function and discontinuation of the immunosuppressant drug. Data on the development of cytotoxic antibodies against donor HLA in islet allotransplant recipients with failing grafts have been reported from several islet transplant centers[65-68]. In the ITN-sponsored trial of islet transplantation using the Edmonton protocol of steroid-free immunosuppression, 5 of 36 subjects had evidence of elevated PRA post-transplant when measured by flow cytometry. Two of these 5 subjects experienced primary islet non-function. Moreover, data from five participating centers in the current CIT consortium indicate that approximately 25% of the islet alone transplant recipients developed a

PRA >20% while on maintenance immunosuppression. These results are comparable to those reported for recipients of kidney transplant with stable serum creatinine and on maintenance immunsuppression[69-71]. Importantly, the incidence of elevated PRA (>20%) in recipients who had lost their islet transplant function and discontinued their immunosuppression rose to approximately 84%.

The available information suggests that there is a strong correlation between islet allograft failure and a rise in anti-donor HLA sensitization as detected by PRA testing. A potential consequence of high PRA levels in type 1 diabetic recipients with failed islet transplants is that if these individuals develop diabetic nephropathy in the future, it may increase their time waiting on a transplant list to qualify for a suitable kidney[72].

1.5.1.4 ACCELERATION OF RETINOPATHY WITH ACUTE CORRECTION IN GLYCEMIC CONTROL

In the DCCT study [19], about 10% of patients with pre-existing retinopathy receiving intensive treatment experienced a transient worsening of their retinopathy during the first year, but nonetheless had a lower cumulative incidence of sustained progression when compared to the conventional group after the third year. A transient worsening of retinopathy has not been formally documented in islet transplantation trials, but it is assumed that a similar process might occur. Exclusion of patients with unstable retinopathy and careful post transplant follow-up will help to minimize the incidence of such occurrences and their morbidity should they occur.

When 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[73]. Long-term follow-up of both groups suggested that successful pancreas transplantation may have a late beneficial effect that becomes evident only after 36 months.

1.5.1.5 PSYCHOLOGICAL IMPACT OF SUCCESSFUL OR FAILED ISLET TRANSPLANTATION

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

1.5.2 Risks of Secondary Investigational Agent: Belatacept

NULOJIX® (belatacept) is contraindicated in transplant recipients who are Epstein-Barr virus (EBV) sero negative or with unknown EBV serostatus due to the risk of post-transplant lymphoproliferative disorder (PTLD), predominantly involving the central nervous system (CNS).

Potential Risks in Renal Transplantation

Post-transplant Lymphoproliferative Disorder

In the combined BMS-sponsored Phase 2 (median exposure 74 to 88 months) and Phase 3 studies (median exposure of approximately 39 months) in de novo renal transplantation, post-transplant lymphoproliferative disorder (PTLD) developed more frequently in patients who received belatacept (14 cases out of 949; 1.5% of subjects) than those who received cyclosporine (3 cases out of 476; 0.6%). Of the PTLD cases reported with belatacept, all but 1 occurred during the first 18 months post transplant. More than half of the PTLD cases in belatacept-treated patients involved the CNS (9 cases out of 14; 65% of belatacept patients). A total of 8 out of 14 patients with PTLD in the belatacept group and 3 out of 3 in the cyclosporine group have died.

The excess risk of PTLD with belatacept was concentrated in EBV negative recipients (approximately 10-fold higher than that observed in EBV positive recipients). While there was also an increased risk in EBV positive subjects with belatacept compared to CsA within the studies, the absolute risk in this population was low. In addition to EBV-negative serostatus, CMV disease, and use of lymphocyte depleting therapy for treatment of AR were also associated with an increased risk of PTLD in the core belatacept studies. Nonetheless, the highest risk of PTLD with belatacept was observed in EBV-negative subjects. Thus, belatacept should not be administered to belatacept naïve patients who are EBV-negative or have unknown EBV serostatus. PTLD should be considered in subjects who develop new neurologic signs or symptoms.

Malignancy

An increased incidence of malignancy is a recognized complication of immunosuppression in recipients of organ transplants. In the Phase 3 studies, overall malignancy rates were similar across all treatment groups, with the exception of PTLD.

Infection

Increased susceptibility to infection, including serious and fatal infections may result from the use of belatacept, as with all immunosuppressive therapies. Overall incidences of infections, including serious fungal and viral infections, were similar across all treatment groups in the Phase 3 studies over the 36 month period of observation. The most common serious infections across treatment groups were urinary tract infection (UTI) and CMV infections.

Progressive Multifocal Leukoencephalopathy (PML)

One (1) case of progressive multifocal leukoencephalopathy (PML) has been reported in the belatacept renal transplantation program, in a subject receiving the more intensive regimen in study IM103027. PML should be considered in subjects who develop new neurologic signs or symptoms.

Infectious Disease

Tuberculosis has been more frequently reported in belatacept-treated patients than CsA-treated patients. There were a total of 13 TB cases (12 with belatacept and 1 with CsA) reported in the Phase 3 studies over 36 months. Nearly all cases of TB were reported in subjects who currently or previously resided in countries with a high prevalence of TB.

Other Potential Risks

Other potential risks include graft thrombosis, infusion-related reactions, proteinuria, congestive heart failure, and autoimmune disorders. These events have been observed infrequently in belatacept-treated subjects but are being closely monitored in all belatacept clinical trial.

Potential Risks in Liver Transplantation

A total of 250 subjects who received a liver transplant were randomized and treated in 5 treatment groups (3 belatacept-containing groups and 2 tacrolimus-containing groups): Group 1): Basiliximab + Belatacept MI + MMF; Group 2): Belatacept MI + MMF; Group 3): Belatacept LI + MMF; Group 4): Tacrolimus + MMF; and Group 5): Tacrolimus. Of these patients, 147 received belatacept. All subjects received corticosteroids that could be tapered or discontinued after Month 3 according to institutional practice.

Over the first 12 months of the study, there were 2 cases of post-transplant lymphoproliferative disorder (PTLD) reported in the belatacept groups; 1 patient died due to PTLD. There was 1 fatal case of progressive multifocal leukoencephalopathy (PML) in the belatacept more intensive (MI) group. The overall frequency of serious infections was not different between the groups, but there was an increase in viral and fungal infections in the belatacept groups versus the tacrolimus groups.

During the long-term extension phase of the study (beyond 12 months post-transplant), a higher number of deaths was observed in 2 of the 3 belatacept groups (belatacept MI+MMF and belatacept LI+MMF) when compared to the tacrolimus+MMF group. The frequencies of death were 12%, 21%, and 22% in the basiliximab+ belatacept MI+MMF, belatacept MI+MMF, and belatacept LI+MMF groups, respectively, in comparison to 6% in the tacrolimus+MMF group and 14% in the tacrolimus group. A causal relationship to belatacept could not be clearly established, but likewise could not be rejected. BMS in consultation with the Independent Data Monitoring Committee decided to terminate the study and recommend that all belatacept patients be switched to local standard of care.

1.5.3 Risk of Immunosuppressive Medication

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

1.5.3.1 BASILIZUMAB (SIMULECT®)

Basiliximab is an anti-IL-2R 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. 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. Additional information about basiliximab can be found in the package insert at: http://www.pharma.us.novartis.com/product/pi/pdf/simulect.pdf

1.5.3.2 MYCOPHENOLATE MOFETIL (CELLCEPT®)

Mycophenolate mofetil (Cellcept®) 1 g - 1.5 g BID is approved (in combination with cyclosporine and corticosteroids) as an immunosuppressive agent for renal, cardiac, and hepatic solid organ transplantation. Adverse events reported in > 30% of renal, cardiac or liver transplant patients receiving CellCept® were pain, fever, headache, asthenia, anemia, leukopenia, thrombocytopenia, leukocytosis, urinary tract infection, hypertension, hypotension, peripheral edema, hypercholesteremia, hypokalemia, hyperglycemia, increased creatinine and BUN, cough, hypomagnesemia, diarrhea, constipation, nausea, vomiting, respiratory infection, dyspnea, lung disorder, pleural effusion, tremor and insomnia.

There is an increased risk of developing lymphoproliferative disease, lymphomas, and other malignancies, particularly of the skin. Lymphoproliferative disease or lymphoma developed in 0.4% to 1% of patients receiving CellCept® 1 - 1.5 mg BID. Severe neutropenia developed in up to 2% of renal transplant recipients receiving CellCept® 1.5 mg BID. Mycophenolate mofetil (MMF) can cause fetal harm when administered to a pregnant woman. Cases of progressive multifocal leukoencephalopathy, sometimes fatal, and pure red cell aplasia have been reported in patients treated with CellCept®. Gastrointestinal bleeding (requiring hospitalization) has been observed in approximately 3% of renal, in 1.7% of cardiac, and in 5.4% of hepatic transplant patients treated with CellCept® 1.5 g BID. Additional information about CellCept® can be found in the package insert at:

http://www.rocheusa.com/products/cellcept/pi.pdf

1.5.3.3 TACROLIMUS (PROGRAF®)

Side effects of tacrolimus include hypertension, glucose intolerance, peripheral neuropathy, renal insufficiency, abnormal liver function studies, seizures, nausea, vomiting, confusion, hypomagnesemia, tremulousness, neurotoxicity, posterior reversible encephalopathy syndrome (PRES), progressive multifocal leukoencephalopathy (PML), interstitial lung disease, BK

nephropathy, and increased risk of secondary malignancies. Additional information about tacrolimus can be found in the package insert at:

http://www.prograf.com/pdf/prograf_full_prescribing_information.pdf

1.5.4 Risk of Study Procedures

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

1.5.4.1 BLOOD DRAW TESTING

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

1.5.4.2 METABOLIC STIMULATION TESTING

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

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

1.5.4.3 THE PROCEDURAL RISKS OF ISLET TRANSPLANTATION

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

Open Surgical Approach

This procedure is usually carried out under general anesthesia, but can be performed occasionally under local anesthesia if required. The potential risk of acute bleeding is anticipated to be less with a controlled operative approach as opposed to a percutaneous approach, especially where a transplant site does not have access to local expertise in advanced interventional radiological procedures. Access to a tributary of the portal vein using the open technique requires a surgical incision for exposure, and direct cannulation of a branch of the middle colic vein, the inferior mesenteric vein, a tributary of the superior mesenteric vein or direct cannulation of a small omental vein. Potential acute surgical risks include bleeding at the surgical site, portal thrombosis, hepatic abscess, hepatic infarction, mesenteric ischemia and mesenteric thrombosis. The general risks of surgery include wound infection, wound hernia, adhesional bowel obstruction, deep vein thrombosis and pulmonary embolism. Risks associated with anesthesia include difficulties with airway management, cardiac arrhythmias

and drug-related anaphylactic reactions. Pain and discomfort at the surgical site is expected in the early period following surgery, and may be reduced by administration of opiate, opioid or non-steroidal analgesic medications. If an ileus develops, a prolonged hospital stay may be anticipated.

Percutaneous Transhepatic Approach

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

Risk of Bleeding after Percutaneous Islet Transplantation

In the 158 islet transplant procedures submitted to the Collaborative Islet Transplant Registry (CITR), the reported SAEs associated with bleeding include hemoperitoneum (n=1), intraabdominal bleed (n=2), low hemoglobin (n=1), right hemothorax (n=1), and subcapsular hematoma (n=1) of the liver[74]. 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 [75]. One instance of injury to hepatic artery leading to death during percutaneous transhepatic catheterization of the portal vein has been reported previously to the Islet Transplant Registry [75]. Reports on intra-abdominal (n=1) [76]and intrathoracic bleeding (n=1) [77] have been published. The risk of significant hemorrhage after percutaneous islet transplantation defined as a drop in hemoglobin of more than 25 g/L or the need for transfusion or surgery was 9% in the Edmonton series[78]. 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 [79]. The risk has since been ameliorated by avoidance of pre-transplant aspirin and more effective measures to seal the catheter tract in the liver [79]. 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 [80]. At the University of Minnesota, no bleed-related complications occurred in 20 consecutive subjects when the catheter tract was sealed with combined coils and gelfoam [81].

Hypoglycemia

Severe hypoglycemia is a risk associated with the infusion of islets. Iatrogenic hypoglycemia in the immediate post-transplant period is a rare event. Frequent blood glucose monitoring immediately following islet transplantation is recommended to avoid severe unrecognized hypoglycemia in the early post-transplant period. In longer-term follow-up, life-threatening hypoglycemia (Grade 4) occurred in six of the 236 SAEs reported to CITR[74]. For these six occurrences, the events occurred at the following time intervals; 59 days post the third infusion,

230 days post the second infusion, 296 days post the second infusion, 360 days post the third infusion, 673 days post the third infusion, and 318 days post the second infusion. The local CITR investigators did not attribute any of the six events to the infusion procedure or to the immunosuppression medication.

Hypotension

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

In the era of non-purified islet preparations and high endotoxin collagenase preparations (before the availability of Liberase®), post-islet transplant hypotension requiring transient use of vasopressors was noted in 15% of the islet autograft recipients, of whom 50% required inotropic support with dopamine following injection until the end of surgery [82].

Disseminated Intravascular Coagulation (DIC)

DIC has been documented after autologous islet transplantation of dispersed pancreatic islet tissue in 3 out of about 400 patients expected to have undergone this procedure [83-85]. Consumption of clotting factors from the extensive pancreatectomy surgery as well as the preparation of non-purified islet tissue from a chronic pancreatitis specimen may have contributed to the coagulopathy. DIC following islet allotransplantation has neither been reported in the literature nor communicated to the CITR. Frequent monitoring of coagulation parameters in the post-transplant period will be part of the protocol-regulated safety assessments.

Hepatic Dysfunction and Steatosis

Transient abnormalities in liver enzyme tests have been observed immediately following intraportal islet transplantation [86, 87]. Three of the 86 islet transplant recipients reported to CITR have experienced transient elevations of liver enzymes requiring prolongation of post-transplant hospitalization or admission [74]. 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 [86]. 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 [88, 89] 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 [88], suggesting that steatosis may be associated with insulin resistance and graft dysfunction. The clinical relevance of steatosis associated with intrahepatic islet transplantation remains questionable. To the best of our knowledge, there is no evidence of clinically significant, persistent liver dysfunction following intraportal islet transplantation.

Portal Hypertension

Portal hypertension following intraportal infusion of unpurified allogeneic islet tissue resulted in a tear of the splenic capsule requiring splenectomy in one case [75]. The elevation in portal pressure following intraportal islet transplantation is temporary in most instances. In 1981, Cameron et al. reported on 4 patients with chronic pancreatitis who developed portal hypertension during intraportal infusion of only partially-purified auto-islet preparations, and in whom direct or indirect measurements of portal pressure were performed 3 to 12 months later [90]. In all patients, the portal pressure had returned to normal and portal venograms were normal. Casey *et al.* reported on changes in portal pressure following sequential islet transplants at the University of Alberta, and found that third islet transplants were associated with significantly greater final portal pressures (18mmHg) than first or second transplants (12mmHg) [91]. The baseline pressures were normal in all cases, suggesting absence of chronic portal hypertension [91].

Portal Vein Thrombosis

Transplanted islets release tissue factor and exhibit prothrombotic properties when infused to an intravascular site such as the portal vein [92]. A partial portal vein thrombosis has been reported in one of six patients transplanted at the intramural National Institutes of Health (NIH) program [76]. In the Edmonton single-center experience, the risk of partial vein thrombosis was 3% in more than 100 intraportal islet transplants [79]. The management of partial vein thrombosis includes anticoagulation therapy which may lead to intra-abdominal hemorrhage requiring transfusion and surgical intervention[93]. There is one published report of complete thrombosis of the portal vein thrombosis after transplantation of partially purified pancreatic islets in a combined islet/liver allograft, which necessitated emergency re-transplantation of the liver[94]. This complication probably related to the transplantation of partially purified islet tissue derived from 4 donors into a freshly transplanted liver. A right upper quadrant ultrasound including Doppler examination of the portal vein is performed on islet transplant recipients on days 1 and 7 post-transplant. Early diagnosis and prompt management of branch vein portal occlusion with systemic heparinization may prevent clot propagation. Repeated intraportal islet transplants are generally contraindicated in patients that have experienced prior portal thrombus.

Injuries to Other Structures

One instance of gall bladder perforation during percutaneous transhepatic catheterization of the portal vein requiring laparoscopic cholecystectomy has been reported to the Islet Transplant Registry [75]. 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 [74]. Gall bladder hematoma (n=1) and gall bladder opacification (n=2) have been observed as well.

1.5.4.4 FOLLOW-UP PROCEDURES

Glomerular Filtration Rate (GFR)

Risks associated with the GFR procedure are minimal and are related to the blood draw process. Rarely, the following will occur: excessive bleeding at blood draw site, syncope, extravasation of injection, hematoma, or infection. Iohexol has been widely used and has an excellent safety record. Very occasionally, allergic reactions to iohexol may occur[95].

1.5.5 Benefits

1.5.5.1 BENEFITS OF ALLOGENIC ISLET TRANSPLANTATION

Successful islet transplantation alleviates T1D patients from life-threatening hypoglycemia and psychosocially debilitating glycemic lability[53]. While the long-term durability of these responses is at present uncertain, they persist for as long as some graft function is maintained, despite the eventual return to insulin therapy in the majority of recipients. This partial function, as indicated by continued c-peptide production, may be present in as many as 80% of recipients after 5 years[60]. Furthermore, as long as graft function is maintained, fear of hypoglycemia and anxiety are significantly lower after islet transplantation. [96] Indeed, T1D subjects in the DCCT who had persistent c-peptide production had a significantly reduced risk of severe hypoglycemia despite intensive insulin therapy. [97] Additionally, while most transplant recipients experience only a temporary reprieve from exogenous insulin therapy, a few have maintained insulin-independent graft function for more than 3 years. Novel strategies aimed at promoting the engraftment or survival of transplanted islets may lead to improved long-term graft function and further the duration of insulin-independence after transplantation, and hopefully lead to reductions in the secondary complications of T1D.

1.5.5.2 BENEFITS OF STUDY REGIMEN

Potential benefits of a belatacept regimen include avoidance of tacrolimus-related side effects, such as nephrotoxicity, hypertension, dyslipidemia, and glucose intolerance, avoidance of sirolimus-related toxicity such as buccal ulcers and dyslipidemia, and minimization of the risk of procedure related AEs if a higher frequency of success with islet transplantation using islets from single donors is achieved by avoidance of tacrolimus and sirolimus.

Tacrolimus is known to be directly beta-cell toxic in vitro and in vivo, and has been associated with new onset immunosuppression-related diabetes in non-diabetic recipients of solid organ transplants. Islet transplants may be particularly sensitive to early exposure to calcineurin inhibitors (CNI's), as intraportally delivered islets are exposed to high peak levels of CNI's when given orally and absorbed via the portal vein[98] [99]. The current protocol also eliminates exposure to sirolimus. Sirolimus may have a negative impact on islet neovascularization and engraftment[100]. For these reasons we hypothesize that the proposed trial will optimize early islet engraftment and function, as well as minimizing risk of islet allograft rejection or autoimmune recurrence.

2. OBJECTIVES

2.1 Primary Objective

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

2.2 Secondary Objectives

The secondary objective is to assess islet graft function in the absence of calcineurin inhibitor drugs, with determination of success being the proportion of patients attaining and maintaining insulin independence after receiving a maximum of 3 islet transplants.

Additional objectives are to obtain samples for the islet and immune function mechanistic studies which are described in Section 9.

3. SELECTION OF SUBJECTS

3.1 Inclusion Criteria

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

1. Male and female patients age 18 to 65 years of age.
2. Ability to provide written informed consent.
3. Mentally stable and able to comply with the procedures of the study protocol.
4. Clinical history compatible with T1D with onset of disease at < 40 years of age, insulin-dependence for ≥ 5 years at the time of enrollment, and a sum of patient age and insulin dependent diabetes duration of ≥ 28.
5. Absent stimulated c-peptide (<0.3ng/mL) in response to a mixed meal tolerance test (MMTT; Boost® 6 mL/kg body weight to a maximum of 360 mL; another product with equivalent caloric and nutrient content may be substituted for Boost®) measured at 60 and 90 min after the start of consumption.
6. Involvement in intensive diabetes management defined as self monitoring of glucose values no less than a mean of three times each day averaged over each week and by the administration of three or more insulin injections each day or insulin pump therapy. Such management must be under the direction of an endocrinologist, diabetologist, or diabetes specialist with at least 3 clinical evaluations during the 12 months prior to study enrollment.
7. At least one episode of **severe hypoglycemia** in the 12 months prior to study enrollment.
8. Reduced awareness of hypoglycemia as defined by a Clarke score of 4 or more OR a HYPO score greater than or equal to the 90th percentile (1047) during the screening period and within the last 6 months prior to randomization;

   OR

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

   OR

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

3.2 Exclusion Criteria

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

1. Body mass index (BMI) >30 kg/m2 or patient weight ≤ 50kg.
2. Insulin requirement of > 1.0 IU/kg/day or <15 U/day.
3. HbA1c >10%.
4. Untreated proliferative diabetic retinopathy.
5. Blood Pressure: SBP > 160mmHg or DBP > 100mmHg.
6. Measured glomerular filtration rate (using iohexol) of <80 mL/min/1.73m^2 (or for subjects with an iodine allergy, calculated using the subject's measured serum creatinine and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation[1]). Strict vegetarians (vegans) with a calculated GFR < 70 mL/min/1.73m^2 are excluded. The absolute (raw) GFR value will be used for subjects with body surface areas > 1.73 m^2.
7. Presence or history of macroalbuminuria (>300mg/g creatinine).
8. Presence or history of panel-reactive anti-HLA antibodies above background by flow cytometry.
9. For female subjects: a) Positive serum pregnancy test (minimum sensitivity 25 IU/L or equivalent units of human chorionic gonadotropin [HCG]) within 72 hours prior to the start of study medication; b) presently breast-feeding; c) unwillingness to use effective contraceptive measures to avoid pregnancy in such a manner that the risk of pregnancy is minimized 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. All participants must use two acceptable methods of contraception while taking mycophenolate mofetil (MMF). For females of child bearing potential, the two methods should be started 4 weeks prior to the first dose of MMF. Oral contraceptives, Norplant®, Depo-Provera®, and barrier devices with spermicide are acceptable contraceptive methods; condoms used alone are not acceptable.
10. All women ≥ 35 years and women of any age who have first degree relatives with a history of breast carcinoma, or who have other risk factors of breast carcinoma, must have a screening mammogram, or provide results of a screening mammogram performed within 6 months of enrollment. Subjects with a mammogram that is suspicious for malignancy and in whom the possibility of malignancy cannot be reasonably excluded following additional clinical, laboratory, or other diagnostic evaluations will be excluded.
11. Active infection including hepatitis B, hepatitis C, or HIV.
12. Presence or history of active tuberculosis (TB). Subjects with laboratory evidence of active infection are excluded even in the absence of clinical evidence of active infection.
13. Negative screen for Epstein-Barr Virus (EBV) by IgG determination.

14. Invasive aspergillus, histoplasmosis, or coccidioidomycosis infection within one year prior to study enrollment.
15. Any history of malignancy except for completely resected squamous or basal cell carcinoma of the skin.
16. Known active alcohol or substance abuse.
17. Baseline Hb below the lower limits of normal at the local laboratory; lymphopenia (<1,000/μL), neutropenia (<1,500/μL), or thrombocytopenia (platelets <100,000/ μL). Participants with lymphopenia are allowed if the investigator determines there is no additional risk and obtains clearance from an independent hematologist.
18. A history of Factor V deficiency.
19. Any coagulopathy or medical condition requiring long-term anticoagulant therapy (e.g., warfarin) after islet transplantation (low-dose aspirin treatment is allowed) or patients with an International normalized ration (INR) >1.5. The use of Plavix is allowed only when portal vein access is obtained using a mini-laparotomy procedure at the time of islet transplant.
20. Severe co-existing cardiac disease, characterized by any one of these conditions:
    a) Recent myocardial infarction (within past 6 months).
    b) Evidence of ischemia on functional cardiac exam within the last year.
    c) Left ventricular ejection fraction <30%.
21. Persistent elevation of liver function tests at the time of study entry. Persistent serum glutamic-oxaloacetic transaminase (SGOT [AST]), serum glutamate pyruvate transaminase (SGPT [ALT]), Alk Phos or total bilirubin, with values >1.5 times normal upper limits will exclude a patient. Known cirrhosis of the liver or portal hypertension.
22. Symptomatic cholecystolithiasis.
23. Acute or chronic pancreatitis.
24. Symptomatic peptic ulcer disease.
25. Severe unremitting diarrhea, vomiting or other gastrointestinal disorders potentially interfering with the ability to absorb oral medications.
26. Hyperlipidemia despite medical therapy (fasting low-density lipoprotein [LDL] cholesterol > 130 mg/dL, treated or untreated; and/or fasting triglycerides > 200 mg/dL).
27. Receiving treatment for a medical condition requiring chronic use of systemic steroids except for the use of ≤ 5 mg prednisone daily, or an equivalent dose of hydrocortisone, for physiological replacement only.
28. Treatment with any anti-diabetic medication other than insulin within 4 weeks of enrollment.
29. Use of any other investigational agents within 4 weeks of enrollment.
30. Subjects previously treated with belatacept.

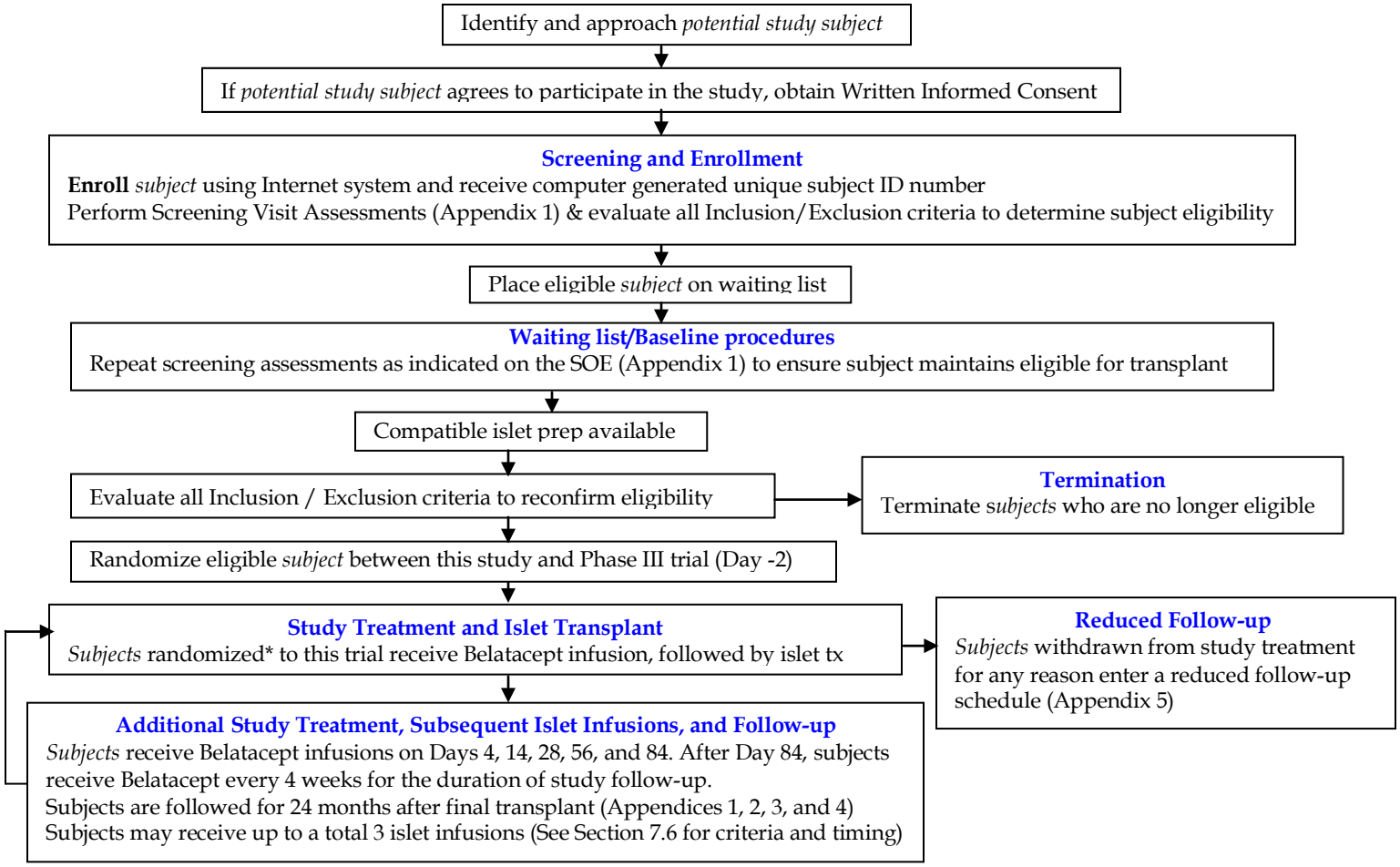
31. Administration of live attenuated vaccine(s) within 2 months of enrollment.
32. Any medical condition that, in the opinion of the investigator, will interfere with safe participation in the trial.
33. Prisoners or subject who are compulsorily detained (involuntarily incarcerated) for treatment of either a psychiatric or physical (e.g., infectious disease) illness.
34. Treatment with any immunosuppressive regimen at the time of enrollment, or subjects with comorbidities for which treatment with such agents are likely during the trial.
35. A previous islet transplant.
36. A previous pancreas transplant, unless the graft failed within the first week due to thrombosis, followed by pancreatectomy and the transplant occurred more than 6 months prior to enrollment.
37. Known hypersensitivity to mycophenolate mofetil or any of the drug's components.
38. Rare hereditary deficiency of hypoxanthine-guanine phosphoribosyltransferase (HGPRT) such as Lesch-Nyhan and Kelly-Seegmiller syndrome.
39. Dietary restriction of phenylalanine.

4. STUDY DESIGN

This trial is a prospective, two-center, open-label, pilot study of islet transplantation assessing the safety and efficacy of a steroid-free, calcineurin inhibitor-free beletacept based immunosuppressive medication in subjects with long-standing T1D that is refractory to intensive insulin therapy. The two centers participating in this phase 2 study will also undertake a separate, phase 3 study in islet transplantation, using a standard manufacturing and immunosuppressive regimen. The phase 3 trial, Protocol CIT07, will have inclusion/exclusion criteria and endpoint measures that are identical to those in this phase 2 trial. In order to avoid bias in selection of subjects, eligible subjects will be randomized, prior to transplantation, to participate either in this phase 2 or the multi-center phase 3 study.

Subjects who meet the general inclusion/exclusion criteria will be approached regarding their participation. Subjects who sign informed consent will be enrolled and assigned a unique subject identification number. Subjects will then be formally evaluated for eligibility through the performance of screening visit procedures. More than one visit may be necessary to complete all of the screening procedures. The two participating centers will accrue subjects over a 24 month period and will treat a total of 10 study subjects. No one center will treat more than 12 subjects.

Figure 1: Study Design Schema.



4.1 Study Endpoints

4.1.1 Primary Endpoint

The primary endpoint for this study is the proportion of **insulin-independent** subjects at day 75 (± 5 days) following the first islet transplant.

4.1.2 Secondary Endpoints

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

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

4.1.2.1 SECONDARY EFFICACY ENDPOINTS

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

- The percent reduction in insulin requirements
- HbA1c
- Mean amplitude of glycemic excursions (MAGE)
- Glycemic lability index (LI)
- Ryan hypoglycemia severity (HYPO) score
- Basal (fasting) and 90-min glucose and c-peptide derived from the mixed-meal tolerance test (MMTT)
- β-score[4]
- C-peptide: (glucose X creatinine) ratio
- Acute insulin response to glucose (AIR_{glu}), insulin sensitivity, and disposition index (DI) derived from the insulin-modified frequently-sampled IV glucose tolerance (FSIGT) test
- Glucose variability and hypoglycemia duration derived from the continuous glucose monitoring system® (CGMS)
- Quality of life (QOL) measures

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

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

- The percent reduction in insulin requirements
- HbA1c
- MAGE
- LI
- Clarke score
- HYPO score
- Basal (fasting) and 90-min glucose and c-peptide (MMTT)

- β-score
- C-peptide: (glucose X creatinine) ratio
- AIR_{glu} insulin sensitivity, and disposition index (DI) derived from the FSIGT test
- Glucose variability and hypoglycemia duration derived from the continuous glucose monitoring system® (CGMS)
- QOL measures
- The proportion of subjects receiving a second islet transplant
- The proportion of subjects receiving a third islet transplant
- Rate of favorable outcome at each center preparing islets (rate of subjects with an HbA1c < 7.0% and free of severe hypoglycemic events)

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

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

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

4.1.2.2 SECONDARY SAFETY ENDPOINTS

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

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

- The incidence and severity of AEs related to the islet transplant procedure including: bleeding (> 2 g/dL decrease in hemoglobin concentration); segmental portal vein thrombosis; biliary puncture; wound complication (infection or subsequent hernia); and increased transaminase levels (> 5 times upper limit of normal [ULN])
- The incidence and severity of AEs related to the immunosuppression including: allergy; reduction in GFR; addition or intensification of anti-hypertensive therapy;

gastrointestinal toxicity; neutropenia, anemia, or thrombocytopenia; viral, bacterial, or fungal infections; and benign or malignant neoplasms
- The incidence of a change in the immunosuppression drug regimen
- The incidence of immune sensitization defined by presence of anti-HLA antibodies absent prior to transplantation
- The incidence of discontinuation of immunosuppression

At 365 ± 14 days following the first islet transplant:

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

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 medication(s).

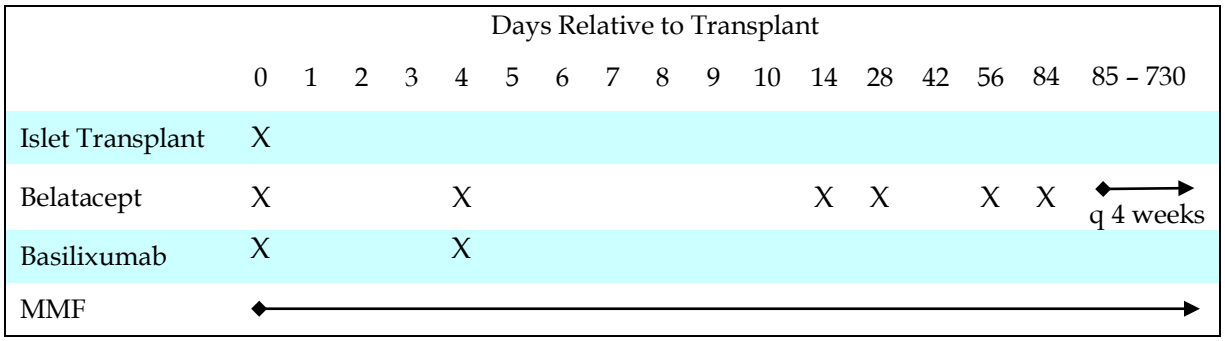


Figure 2: Study Treatment Regimen

5.1 Investigational Agents

The investigational agents for this study:

- Allogeneic islets: The allogeneic islets are considered the primary investigational agent being regulated by the FDA under DAIT, NIAID's BB-IND 9336.
- NUJOLIX® (belatacept) : Belatacept is considered the secondary investigational agent in this study.

5.1.1 Allogeneic Islets

5.1.1.1 FORMULATION, DOSAGE, AND ADMINISTRATION

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

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

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

Administration:

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

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

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

5.1.2 NUJOLIX® (belatacept)

5.1.2.1 FORMULATION, PACKAGING, AND LABELING

NUJOLIX® (belatacept) for injection, 100 mg/vial or 250 mg/vial is a sterile non-pyrogenic lyophilized powder. Each 100-mg vial contains 110 mg of belatacept, 220 mg of sucrose, 15.18 mg of sodium phosphate monobasic monohydrate, 2.55 mg sodium chloride, and 1N sodium hydroxide/1N hydrochloric acid solution sufficient to adjust the pH to 7.5; each 250-mg vial contains 275 mg of belatacept, 550 mg of sucrose, 38.0 mg of sodium phosphate monobasic monohydrate, 6.4 mg of sodium chloride, and 1 N sodium hydroxide/ 1 N hydrochloric acid solution sufficient to adjust the pH to 7.5. The product includes a 10% overfill to account for vial, syringe and needle holdup. The lyophilized powder is provided in Type 1 glass vials, stoppered with gray butyl stoppers and sealed with aluminum seals.

NUJOLIX® (belatacept) for injection, 100 mg/vial or 250 mg/vial will be provided as open-label supplies packaged in boxes. Each box of 100 mg/vial will contain 16 vials; each box of 250 mg/vial will contain 8 vials. Each box will be labeled with a 1-panel, open label printed in black. The protocol number (CIT04), product identity and strength, container number range, batch number, the number of vials, directions for use, route of administration and storage conditions will be indicated.

5.1.2.2 PREPARATION, ADMINISTRATION, AND DOSAGE

All constitution and dilution of belatacept 100mg or 250mg vials must be performed using silicone free disposable (Norm-Ject®) syringes manufactured by Henke Sass Wolf in Germany, and administered through a sterile, non-pyrogenic, low protein binding in-line filter.

NOTE: It is recommended that a separate needle and syringe be used to withdraw the drug product solution from each vial.

Belatacept 100 mg vials (are sealed under vacuum. If any vials are found without this vacuum, they should be segregated and destroyed. Belatacept 250 mg vials are not sealed under vacuum.

Each 100mg vial of belatacept should be constituted with 4.2 mL of Sterile Water for injection to yield an approximate concentration of 25 mg/mL of belatacept; each 250 mg vial of belatacept should be constituted with 10.5 mL of Sterile Water for injection to yield an approximate concentration of 25 mg/mL of belatacept. To avoid foam formation, the stream of sterile water for injection should be directed to the sides of vial and should be constituted with gentle swirling until a clear solution is obtained. A sufficient excess of belatacept is incorporated into each vial to account for withdrawal losses so that 100 mg of belatacept can be withdrawn from the vial as 4 mL of a 25-mg/mL solution, or 250 mg of belatacept can be withdrawn from the vial as 10 mL of a 25-mg/mL solution. After initial constitution of the product to a concentration of 25 mg/mL, the solution may be diluted further with 5% Dextrose for Injection or 0.9% Normal Saline Solution to final belatacept concentrations as low as 1 mg/mL.

The final belatacept solution should be visually inspected for particulate matter prior to administration.

The continuous infusion solution must be filtered upon administration using an in-line, sterile, non-pyrogenic, low protein-binding filter with a pore size of 1.2 μm (to be provided by BMS). This infusion should be administered over a period of approximately 30 minutes. Any unused portion of the infusion solution should not be stored for reuse.

No incompatibilities have been observed with glass bottles or polyvinyl chloride bags and administration sets.

No data are available on the compatibility of belatacept with other IV substances. Other drug substances should not be added or infused simultaneously through the same IV line. Assure adequate, appropriate flushing between each drug substance if multiple drugs are administered through the same line sequentially.

Recommended safety measures for preparation and handling include protective clothing, gloves, and safety cabinets.

Subjects will receive NULOJIX® (belatacept)10mg/kg through a peripheral vein on Day 0 and post-operative days 4, 14, 28, 56, 84. After Day 84 subjects will receive belatacept at a maintenance dose of 5 mg/kg every 4 weeks for the duration of study follow-up (2 years after the final islet transplant). Infusion of the Day 0 dose should be started after the islet product is deemed suitable for transplant and completed prior to beginning the transplant. Infusion doses will be based upon the subject's actual body weight at study Day 0 and will not be modified

during the course of the study unless there is a change in body weight ±10% of the Day 0 weight. For second or third islet transplants performed after day 85, subjects will remain on the current maintenance dose and schedule of belatacept (5 mg/kg monthly), without modification. In addition to the maintenance doses, subjects will receive a single supplementary dose of belatacept 10 mg/kg. If the second or third transplant is performed within 14 days after the last dose of belatacept, the supplementary dose will be administered approximately 14 days (within the +/- 4 day visit window) after the last dose of belatacept. If the transplant is performed more than 14 days of the last dose of belatacept, the supplementary dose will be administered within 24 hours of the transplant. This supplementary dose was chosen so as to approximate serum trough concentrations that are attained in the first 6-8 weeks after the initial transplant.

NOTE: If the maintenance dose is scheduled to be given within four days of a supplemental dose, the maintenance dose should not be given. Subjects will resume maintenance dosing at their next scheduled visit.

Currently, there is no existing long-term extension study for subjects who complete the study (CIT04) and receive belatacept (LEA29Y). Therefore, subjects may not be able to continue taking belatacept upon completion of the study. It is possible that a long-term extension study with belatacept may be conducted in the future and subjects may be eligible for this study if all inclusion and exclusion criterion are met. This will be detailed in a separate protocol, and a separate informed consent will be required. In the event a subject is not eligible, or the study is not implemented, clinical care beyond the completion of this study (CIT04) should be discussed between the subject and his/her islet transplant physician.

5.1.2.3 HANDLING, DISPENSING, AND DESTRUCTION OF BELATACEPT

Care should be taken when handling the injectable drug products found in the protocol. Proper aseptic techniques should be used when preparing and administering sterile products such as belatacept.

The belatacept for injection 100 mg or 250 mg vial should be stored under refrigeration (2-8°C), and should be protected from long term exposure to light. Intact vials are stable for at least 1 year under these conditions.

Constituted solutions of belatacept at a concentration of 25 mg/mL are stable for 24 hours in the vials if stored at room temperature (15-25°C) and ambient lighting conditions, or under refrigeration. When further diluted with 5% Dextrose for Injection or 0.9% Normal Saline Solution to a belatacept concentration as low as 1 mg/mL, solutions may be stored in plastic, non-siliconized IV bags for up to 24 hours at room temperature and ambient lighting conditions or under refrigeration.

Belatacept should be stored in a secure area according to local regulations. It is the responsibility of the Investigator to ensure that belatacept is only dispensed to study subjects. Belatacept must be dispensed only from official study sites by authorized personnel according to local regulations. The Investigator should ensure that belatacept is stored in accordance with the environmental conditions (temperature, light and humidity) as determined by the Sponsor and defined in the Investigator Brochure or SmPC/reference label.

If an investigational product is destroyed at the site, it is the investigator's responsibility to ensure that arrangements have been made for the disposal, procedures for proper disposal have been established according to applicable regulations and guidelines and institutional procedures, and appropriate records of the disposal have been documented. The unused investigational products can only be destroyed after being inspected and reconciled by the responsible study monitor.

5.1.3 Drug Accountability

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

Records for receipt, storage, use, and disposition must comply with applicable regulations and guidelines and will be maintained by the study site. Records should include:

- The amount of study drug received and placed in the storage area;
- The amount of drug currently in the storage area;
- Label identification number or batch number and use data or expiry date;
- Amount dispensed to and returned by each subject, including unique subject identifiers
- Non-study disposition (e.g., lost, wasted, broken)
- Amount returned to the sponsor
- Amount destroyed at study site, if applicable
- Retained samples sent to a third party for bioavailability/bioequivalence, if applicable.
- Dates and initials of person responsible for each investigational product inventory entry/movement; and
- Amount transferred to another area for dispensing or storage.

In addition, a drug-dispensing log will be kept current for each subject. This log will contain the identification of each subject and the date and quantity of drug dispensed.

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

5.2 Immunosuppression Medications

The immunosuppressive agents used for the initial and subsequent islet transplants will be identical.

5.2.1 Mycophenolate Mofetil (Cellcept®)

Subjects will receive mycophenolate mofetil starting immediately pre-transplant on Day 0 at a dose of 1g PO BID.

If a subject experiences severe neutropenia (absolute neutrophil count <1x10^9/L), gastrointestinal toxocity, or other side effects requiring a dose reduction, then the MMF dose may be adjusted at the investigator's discretion (see section 5.7.1 for guidelines). If the dose is less than 500 mg BID, then a mycophenolic acid (MPA) blood trough level will be drawn. If the MPA level is below therapeutic range (reference range < 1 µg/ml or lower end of reference range at institution) in the absence of infectious complications, then tacrolimus will be added to the immunosuppressive regimen.

5.2.2 Basiliximab (Simulect®)

Two IV doses of basiliximab, a monoclonal antibody IL-2 receptor blocker, will be given with the first and second (if necessary) transplants. The first dose will be 20 mg and will be given within two hours prior to islet transplant on the day of islet transplantation. The second 20 mg dose will be given on Day 4 after the transplant.

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

If a third islet transplant is deemed necessary and performed more than 70 days after the second transplant (see Section 7.6 for indications for subsequent transplants), both doses of basiliximab will be repeated.

5.2.3 Tacrolimus (Prograf®)

Tacrolimus may be used only as a supplement to MMF in those cases where the trough level is below the therapeutic range as outlined in section 5.2.1. Tacrolimus will be administered orally twice a day to maintain trough levels of 3-5 ng/mL. Generic equivalents of Prograf® will not be permitted.

5.3 Concomitant Medications

5.3.1 Antibacterial, Antifungal, and Antiviral Prophylaxis

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

5.3.1.1 TRIMETHOPRIM/SULFAMETHOXAZOLE (SEPTRA SS®/BACTRIM®)

Trimethoprim/sulfamethoxazole will be administered at a dose of 80 mg/400 mg PO QD starting on Day +1 for 6 months after each transplant. In the event that a subject is unable to take trimethoprim/sulfamethoxazole, he/she will be treated on a case-by-case basis as is medically indicated. Dapsone is contraindicated in the setting of this trial due to its known

interaction with the HbA1c assay, which would have an effect on interpretation of endpoint outcomes for the trial.

5.3.1.2 VALGANCICLOVIR (VALCYTE®)

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

5.3.2 Anticoagulation Prophylaxis / Hematological Agents

5.3.2.1 HEPARIN

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

5.3.2.2 ENOXAPARIN (LOVENOX®)

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

5.3.2.3 ASPIRIN

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

5.3.2.4 PENTOXIFYLLINE

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

5.3.3 Updated Vaccinations

Subjects will remain up to date on CDC-recommended adult vaccinations; please refer to the MOP for guidance. Live vaccines should be avoided while taking belatacept.

5.3.4 Insulin Therapy

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

5.3.5 Other Standard Therapies

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

5.4 Rescue Medications

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

5.5 Prohibited Medications

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

- steroid medication (save topicals and prednisone at a dose of ≤ 5 mg daily, or an equivalent dose of hydrocortisone, for physiological replacement only)
- any medications in the macrolide antibiotic class other than Zithromax
- other investigational products
- other immunosuppressive therapies
- immunomodulatory agents
- other anti-diabetic agents
- Dapsone
- azathioprine
- cholestyramine or other agents that may interfere with enterohepatic recirculation
- live vaccines
- sevelamer or other calcium free phosphate binders

5.6 Assessment of Compliance with Study Treatment

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

5.7 Modification or Discontinuation of Study Treatment

5.7.1 Modification of Consensus Immunosuppression

5.7.1.1 INTOLERANCE OF PROTOCOL MEDICATIONS

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

5.7.1.2 GRAFT FAILURE

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

5.7.1.3 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 the prophylactic use of valganciclovir from 900 mg per day to 450 mg per day or hold valganciclovir.
- Reduce trimethoprim/sulfamethoxazole to 80/400 mg on Monday, Wednesday, and Friday or hold trimethoprim/sulfamethoxazole.
- Consider reducing the dose of mycophenolate mofetil.

- Consider administration of G-CSF.
- Monitor temperature BID.
- Follow-up within 48-72 hours to obtain: repeat complete blood count (CBC) with differential, patient symptoms, and measured temperatures.

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

- Obtain Infectious Disease Consult.
- Hold valganciclovir and trimethoprim/sulfamethoxazole.
- Consider reducing the dose of mycophenolate mofetil.
- Obtain CMV antigenemia or PCR for CMV.
- Administer G-CSF.
- Monitor temperature BID.
- Follow-up within 48-72 hours to obtain: repeat CBC with differential, subject symptoms, and measured temperatures.

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

- Hold administration of trimethoprim/sulfamethoxazole and/or valganciclovir.
- Consider holding dose of mycophenolate mofetil.
- Obtain CMV antigenemia or PCR for CMV.
- Consider fluoroquinolones in afebrile subjects.
- Consider clotrimazole.
- Administer G-CSF.
- Monitor temperature BID.
- Follow up within 24 hours to obtain repeat CBC, subject symptoms, and measured temperatures.

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

- The patient will be hospitalized under neutropenic precautions and Infectious Disease/Hematology consult will be obtained.
- Hold administration of trimethoprim/sulfamethoxazole and/or valganciclovir.
- Consider holding dose of mycophenolate mofetil.
- Obtain CMV antigenemia or PCR for CMV.
- Administer G-CSF.
- Follow up within 24 hours with admitting physician.

5.7.1.4 THROMBOCYTOPENIA

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

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

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

1. The subject is unwilling or unable to comply with the protocol.
2. The investigator believes that the study treatment is no longer in the best interest of the subject.
3. Graft Failure (see Study Definitions).
4. Any clinical AE, laboratory abnormality, or intercurrent illness which, in the opinion of the investigator, indicates that continued treatment with study therapy is not in the best interest of the subject. The agent(s) to which the event is attributed will be discontinued.
5. The subject becomes pregnant
6. Missing 2 consecutive belatacept infusions
7. The development of belatacept is terminated by the manufacturer (BMS)
8. The subject is imprisoned or compulsorily detained for the treatment of either a psychiatric or physical illness (e.g., infectious disease).

Subjects who prematurely discontinue study treatment will remain in the study until normal termination, for the purpose of monitoring safety and efficacy parameters and will enter the reduced follow-up scheduled outlined in Appendix 5. Data from these subjects will be used in the intent-to-treat analysis. These subjects are permitted to simultaneously enroll in a CIT or site-specific graft failure follow-up protocol, if available.

6. CRITERIA FOR SUBJECT WITHDRAWAL AND PREMATURE TERMINATION OF THE STUDY

6.1 Subject Withdrawal Criteria

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

1. The subject elects to withdraw consent from all future study activities, including follow-up.
2. The subject is "lost to follow-up" (i.e., no further follow-up is possible because attempts to reestablish contact with the subject have failed).
3. The subject develops a clinical AE, laboratory abnormality, or intercurrent illness which, in the opinion of the investigator, indicates that continued treatment with study therapy and further participation in the study (including obtaining vital status of the subject and islet graft) is not in the best interest of the subject.
4. The development of belatacept is terminated by the manufacturer (BMS).
5. The subject becomes a prisoner or becomes involuntarily incarcerated for treatment of either a psychiatric or physical (e.g., infectious disease) illness.
6. The subject dies.

6.2 Study Stopping Rules

6.2.1 Protocol Suspension and Review

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

1. Any unexpected fatal or life-threatening AE that is possibly, probably, or definitely related to the study treatment regimen (Section 5);
2. **Primary non-function** occurs in 3 or more subjects;
3. There are 6 consecutive study subjects with a c-peptide less than 0.3ng/mL (on random testing, at baseline and 1-3 hrs post MMTT) at 75 days post-transplant;
4. Any event(s) which in the opinion of the Medical Monitor or Protocol Chair indicates the need for DSMB review; or
5. The DSMB recommends termination of protocol enrollment and further transplants on a study-wide basis based on a review of the data and finding evidence that such action is necessary.

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

person or by conference call to review in depth the results and circumstances surrounding the islet functional failure or SAE to determine whether the trial enrollment of new subjects and conduct of additional transplants could be safely resumed.

6.2.2 Site Suspension and Review

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

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

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

In all cases of PNF, no further belatacept will be administered and subjects will be asked to temporarily continue on the standard immunosuppression to decrease the risk of sensitization that could increase the risk of poor outcome should future transplants occur. A tapering schedule will be applied until immunosuppressants are completely discontinued.

7. STUDY PROCEDURES

7.1 Enrollment and Screening

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

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

Table 2: Time frames for screening assessments

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

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

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

7.2 Waiting List / Baseline

After completion of the screening assessments required to confirm eligibility for the study, he/she will be listed for an islet transplant. During this period when subjects are awaiting their first transplant, the remaining screening assessments – FSIGT, CGMS, and retinal photos – should be completed as time allows. If retinal photos cannot be obtained at WL/BL, they should not be collected post-randomization. Waitlist assessments will be repeated at pre-defined intervals as detailed in Appendix 1. Results from assessments done closest to the start of immunosuppression will be used as the subject's baseline values. All one-time waitlist/baseline assessments should be completed on Day -2, whenever possible, but always

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

7.3 Randomization, Islet Transplant, and Study Treatment

Once a compatible islet prep becomes available, subject eligibility will be re-confirmed. Eligible subjects will be randomized on Day -2 relative to transplant, between this Phase 2 trial and the multi-center Phase 3 trial. Randomizations will occur at a ratio of 2:1, where 2 participants are assigned to CIT07 for every subject assigned to the site-specific Phase 2 trial. Subjects randomized to this Phase 2 trial will receive their initial study drug infusion of belatacept 10mg/kg by peripheral IV over 30 minutes, followed by the islet transplant. The infusion of islets must be initiated within 24 hours of completion of the belatacept infusion.

7.4 Post-transplant Study Treatment Visits

Subjects will receive NULOJIX® (belatacept)10mg/kg by peripheral IV over 30 minutes on post-operative days 4, 14, 28, 56, 84. After Day 84 subjects will receive belatacept at a maintenance dose of 5mg/kg every 4 weeks for the duration of study follow-up (24 months after the final transplant). Infusion doses will be based upon the subject's actual body weight at study Day 0 and will not be modified during the course of the study unless there is a change in body weight ±10% of the Day 0 weight. For second or third islet transplants performed after day 85, subjects will remain on the current maintenance dose and schedule of belatacept (5 mg/kg monthly), without modification. In addition to the maintenance doses, subjects will receive a single supplementary dose of belatacept 10 mg/kg. If the second or third transplant is performed within 14 days of the last dose of belatacept, the supplementary dose will be administered approximately 14 days (within the +/- 4 day visit window) after the last dose of belatacept. If the transplant is performed more than 14 days of the last dose of belatacept, the supplementary dose will be administered within 24 hours of the transplant. This supplementary dose was chosen so as to approximate serum trough concentrations that are attained in the first 6-8 weeks after the initial transplant.

NOTE: If the maintenance dose is scheduled to be given within four days of a supplemental dose, the maintenance dose should not be given. Subjects will resume maintenance dosing at their next scheduled visit.

7.5 Follow-up Visits

Subject will undergo a 24-month follow-up period following their final islet transplant. Please refer to the Schedule of Events (Appendices 1, 2, 3, and 4), for the clinical time points of specific follow-up study procedures.

All subjects will follow the Year One Schedule of Events (Appendix 1) in its entirety. Subjects who receive a subsequent islet transplant will continue onto Appendix 2, Continuation of Appendix 1 Schedule of Events (Subjects with Subsequent CIT Transplants) until they reach their one year visit after the final islet transplant. In addition to following Appendix 2, all

subjects who receive a subsequent transplant should complete the assessments listed on the Subsequent Transplant Schedule of Events (Appendix 3), which provides immediate post-transplant and endpoint day assessments. After the one year post-final transplant visit, subjects will move to Appendix 4, Schedule of Events for 1-Year Additional Follow-up.

Subjects are ineligible for subsequent islet transplantation in the CIT04 protocol as of 8 months following their initial transplant (between visits 16 and 17). After visit 21 (365 days following transplant) has been completed, study subjects may obtain a subsequent islet transplant outside of CIT04. Subjects will be followed for adverse events only until until 24 months after their final CIT islet transplant.

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 5).

7.6.1 Second Islet Transplant

Islet transplant recipients with partial islet graft function (see Study Definitions) will be considered for a second islet transplant in the interim between 85 days and 8 months post-initial infusion. Islet transplant recipients with **graft failure** will be considered for a second islet transplant before 85 days post-initial infusion. In addition to meeting the criteria outlined below, approval from the Steering Committee must be obtained in advance. Please refer to the MOP for details on this process, which includes review of the potency testing from the first transplant product and post-transplant clinical data.

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

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

If graft failure occurs after the second islet transplant, these recipients will be considered **treatment failures** with no islet graft function, and immunosuppression will be withdrawn.

7.6.2 Third Islet Transplant

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

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

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

7.7 Visit Windows

Study visits should take place within the time limits specified on the Schedule of Events (Appendices 1, 2, 3, and 4).

8. SAFETY MONITORING

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

8.1 Definitions

8.1.1 Adverse Event

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

8.1.2 Serious Adverse Event

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

1. Death. A death that occurs during the study or that comes to the attention of the investigator during the protocol-defined follow-up after the completion of therapy must be reported whether it is considered to be treatment related or not.
2. A life-threatening event. A life-threatening event is any adverse therapy experience that, in the view of the investigator, places the patient or subject at immediate risk of death from the reaction as it occurred.
3. Inpatient hospitalization or prolongation of existing hospitalization. Please note that hospital admissions for the purpose of conducting protocol-mandated procedures do not need to be reported as SAEs, unless the hospitalization is prolonged due to complications.
4. Persistent or significant disability.
5. Congenital anomaly or birth defect.
6. An event that required intervention to prevent permanent impairment or damage. An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based on appropriate

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

7. Other conditions specified in the protocol.

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

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

Following the subject's written consent to participate in the study, all SAEs should be collected, including those thought to be associated with clinical trial procedures. Following study completion, any SAE thought to be related to study drug or clinical trial procedures should also be reported to the sponsor.

8.1.3 Unexpected Adverse Event

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

8.2 Adverse Events

8.2.1 Collecting Procedure

AEs that are associated with a protocol mandated procedure, which is not part of the normal standard of care of the participant, and **severe hypoglycemic events** (see study definitions) will be collected beginning immediately after the enrollment consent has been obtained. All other AEs will be collected beginning immediately after randomization. All AEs will continue to be collected until study completion, or for 56 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. For transplants that occur as a standard of care procedure at the University of Alberta, adverse events will be collected and submitted by the site investigator until all CIT04 study visits have been completed. If a subject enrolls in a non-CIT islet transplant study, adverse events will no longer be collected in CIT starting at the time of the non-CIT study intervention. All adverse event reporting from that point on will be done through the non-CIT study.

AEs may be discovered through any of these methods:

- Observing the subject.
- Questioning the subject, which should be done in an objective manner.
- Receiving an unsolicited complaint from the subject.
- An abnormal value or result from a clinical or laboratory evaluation (e.g., a radiograph, an ultrasound, or an electrocardiogram) can also indicate an AE. If this is the case, then the evaluation that produced the value or result should be repeated

until the value or result returns to normal or can be explained and the subject's safety is not at risk. If an abnormal value or result is determined by the investigator to be clinically significant, it must be indicated as such on the appropriate laboratory evaluation form(s), and must also be reported as an adverse event on the adverse event formAE.

AEs should be followed to resolution or stabilization, and reported as SAEs if they become serious. This also applies to subjects experiencing AEs that cause interruption or discontinuation of investigational product, or those experiencing AEs that are present at the end of their participation in the study; such subjects should receive post-treatment follow-up as appropriate. If an ongoing AE changes in its severity or in its perceived relationship to study drug, a new AE entry for the event should be completed.

8.2.2 Recording Procedure

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

Reporting of AE Information Following Study Completion

Collection of safety information following the end of investigational product administration is important in assisting in the identification of possible delayed toxicities or withdrawal effects. In this trial, all SAEs must be collected that occur within 56 days following discontinuation of dosing. In addition, the investigators should report any SAE that may occur after this time period which they believe to be certainly, probably, or possibly related to the investigational product. Finally, all events of death, graft loss, malignancy, PTLD, and serious infections (i.e., otherwise meeting SAE reporting requirements) must be reported for all randomized subjects until the end of the study, irrespective of study drug discontinuation or investigator-deemed causality.

8.2.3 Grading and Attribution

8.2.3.1 GRADING CRITERIA

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

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

Grade 1 = Mild AE.

Grade 2 = Moderate AE.

Grade 3 = Severe and undesirable AE.

Grade 4 = Life-threatening or disabling AE.

Grade 5 = Death.

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

Table 3: General severity definition of adverse event

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

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

8.2.3.2 DEFINITION OF ATTRIBUTION

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

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

Table 4.: Attribution of adverse events

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

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

8.3 Serious Adverse Events

8.3.1 Collecting Procedure

SAEs will be collected following the subject's signing of the enrollment consent to participate in the study until 56 days after the subject completes or withdraws from the study. SAEs will be followed until the time the event is resolved, stabilized, or until 56 days after the subject completes or withdraws from the study, whichever comes first.

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

For transplants that occur as a standard of care procedure at the University of Alberta, serious adverse events will be collected and submitted by the site investigator until all CIT04 study visits have been completed. If a subject enrolls in a non-CIT islet transplant study, serious adverse events will no longer be collected in CIT starting at the time of the non-CIT study

intervention. All serious adverse event reporting from that point on will be done through the non-CIT study.

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

8.3.2 Recording Procedure

SAEs will be recorded on the AE eCRF.

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

8.3.3 Reporting Procedure

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

8.3.3.1 REPORTING CRITERIA FROM SPONSOR TO HEALTH AUTHORITY

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

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

  Serious, expected, and drug related.

  Serious, expected, and *not* drug related.

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

8.3.3.2 REPORTING TIMELINE– FROM THE SITE TO THE DCC

When an investigator identifies an SAE (as defined in section 8.1.2), he or she must notify the DCC Safety Reporting Center within 24 hours of discovering the event by submitting an initial

electronic SAE CRF. In the event that the eCRF cannot be submitted (i.e., computer failure), the site must fax a paper SAE report to the DCC within 24 hours of discovering the event.

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

8.3.3.3 REPORTING TIMELINE – FROM THE DCC TO 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, all investigators in the study, and the manufacturer of the secondary study drug(s). SAEs per 21 CFR §312.32 definitions, except elective hospitalizations, will be reported to the Health Authority by the study sponsor (NIAID) in accordance with applicable regulations.

8.3.3.4 NOTIFYING THE DATA AND SAFETY MONITORING BOARD

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

8.3.3.5 NOTIFYING THE INSTITUTIONAL REVIEW BOARD AND ETHICS COMMITTEE

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

8.3.3.6 PREGNANCY

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

Pregnancy testing must also be performed throughout the study prior to the infusion with NULOJIX® (belatacept)and the results of all pregnancy tests (positive or negative) recorded on the CRF. All WOCBP MUST have a negative serum pregnancy test within 72 hours prior to receiving the investigational product (belatacept). The minimum sensitivity of the pregnancy test must be 25 IU/L or equivalent units of HCG. If the pregnancy test is positive, the subject must not receive the investigational product, and must not be enrolled in the study.

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

If following initiation of study treatment, it is subsequently discovered that a subject is pregnant or may have been pregnant at the time of investigational product exposure, including during at least 56 days after product administration, the investigational product will be permanently discontinued. The investigator will be provided with Pregnancy Surveillance Forms. Upon completion, the form should immediately be sent to the DCC, from where it will be forwarded to NIH and BMS.

Protocol-required procedures for study discontinuation and follow-up must be performed on the subject unless contraindicated by pregnancy (e.g., x-ray studies). Other appropriate pregnancy follow-up procedures should be considered if indicated. In addition, the investigator must report on the appropriate BMS Pregnancy Surveillance Forms(s), follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome. Infants should be followed for a minimum of 8 weeks. This pregnancy surveillance procedure includes male subjects who fathered a child while receiving study medication; however, male subjects do not need to discontinue study medication.

8.3.3.7 REPORTING PREGNANCY AS A SERIOUS ADVERSE EVENT

Any pregnancy that occurs during a clinical study that is using an investigational drug must be reported to the DCC utilizing the SAE report form. This report is *for tracking purposes only*. All pregnancies that are identified during the study must be followed to conclusion and the outcome of each must be reported. The investigator will inform the subject if there are any study medications they must stop taking in addition to the belatacept. The investigator should report all pregnancies within 24 hours (as described in section 8.3.3.2) using the SAE report form. The investigator should counsel the subject and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. A woman who becomes pregnant or wishes to while on the study will be counseled as to her choices and will be encouraged to discuss those choices with her obstetrician. Monitoring of the subject should continue until the conclusion of the pregnancy, and a follow-up SAE report form detailing the outcome of the pregnancy should be submitted.

8.3.4 Updating Source Documentation

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

9. MECHANISTIC ASSAYS

9.1 Metabolic Testing

9.1.1 Study Endpoints

Because the assessment of islet graft function is dependent on complex physiologic relationships between the graft and its recipient, no single test adequately addresses the viability of the transplant. Therefore, insulin-independence will be used as a clinically relevant measure of islet graft function for the primary endpoint, and additional stimulatory tests of islet graft function utilizing meal (MMTT) and glucose (FSIGT) challenges will be performed to assess secondary endpoints. Also, the effect of islet graft function on glycemic control (HbA1c), glycemic lability (MAGE and LI), hypoglycemia (Clarke and HYPO scores), glucose variability (CGMS), and QOL) will be assessed as additional secondary endpoints (see Section 4.1.2 for endpoint description).

9.1.2 Metabolic Assessments

All subjects will use a study provided One Touch® Ultra glucometer or an approved glucometer or CGMS unit identified in the MOP for measuring capillary glucose levels until one year after their final islet transplant. The timing of these metabolic assessments is provided in Appendices 1 - 3.

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

9.1.2.1 INSULIN REQUIREMENTS

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

9.1.2.2 GLYCEMIC CONTROL

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

9.1.2.3 GLYCEMIC LABILITY

Glycemic lability will be assessed by both the MAGE[49] and the LI[53] measured every 3 months following transplant, including 365 days post-initial transplant.

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

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

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

9.1.2.4 HYPOGLYCEMIA

An episode of severe hypoglycemia is defined as an event with one of the following symptoms: memory loss; confusion; uncontrollable behavior; irrational behavior; unusual difficulty in awakening; suspected seizure; seizure; loss of consciousness; or visual symptoms, in which the subject was unable to treat him/herself and which was associated with either a blood glucose level < 54 mg/dL (3.0 mmol/L) or prompt recovery after oral carbohydrate, IV glucose, or glucagon administration.[19]

In addition, composite indices of hypoglycemia frequency, severity, and symptom recognition will be assessed by both the Clarke survey[101] and the HYPO score[53].

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

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

9.1.2.5 MIXED-MEAL TOLERANCE TEST (MMTT)

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

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

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

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

The β-score will be determined from the HbA1c, insulin requirements, fasting (basal) glucose, and stimulated c-peptide as developed by Ryan et al.[102] The score may range from 0 (no graft function) to 8, with all subjects reported with a score of 8 also having 90-minute glucose levels during a MMTT that are ≤ 10.0 mmol/L (180 mg/dL), indicative of excellent graft function.

9.1.2.7 THE C-PEPTIDE (GLUCOSE X CREATININE) RATIO

The c-peptide: (glucose X creatinine) ratio (CPGCR) will be determined from the fasting (basal) glucose and c-peptide, and a simultaneous serum creatinine. This measure accounts for both the dependence of c-peptide secretion on the ambient glucose concentration and the dependence of c-peptide clearance on kidney function.[103, 104] 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.

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

The AIR_{glu}, insulin sensitivity, and disposition index (DI) will be determined using the FSIGT test. This assessment provides a composite measure of β-cell function, the disposition index (DI), which relates the effect of insulin sensitivity on first-phase insulin secretion (AIR_{glu}). Understanding the effect of insulin sensitivity on insulin secretory dynamics post-transplant is important because insulin resistance imposes an increased demand on β-cell function to maintain the same level of glycemia. Whether insulin resistance, possibly attributable to immunosuppressive drugs, is an important problem post-transplant is not known. Preliminary data indicate that insulin sensitivity may actually be improved post-transplant, despite immunosuppression, possibly due to the improved glycemia that occurs with transplantation[105]. These results require confirmation by longitudinal analysis.

The insulin-modified FSIGT test[106] involves blood sampling at baseline (t = -10, -5, and -1 min) and at t = 1, 2, 3, 4, 5, 7, 10, 12, 14, 16, 18, 20, 22, 25, 30, 40, 50, 70, 100, 140, & 180 minutes post-injection of glucose at t = -30 seconds with an injection of insulin at t = 20 min. Each pre-transplant blood sample will be used for insulin and glucose determination. Each post-transplant blood sample will be used for insulin and glucose determination; in addition, the

baseline (t = -10, -5, and -1 min) and t = 1, 2, 3, 4, 5, 7, and 10 minutes post-glucose injection samples will be used for c-peptide determination.

All samples will be drawn according to University of Washington (Seattle, WA) SOP and will be shipped frozen to U of W for measurement in the core laboratory. The AIR_{glu} is calculated as the incremental area-under-the-curve for insulin between 0 and 10 minutes post-injection (the same calculation can be performed for c-peptide). Glucose effectiveness (SG), a measure of insulin-independent glucose disposal, and insulin sensitivity, a measure of insulin-dependent glucose disposal, are derived from Bergman's minimal model using MinMod Millenium® software, and further allow for determination of the disposition index ($DI = AIR_{glu} \bullet SI$).

9.1.2.9 CONTINUOUS GLUCOSE MONITORING SYSTEM® (CGMS)

Glucose variability and hypoglycemia duration will be determined using CGMS® (Medtronic Minimed, Northridge, CA). CGMS® involves the SC placement of a glucose sensor connected by tubing to a pager-sized monitoring device that stores glucose data over a 72-hour period. Subjects will have the sensor placed in the diabetes clinic and wear it continuously for 72 – 84 hours. Then they will drop the monitoring device off or ship it to the clinic 4 days later for analysis. Subjects will need to calibrate the sensor to their capillary BG readings 4 times daily with no interval between readings exceeding 12 hours. Data from each 72-hour period will be analyzed for mean glucose concentration, mean glucose variability (absolute value of measured glucose minus 5.5 mmol/L [100 mg/dL]), number and duration of hyper- (> 10.0 mmol/L [180 mg/dL]) and hypo- (< 3.0 mmol/L [54 mg/dL]) glycemic episodes, and total duration of hypoglycemia.[51, 107]

9.1.2.10 QUALITY OF LIFE (QOL)

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

Generic Measures

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

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

EQ-5D (EuroQoL)

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

Disease-targeted Measures

Diabetes Distress Scale

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

Hypoglycemic Fear Survey

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

9.2 Immunologic Testing

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

maintenance of protective immunity in the setting of immunosuppression will be addressed, as will the role of innate immune reactions in the early post-transplant period.

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

9.2.1 Immune Assays

9.2.1.1 HLA TYPING OF DONORS AND RECIPIENTS, CROSSMATCHING

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

9.2.1.2 ALLOANTIBODY

Development of alloantibody is generally associated with longer term graft loss. Development of alloantibody specific for 1 or 2 HLA antigens can now be defined using assays that incorporate HLA specific monoclonal antibodies. Malek Kamoun at Penn will provide core lab service for alloantibody assessments.

9.2.1.3 AUTOANTIBODY

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

9.2.1.4 ARCHIVED SAMPLES

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

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

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

9.2.1.5 IMMUNOGENICITY DETERMINATION

Anti-belatacept antibody and immunogenicity testing will be performed during the study. Immunogenicity samples should also be taken 4 and 8-weeks post last dose for subjects discontinued from belatacept. Samples must be taken prior to beginning the infusion of belatacept.

10. STATISTICAL CONSIDERATIONS AND ANALYTICAL PLAN

10.1 Analysis Samples

The details of the analyses will be provided in the statistical analysis plan (SAP). All primary and secondary analyses will be done using the intention to treat principal. Every subject who receives belatacept or islets will be accounted for in the analysis.

10.2 Study Endpoint Assessment

10.2.1 Primary Endpoint

The primary objective of the analysis is to estimate the rate of insulin independence at 75 days after the first islet transplant. The primary endpoint is insulin independence (yes/no) at day 75 after islet transplant as defined in section 4.1.1. The primary analysis will compute an exact binomial estimate and a 95% confidence interval for the true rate. If the results of this trial show at least 10% insulin independence in the context of evaluation all of the secondary endpoints, the investigators will consider this grounds for further investigation.

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

10.2.2 Secondary Endpoints

Secondary endpoints are defined in section 4.1.2. There are a very large number of secondary endpoints so there will be no adjustment for multiplicity. We will not impute values for secondary endpoints but will conduct a sensitivity analysis to determine the potential magnitude of the contributions of the missing values. Details of the sensitivity analysis will be included in the SAP.

Analysis of secondary endpoints will use methods similar to those defined for the primary outcome. When the endpoint is a proportion such as patients achieving insulin independence at one year after completing their first islet transplant then the observed rate will be used as the point estimate and an exact 95% binomial confidence interval will be reported. Continuous variables will be treated in a similar fashion. If the necessary normality assumption is valid then the sample mean will be used as the point estimate and the usual 95% normal confidence intervals will be computed. Where the normality assumptions are not valid and an appropriate transform will achieve normality then the inverse of the mean of the transformed data will be used as the point estimate and the inverse of the endpoints for a standard 95% confidence interval for the transformed mean will be reported for the confidence interval. If no valid

transformation can be found then we will use the bootstrap method to construct a point estimate and a 95% confidence interval.

10.3 Patient and Demographic Data

10.3.1 Baseline Characteristics and Demographics

Summary descriptive statistics for baseline and demographic characteristics will be provided for the intent-to-treat population. Demographic data will include age, sex, race, ethnicity, sex, height and body weight; these data will be presented in the following manner:

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

10.3.2 Medical History

Medical history will be collected, including the existence of current signs and symptoms and clinical significance for each body system. Numbers and rates of treated subjects with previous history of each condition will be reported.

10.3.3 Use of Medications

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

10.3.4 Study Completion

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

10.4 Sample Size and Power Calculations

The purpose of this pilot study is to estimate the true rate of insulin independence at 75 days. The selected sample size is 10 subjects. The point estimate of the true insulin independence rate will be the proportion of the 10 patients that achieve insulin independence. The precision of the estimate depends on the observed number of subjects achieving insulin independence. The following table displays the confidence intervals that would be computed for each possible outcome. If 5 of the 10 subjects achieve insulin independence then the estimated rate will be 50% and a 95% confidence interval will be 0.1871 to 0.8129. That is, we are 95% confident that the true rate is at least 18.71% and no more than 81.29%. The confidence interval rules out any rate less than 18.71% or greater than 81.29%. If 3 patients (30%) achieve insulin independence, then the confidence interval will rule out any rate less than 6.67% or greater than 65.25%.

Table 5: Confidence Intervals for each possible outcomes

Number of Subjects insulin Independent at 75 Days	Estimated Rate	Exact 95% Confidence Interval	
		Lower Bound	Upper Bound
0	0	0	0.3085
1	0.1	0.0025	0.445
2	0.2	0.0252	0.5561
3	0.3	0.0667	0.6525
4	0.4	0.1216	0.7376
5	0.5	0.1871	0.8129
6	0.6	0.2624	0.8784
7	0.7	0.3475	0.9333
8	0.8	0.4439	0.9748
9	0.9	0.555	0.9975
10	1	0.6915	1

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 and in the subsequent SAP. Any changes in these principal features will require a protocol or an SAP amendment, which would be subject to review by the Steering Committee, the independent DSMB, the study sponsor, and the health authorities. These changes will be described in the final report as appropriate.

11. IDENTIFICATION AND ACCESS TO SOURCE DATA

11.1 Identifying Source Data

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

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

11.2 Permitting Access to Source Data

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

12. QUALITY CONTROL AND QUALITY ASSURANCE

The investigator is required to keep accurate records to ensure that the conduct of the study is fully documented.

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

12.1 Data Handling

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

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

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

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

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

13. ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE

13.1 Ethical Considerations

The rights, safety and well-being of the trial subjects are the most important considerations and should prevail over interests of science and society.

Study personnel involved in conducting this trial will be qualified by education, training, and experience to perform their respective task(s).

This trial will not use the services of study personnel where sanctions have been invoked or where there has been scientific misconduct or fraud (e.g., loss of medical licensure, debarment).

Systems with procedures that assure the quality of every aspect of the study will be implemented.

13.2 Statement of Compliance

This clinical study will be conducted using cGCP, as delineated in *Guidance for Industry: E6 Good Clinical Practice Consolidated Guidance*[5], and according to the criteria specified in this study protocol. Before study initiation, the protocol and the informed consent, subject recruitment materials/process (e.g., advertisements), and any other written information to be provided to subjects will be reviewed and approved by an appropriate EC or IRB, and NIAID/NIDDK. The investigator or sponsor should also provide the IRB/EC with a copy of the IB or product labeling and information to be provided to subjects and any updates. Any amendments to the protocol or to the consent materials must also be approved by the IRB/EC and submitted to the applicable Health Authorities before they are implemented.

13.3 Informed Consent and Assent

Preparation of the consent form is the responsibility of the investigator, and must include all elements required by ICH, GCP, and applicable regulatory requirements, and must adhere to GCP and to the ethical principles that have their origin in the Declaration of Helsinki. The consent form must also include a statement that the sponsor and regulatory authorities have direct access to subject records. Consent materials for subjects who do not speak or read English must be translated into the subjects' appropriate language.

The informed consent form must be revised whenever important new safety information is available, whenever the protocol is amended, and/or whenever any new information becomes available that may affect participation in the trial.

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

13.4 Privacy and Confidentiality

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

13.5 Mentally Impaired or Incapacitated Subjects

Investigators should determine whether or not a mentally impaired or incapacitated subject is capable of giving informed consent. If the subject is deemed mentally competent to give informed consent, the investigator should follow standard procedures. If the subject is deemed not to be mentally competent to give informed consent, he/she is not eligible to participate in this study.

Subjects who are involuntarily hospitalized because of mental illness will not be enrolled in this clinical trial.

13.6 Other Circumstances

Prisoners or individuals who are compulsorily detention for treatment of either a psychiatric or physical (e.g., infectious disease) illness will not be enrolled in this clinical trial.

13.7 Illiterate Subjects

If the subject or legally acceptable representative is unable to read, a reliable and independent witness should be present during the entire informed consent discussion. The choice of the witness must not breach the subject's rights to confidentiality. A reliable independent witness is defined as one not affiliated with the institution or engaged in the investigation. A family member or acquaintance is an appropriate independent witness. After the subject or legally acceptable representative orally consents and has signed, if capable, the witness should sign and personally date the consent form attesting that the information is accurate and that the subject or legally acceptable representative has fully understood the content of the informed consent agreement and is giving true informed consent.

13.8 Records Retention

The investigator must retain investigational product disposition records, copies of CRFs (paper or electronic files), and source documents for the maximum period required by applicable regulations and guidelines, or institution procedures, or for the period specified by the sponsor, whichever is longer. The investigator must contact the Sponsor prior to destroying any records associated with the study.

If the investigator withdraws from the study (e.g., relocation, retirement), the records shall be transferred to a mutually agreed upon designee (e.g., another investigator, IRB).

14. PUBLICATION POLICY

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

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Appendix 1: Year One Schedule of Events

Time points (in days relative to transplant)	SCR	WL / BL[1]	0[2]	3	4	7	14	21	28	56	75	84	112	140	168	196	224	252	280	308	336	365
Visit Number	01	02	03	03a	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21
Visit Windows (in days)	N/A	N/A	N/A	N/A	N/A	+/-2	+/-2	+/-2	+/-3	+/-3	+/-5	+/-3	+/-3	+/-3	+/-3	+/-5	+/-5	+/-5	+/-5	+/-5	+/-5	+/-5
Equivalent Week	N/A	N/A	N/A	N/A	N/A	W1	W2	W3	W4	W8	N/A	W12	W16	W20	W24	W28	W32	W36	W40	W44	W48	W52
GENERAL ASSESSMENTS																						
Informed Consent	X[3]	X[4]																				
Med/Diabetes Hx & Demographics	X																					
Eval of Inclusion / Exclusion	X	X																				
Mammogram (females >35)	X	X-yrly																				
Retinopathy Evaluation[5]	X	X-yrly[6]																				X
Physical Exam	X	X-yrly	X			X	X	X	X	X	X		X	X		X			X			X
QOL		X-q3mo									X					X			X			X
Chest X-Ray	X	X-yrly																				X
Abdominal US (Pelvis/Liver	X	X-yrly				X																X
ECG	X	X-yrly																				X
Cardiac Stress Test or Angiogram	X																					
PPD	X	X-yrly																				X
AE/Hypo Event/Toxicity Assess		X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
LOCAL LABORATORY ASSESSMENTS																						
CBC (WBC + Diff & Plat)	X	X-q6mo	X			X	X	X	X	X	X		X	X		X			X			X
Chemistry[7]	X	X-yq6mo	X			X	X	X	X	X	X		X	X		X			X			X
Lipids	X	X-q6mo									X					X			X			X
Thyroid Function (TSH)	X	X-yrly																				
Pregnancy test (WOCBP)	X	X[8]			X[9]		X[9]		X[9]	X[9]		X[9]	X[9]	X[9]	X[9]	X[9]	X[9]	X[9]	X[9]	X[9]	X[9]	X[9]
Serology[10] (Hep B/C, HIV,)	X	X-yrly																				X

Time points (in days relative to transplant)	SCR	WL / BL[1]	0[2]	3	4	7	14	21	28	56	75	84	112	140	168	196	224	252	280	308	336	365
Visit Number	01	02	03	03a	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21
Visit Windows (in days)	N/A	N/A	N/A	N/A	N/A	+/-2	+/-2	+/-2	+/-3	+/-3	+/-5	+/-3	+/-3	+/-3	+/-3	+/-5	+/-5	+/-5	+/-5	+/-5	+/-5	+/-5
Equivalent Week	N/A	N/A	N/A	N/A	N/A	W1	W2	W3	W4	W8	N/A	W12	W16	W20	W24	W28	W32	W36	W40	W44	W48	W52
LOCAL LABORATORY ASSESSMENTS (Con't)																						
EBV IgG	X																					
CMV IgG, CMV IgM		X-yrly[11]																				X[11]
Coagulation (PT, PTT, INR)	X	X-yrly	X																			
Blood Type		X[12]																				
HLA		X																				
Crossmatch		X[13]																				
Fasting & post-prandial c-pep[14]				X		X																
Glucose (immediately post-tx)			X[15]																			
PRA by flow cytometry		X[16]																				
CMV by PCR		X									X				X							
EBV by PCR[17]		X																				
CENTRAL LABORATORY ASSESSMENTS																						
First morning spot urine[18]	X	X							X		X											X
GFR	X	X-yrly							X		X											X
HbA1c	X	X-q3mo									X					X			X			X
Fasting serum gluc/c-pep & creat[19]	X	X							X	X	X		X	X	X	X	X	X	X	X	X	X
Insulin modified FSIGT[19]		X-yrly6									X											X
90 min[20] c-pep/gluc (MMTT)[19]	X										X					X			X			X
Atherogenic Profile[21]		X																				X
LOCAL METABOLIC ASSESSMENTS																						
Glycemic Stability (CGMS)[19]		X6									X											X
BSR eCRFs[19,22]	X	X-q6mo									X					X			X			X

Time points (in days relative to transplant)	SCR	WL / BL[1]	0[2]	3	4	7	14	21	28	56	75	84	112	140	168	196	224	252	280	308	336	365
Visit Number	01	02	03	03a	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21
Visit Windows (in days)	N/A	N/A	N/A	N/A	N/A	+/-2	+/-2	+/-2	+/-3	+/-3	+/-5	+/-3	+/-3	+/-3	+/-3	+/-5	+/-5	+/-5	+/-5	+/-5	+/-5	+/-5
Equivalent Week	N/A	N/A	N/A	N/A	N/A	W1	W2	W3	W4	W8	N/A	W12	W16	W20	W24	W28	W32	W36	W40	W44	W48	W52
CALCULATED METABOLIC ASSESSMENTS																						
MAGE		X-q6mo									X					X			X			X
LI	X	X-q6mo									X					X			X			X
Clarke Score	X	X-q6mo														X						X
HYPO	X	X-q6mo									X					X			X			X
Beta Score		X									X					X			X			X
C-peptide (gluc X creat) ratio	X	X							X	X	X		X	X	X	X	X	X	X	X	X	X
IMMUNOSUPPRESSION LEVELS																						
Belatacept trough levels[23]					X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
MECHANISTIC ASSAYS																						
Alloantibody	X	X-q6mo[24]									X					X			X			X
Autoantibody[25]		X									X					X			X			X
Immunogenicity samples[26]			X[27]												X							X
ARCHIVED SAMPLES																						
Serum		X									X					X			X			X
Plasma		X									X					X			X			X

[1] WL = Waiting List. BL = Baseline. Repeat assessments as indicated (i.e. yrly, q3mo), while subject is on the waiting list. All one-time WL/BL assessments should be completed on Day -2 whenever possible, but always prior to start of immunosuppression. For WL/BL repeat assessments, record results from test done closest to the start of immunosuppression will be used as the baseline value.
[2] Day 0 = the day of transplant.
[3] *Informed consent #1* includes information on CIT04 and the multi-center Phase 3 protocol (CIT07)
[4] *Informed Consent #2* includes information specific to CIT04. IC # 2 must be signed immediately after randomization.
[5] Retinopathy eval includes fundoscopic pictures for WL/BL assessments and Y1. Screening retinopathy evaluation should be done per site-specific standards. If pupils cannot be dilated, then a manual ophthalmologic evaluation can be substituted.
[6] These can be collected after subject is considered protocol eligible and has been moved to the transplant wait list, as time allows.
[7] Chemistry includes: Sodium, albumin, magnesium, chloride, potassium, alk phosphatase, total bilirubin, CO_2, creatinine, ALT (SGPT), BUN, gamma GT, glucose, AST (SGOT), calcium, phosphorus

[8] Complete serum pregnancy tests within 72 hours prior to initiation of study medication.
[9] Complete test prior to Belatacept infusion. Confirm negative result prior to administering Belatacept
[10] Serology includes: HBc Ab, HBs Ab, HBs Ag, HCV Ab, and HIV. Do not repeat Hepatitis B tests if HBs Ab was previously positive.
[11] Repeat only if previous test was negative.
[12] Repeat for subsequent transplant(s)
[13] Sample used for crossmatch may be obtained up to 60 days prior to the start of immunosuppression, as long as there is no evidence of infections or transfusions since the time the sample was drawn. Repeat crossmatch for subsequent transplants.
[14] C-peptide should be done locally and drawn fasting, and twice between 1-3 hrs post-prandial on Day 3 and Day 7 post-transplant.
[15] Finger stick glucose should be done locally and drawn every hour for the first 6 hours immediately post-transplant.
[16] Subsequent transplants only. Local result used to determine eligibility for subsequent transplants only.
[17] EBV by PCR should only be done post-randomization if reactivation is suspected.
[18] First morning spot urine includes: albumin, protein, and creatinine
[19] Do not collect for participants with graft failure. Results of tests performed at the time of graft failure will be used for day 75 endpoint calculations.
[20] MMTT should include 60 and 90 minute c-peptide and glucose measurements for the screening visit and as necessary when determining graft failure.
[21] Atherogenic profile consisting of fasting lipid panel (TG, TC, HDL, LDL, non-HDL), C reactive protein, serum amyloid A, apolipoprotein A1 and apolipoprotein B. If blood is drawn locally, sample should be sent from local lab to study site and then shipped to the central laboratory (Univ of Washington).
[22] Blood Sugar Record (BSR) eCRF is completed using information gathered from subject diary logs, glucometer download data, and insulin requirements
[23] Complete test prior to Belatacept infusion.
[24] For each transplant, complete alloantibody assessment every 6 months and again on Day -2, regardless of the most recent draw. Central PRA result, current within 6 months, is used to determine subject eligibility for first transplant
[25] Autoantibody testing includes GAD, IA-2, and IAA.
[26] Collect immunogenicity samples prior to belatacept infusion. For subjects that discontinue belatacept, collect additional immunogenicity samples at 4 weeks and 8 weeks post last dose.
[27] Collect immunogenicity sample prior to dosing with immunosuppression medications.

Appendix 2: Continuation of Appendix 1 Schedule of Events (Subjects with Subsequent CIT Transplants)

Subjects who receive a subsequent islet transplant should continue their study visits according to this Schedule of Events until one year (365 +/- 5 days) after the final islet transplant. Additional visits - outlined in Appendix 3 - will also need to be completed after each subsequent transplant. After day 365 post final transplant, subjects should stop following this schedule, and complete the follow-up visits outlined in Appendix 4: *Schedule of Events for 1-Year Additional Follow-Up*.

Time points (in days relative to 1st transplant)	392	420	448	476	504	532	560	588	616
Visit Number	22	23	24	25	26	27	28	29	30
Visit Windows (in days)	±5	±5	±5	±5	±5	±5	±5	±5	±5
Equivalent Week	W56	W60	W64	W68	W72	W76	W80	W84	W88
GENERAL ASSESSMENTS									
Physical Exam			X			X			X
QOL			X			X			X
AE/Hypo Event/Toxicity Assessment	X	X	X	X	X	X	X	X	X
LOCAL LABORATORY ASSESSMENTS[1]									
CBC (WBC + Diff & Plat)			X			X			X
Chemistry[2]			X			X			X
Lipids			X			X			X
Pregnancy test (WOCBP)[3]	X	X	X	X	X	X	X	X	X
CENTRAL LABORATORY ASSESSMENT									
HbA1c			X			X			X
Fasting serum glucose & c-peptide & serum creat[4]	X	X	X	X	X	X	X	X	X
90 min[5] c-peptide/glucose (MMTT)[4]			X			X			X
Atherogenic Profile[6]	*1 year post-final transplant*								
LOCAL METABOLIC ASSESSMENTS									
BSR eCRFs4[7]			X			X			X
CALCULATED METABOLIC ASSESSMENTS									
MAGE			X			X			X
LI			X			X			X
Clarke Score						X			
HYPO			X			X			X

Time points (in days relative to 1st transplant)	392	420	448	476	504	532	560	588	616
Visit Number	22	23	24	25	26	27	28	29	30
Visit Windows (in days)	±5	±5	±5	±5	±5	±5	±5	±5	±5
Equivalent Week	W56	W60	W64	W68	W72	W76	W80	W84	W88
CALCULATED METABOLIC ASSESSMENTS (continued)									
Beta Score			X			X			X
C-peptide (glucose X creatinine) ratio	X	X	X	X	X	X	X	X	X
IMMUNOSUPPRESSION LEVELS									
Belatacept trough levels[8]	X	X	X	X	X	X	X	X	X
MECHANISTIC ASSAYS									
Alloantibodies[9]			X			X			X
Autoantibodies[10]			X			X			X
Immunogenicity samples[11]						X			
ARCHIVED SAMPLES									
Serum			X			X			X
Plasma			X			X			X

[1] EBV by PCR should only be done post-randomization if reactivation is suspected.
[2] Chemistry includes: Sodium, albumin, magnesium, chloride, potassium, alk phosphatase, total bilirubin, CO_2, creatinine, ALT (SGPT), BUN, gamma GT, glucose, AST (SGOT), calcium, phosphorus
[3] Confirm negative result prior to administering Belatacept.
[4] Also collect as necessary to perform graft failure. Do not collect after graft failure has been confirmed.
[5] MMTT should include 90 minute c-peptide and glucose measurements, add 60 minute as necessary when determining graft failure.
[6] Atherogenic profile consisting of fasting lipid panel (TG, TC, HDL, LDL, non-HDL), C reactive protein, serum amyloid A, apolipoprotein A1 and apolipoprotein B. If blood is drawn locally, sample should be sent from local lab to study site and then shipped to the central laboratory (Univ of Washington).
[7] Blood sugar Record (BSR) eCRF is completed using information gathered from subject diary logs, glucometer download data, and insulin requirements.
[8] Complete test prior to Belatacept infusion. For pregnancy test, confirm negative result prior to administering Belatacept.
[9] For each transplant, complete alloantibody assessment every 6 months and again on Day -2, regardless of the most recent draw.
[10] Autoantibody testing includes GAD, IA-2, and IAA.
[11] Collect immunogenicity samples prior to belatacept infusion. For subjects that discontinue belatacept, collect additional immunogenicity samples at 4 weeks and 8 weeks post last dose.

Appendix 3: Subsequent Transplant Schedule of Events

The following immediate post-transplant and endpoint assessments should be completed for subjects who receive a subsequent islet transplant. If any of the visits below fall within an acceptable window of a follow-up visit on the Year One Schedule of Events (Appendix 1) or Year Two Schedule of Events (Appendix 2), the assessments may be added to that follow-up visit so that a separate visit does not need to occur.

Time point (in days relative to most recent infusion)	0[1]	3	4	7	75	365
Visit Number	TBD	TBD	TBD	TBD	TBD	TBD
Visit Window (in days)	N/A	N/A	N/A	+/- 3	+/- 5	+/- 14
Equivalent Week (post most recent infusion)	N/A	N/A	N/A	W1	N/A	W52
GENERAL ASSESSMENTS						
Physical Exam	X			X	X	X
QOL					X	X
Chest X-Ray						X
Abdominal US (Pelvis/Liver)				X		X
ECG						X
PPD						X
AE/Hypo Event/Toxicity Assess	X		X	X	X	X
LOCAL LABORATORY ASSESSMENTS						
CBC (WBC + Diff & Plat)	X			X	X	X
Chemistry[2]	X			X	X	X
Lipids					X	X
Coagulation (PT, PTT, INR)	X					
Blood Type & HLA	X[3]					
Crossmatch	X[4]					
PRA by flow cytometry	X[5]					
Fasting & post-prandial c-peptide[6]		X		X		
Glucose (immediately post-transplant)[7]	X					
Pregnancy test (WOCBP)[8]	X					X
CMV by PCR[9]					X	
CENTRAL LABORATORY ASSESSMENTS						
First morning spot urine[10]					X	X
GFR					X	X
HbA1c					X	X
Fasting serum glucose/c-peptide & creat[11]					X	X
Insulin modified FSIGT[11]					X	X
90 min c-pep/glucose (MMTT)[11]					X	X
Artherogenic Profile[12]						X
LOCAL METABOLIC ASSESSMENTS						
Glycemic Stability (CGMS)[11]					X	X
BSR eCRFs[11,13]					X	X
CALCULATED METABOLIC ASSESSMENTS						
MAGE					X	X
LI					X	X
Clarke Score						X
HYPO					X	X
Beta Score					X	X

Time point (in days relative to most recent infusion)	0[1]	3	4	7	75	365
Visit Number	TBD	TBD	TBD	TBD	TBD	TBD
Visit Window (in days)	N/A	N/A	N/A	+/- 3	+/- 5	+/- 14
Equivalent Week (post most recent infusion)	N/A	N/A	N/A	W1	N/A	W52
CALCULATED METABOLIC ASSESSMENTS (continued)						
C-peptide glucose creatinine ratio					X	X
IMMUNOSUPPRESSION LEVELS						
Belatacept trough levels[14]			X	X	X	X
MECHANISTIC ASSAYS						
Alloantibody[15]	X				X	X
Autoantibody[16]					X	X
Immunogenicity samples[17]						X
ARCHIVED SAMPLES						
Serum					X	X
Plasma					X	X

[1] Day 0 = the day of transplant.
[2] Chemistry includes: Sodium, albumin, magnesium, chloride, potassium, alk phosphatase, total bilirubin, CO_2, creatinine, ALT (SGPT), BUN, gamma GT, glucose, AST (SGOT), calcium, phosphorus
[3] Repeat for subsequent transplant(s).
[4] Sample used for crossmatch may be obtained up to 60 days prior to islet infusion, as long as there is no evidence of infections or transfusions since the time the sample was drawn.
[5] PRA by flow cytometry should be performed locally prior to any subsequent transplant. Local result used to determine eligibility for subsequent transplants only.
[6] C-peptide should be done locally and drawn fasting, and twice between 1-3 hrs post-prandial on Day 3 and Day 7 post-transplant.
[7] Finger stick glucose should be done locally and drawn every hour for the first 6 hours immediately post-transplant.
[8] Perform pregnancy test and confirm negative result prior to each belatacept infusion.
[9] EBV by PCR should only be done post-randomization if reactivation is suspected.
[10] First morning spot urine includes: albumin, protein, and creatinine.
[11] Also collect as necessary to confirm graft failure. Do not collect after graft failure has been confirmed.
[12] Atherogenic profile consisting of fasting lipid panel (TG, TC, HDL, LDL, non-HDL), C reactive protein, serum amyloid A, apolipoprotein A1 and apolipoprotein B. If blood is drawn locally, sample should be sent from local lab to study site and then shipped to the central laboratory (Univ of Washington).
[13] Blood Sugar Record (BSR) eCRF is completed using information gathered from subject diary logs, glucometer download data, and insulin requirements.
[14] Complete test prior to belatacept infusion. For pregnancy test, confirm negative result prior to administering belatacept.
[15] For each transplant, complete alloantibody assessment every 6 months and again on Day -2, regardless of the most recent draw.
[16] Autoantibody testing includes GAD, IA-2, and IAA.
[17] Collect immunogenicity samples prior to belatacept infusion. For subjects that discontinue belatacept, collect additional immunogenicity samples at 4 weeks and 8 weeks post last dose.

Appendix 4: Schedule of Events for 1-Year Additional Follow-Up

Time point (Equivalent weeks after 'Day 365 post final transplant' visit)	**W4 (AY)**	**W8 (AY)**	**W12 (AY)**	**W16 (AY)**	**W20 (AY)**	**W24 (AY)**	**W28 (AY)**	**W32 (AY)**	**W36 (AY)**	**W40 (AY)**	**W44 (AY)**	**W48 (AY)**	**W52 (AY)**	**Y2[1] (AY)**
Visit Number (relative to final islet transplant)	**51**	**52**	**53**	**54**	**55**	**56**	**57**	**58**	**59**	**60**	**61**	**62**	**63**	**64**
Visit Window (specified in days)	**±5**	**±5**	**±5**	**±5**	**±5**	**±5**	**±5**	**±5**	**±5**	**±5**	**±5**	**±5**	**±5**	**±30**
GENERAL ASSESSMENTS														
Physical Exam						X							X	X
Telephone Consult			X							X				
QOL													X	X
AE /Hypoglycemic Events/Toxicity Assessment			X			X				X			X	X
LOCAL LABORATORY ASSESSMENTS														
CBC (WBC + Diff & Plat)			X			X				X			X	
Chemistry[2]			X			X				X			X	
Lipids						X							X	
Pregnancy test (WOCBP)[3]	X	X	X	X	X	X	X	X	X	X	X	X	X	
CENTRAL LABORATORY ASSESSMENTS														
First morning spot urine[4]						X							X	
GFR													X	
HbA1c			X[5]			X				X[5]			X	X
90-min c-pep/glucose (MMTT)[2]						X							X	X
Atherogenic Profile													X	
LOCAL METABOLIC ASSESSMENTS														
Glycemic Stability (CGMS)[2]													X	X

[1] Two years post initial transplant.

[2] Also collect as necessary to confirm graft failure. Do not collect after graft failure is confirmed.

Time point (Equivalent weeks after 'Day 365 post final transplant' visit)	**W4 (AY)**	**W8 (AY)**	**W12 (AY)**	**W16 (AY)**	**W20 (AY)**	**W24 (AY)**	**W28 (AY)**	**W32 (AY)**	**W36 (AY)**	**W40 (AY)**	**W44 (AY)**	**W48 (AY)**	**W52 (AY)**	**Y2[1] (AY)**
Visit Number (relative to final islet transplant)	**51**	**52**	**53**	**54**	**55**	**56**	**57**	**58**	**59**	**60**	**61**	**62**	**63**	**64**
Visit Window (specified in days)	**±5**	**±5**	**±5**	**±5**	**±5**	**±5**	**±5**	**±5**	**±5**	**±5**	**±5**	**±5**	**±5**	**±30**
CALCULATED METABOLIC ASSESSMENTS														
Clarke Score													X	
IMMUNOSUPPRESSION LEVELS														
Belatacept Trough Levels	X	X	X	X	X	X	X	X	X	X	X	X	X	
MECHANISTIC ASSAYS														
Autoantibody						X							X	
MECHANISTIC ASSAYS (Cont'd)														
Alloantibody						X							X	
Immunogenicity Samples													X	X

[1] Since belatacept is infused every 28 days, an additional infusion visit will be required during the second year. Since the timing is dependent on final transplant date, this visit can be shifted as needed.
[2] Chemistry includes: Sodium, albumin, magnesium, chloride, potassium, alk phosphatase, total bilirubin, CO2, creatinine, ALT (SGPT), BUN, gamma GT, glucose, AST (SGOT), calcium, phosphorus.
[3] Perform pregnancy test and confirm negative result prior to each belatacept infusion.
[4] First morning spot urine includes: albumin, protein, and creatinine.
[5] Can be drawn locally.

Appendix 5: Reduced Follow-up Schedule of Events

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

REDUCED FOLLOW-UP SCHEDULE

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

- Assess SAEs and hypoglycemic events: q1 month. If subject does not come to the study site for the visit, attempt to obtain information via a phone contact.
- Alloantibody (central lab): q 1 month for the first 3 months and q 3 months thereafter.
- Immunogenicity Samples: for subjects who discontinue belatacept, collect samples at 4 weeks and 8 weeks post last dose

Complete the following assessments at 1 and 2 years (+/- 14 days) post **initial** transplant:

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

Complete the following assessments at 1 and 2 years (+/- 7 days) post **last** transplant:

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

Appendix 6: Study Contacts

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

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

CIT PRINCIPAL INVESTIGATORS

Clinical Islet Transplantation (CIT) Consortium
(as defined in RFA-DK-04-005)

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Confidentiality Statement

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

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

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

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

I confirm that I have read the above protocol in the latest version. I understand it, and I will work according to the principles of Good Clinical Practice (GCP) as described in the United States Code of Federal Regulations (CFR) – 21 CFR Parts 45, 50, 54, 56, and 312, and the International Conference on Harmonization (ICH) document "Guidance for Industry: E6 Good Clinical Practice: Consolidated Guidance" dated April 1996. Further, I will conduct the study in keeping with local, legal, and regulatory requirements.

As the Site Principal Investigator, I agree to conduct protocol CIT-08, "Islet Transplantation in Type 1 Diabetes" according to good clinical practices. I agree to carry out the study by the criteria written in the protocol and understand that no changes can be made to this protocol without written permission of the NIAID and NIDDK.

_____

Site Principal Investigator (Print)

_____ _____

Site Principal Investigator (Signature) **Date**

Protocol Synopsis

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

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

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

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Glossary of Abbreviations

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

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

Study Definitions

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

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

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

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

1. BACKGROUND AND RATIONALE

1.1 Background

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

1.2 CIT Parent Studies

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

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

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

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

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

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

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

1.3 Rationale for Current Protocol

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

1.4 Known and Potential Risks and Benefits to Human Subjects

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

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

1.4.1.1 SIROLIMUS (RAPAMUNE®)

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

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

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

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

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

1.4.1.2 TACROLIMUS (PROGRAF®)

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

1.4.1.3 CYCLOSPORINE (NEORAL®)

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

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

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

Contraception requirements are outlined in the eligibility criteria.

1.4.2 Risk of Study Procedures

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

1.4.2.1 METABOLIC STIMULATION TESTING

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

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

1.4.2.2 BLOOD DRAW TESTING

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

1.4.3 Benefits

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

2. OBJECTIVES

2.1 Primary Objective

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

3. SELECTION OF SUBJECTS

3.1 Inclusion Criteria

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

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

3.2 Exclusion Criteria

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

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

4. STUDY DESIGN

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

4.1 Study Endpoints

4.1.1 Primary Endpoint

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

4.1.2 Secondary Endpoints

Secondary endpoints include the following:

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

5. STUDY TREATMENT REGIMEN

5.1 Immunosuppression Medications

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

5.1.1 Drug Accountability

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

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

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

5.2 Concomitant Medications

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

5.3 Rescue Medications

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

5.4 Prohibited Medications

None.

6. CRITERIA FOR PREMATURE TERMINATION OF THE STUDY

6.1 Subject Withdrawal Criteria

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

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

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

6.2 Subject Stopping Rules

6.2.1 Subject Stopping Rules

None.

6.2.2 Study Stopping Rules

None.

7. STUDY PROCEDURES

7.1 Enrollment and Screening

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

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

7.2 Follow-up Visits

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

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

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

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

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

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

7.3 Visit Windows

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

8. SAFETY MONITORING

8.1 Overview

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

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

8.2 Definitions

8.2.1 Adverse Event

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

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

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

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

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

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

8.2.2 Suspected Adverse Reaction (SAR)

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

8.2.3 Unexpected Adverse Event

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

8.2.4 Serious Adverse Event

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

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

8.3 Grading and Attribution of Adverse Events

8.3.1 Grading Criteria

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

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

Grade 1 = Mild AE.

Grade 2 = Moderate AE.

Grade 3 = Severe and undesirable AE.

Grade 4 = Life-threatening or disabling AE.

Grade 5 = Death.

Table 4: General severity definition of adverse event

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

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

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

8.3.2 Definition of Attribution

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

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

Table 5: Attribution of adverse events

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

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

8.4 Collecting and Recording of Adverse Events

8.4.1 Collection Period

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

8.4.2 Collecting Adverse Events

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

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

8.4.3 Recording Adverse Events

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

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

Adverse events collected on a case report form.

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

8.4.4 Reporting Serious Adverse Events

8.4.4.1 REPORTING OF SERIOUS ADVERSE EVENTS TO SPONSOR

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

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

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

8.4.4.2 REPORTING OF ADVERSE EVENTS TO IRBS

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

8.4.4.3 REPORTING PREGNANCY

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

8.5 Review of Safety Information

8.5.1 Medical Monitor Review

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

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

8.5.2 DSMB Review

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

9. MECHANISTIC ASSAYS

9.1 Metabolic Testing

9.1.1 Study Endpoints

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

9.1.1.1 GLYCEMIC CONTROL

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

9.1.1.2 HYPOGLYCEMIA

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

9.1.1.3 MIXED-MEAL TOLERANCE TEST (MMTT)

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

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

9.2 Immunologic Testing

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

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

9.2.1 Immune Assays

9.2.1.1 ALLOANTIBODY

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

9.2.1.2 ARCHIVED SERUM

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

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

10. STATISTICAL CONSIDERATIONS AND ANALYTICAL PLAN

10.1 Study Endpoint Assessment

10.1.1 Primary Endpoint

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

10.1.2 Secondary Endpoints

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

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

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

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

Life table methods will be used to estimate mortality rates.

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

10.2 Patient and Demographic Data

10.2.1 Baseline Characteristics and Demographics

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

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

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

10.3 Reporting Deviations from Original Statistical Plan

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

11. IDENTIFICATION AND ACCESS TO SOURCE DATA

11.1 Identifying Source Data

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

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

11.2 Permitting Access to Source Data

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

12. QUALITY CONTROL AND QUALITY ASSURANCE

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

12.1 Compliance, Access, Entry and Handling of Study Data

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

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

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

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

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

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

13. ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE

13.1 Statement of Compliance

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

13.2 Informed Consent and Assent

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

The informed consent form must be revised whenever important new safety information is available, whenever the protocol is amended, and/or whenever any new information becomes available that may affect participation in the trial.

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

13.3 Privacy and Confidentiality

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

14. PUBLICATION POLICY

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

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Appendix 1. Schedule of Events for Extended Follow-up

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

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

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

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

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

[5] Also collect as clinically indicated.

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

[7] Do not collect after confirmed graft failure.

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

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

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

Appendix 2. Study Contacts

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