# CLINICAL ISLET TRANSPLANTATION (CIT)

# PROTOCOL NUMBER: CIT-03

## Multi-center, open-label clinical trial of the efficacy of peritransplant administration of deoxyspergualin in promoting restoration of insulin independence after single-donor islet allotransplantation in non-uremic type 1 diabetic recipients

## Peritransplant deoxyspergualin in islet transplantation in type 1 diabetes

**Version 7.0 04 October 2012**

**BB-IND 9336**

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

**Drug Manufacturer:** Nippon Kayaku Co., Ltd.

**Representative in Europe and U.S.:** Disphar International B.V

**CIT PRINCIPAL INVESTIGATORS**

**BERNHARD HERING, MD**
University of Minnesota

**XUNRONG LUO, MD, PHD**
Northwestern University

**ANDREW POSSELT, MD, PHD**
University of California, San Francisco

**MEDICAL MONITOR**

**NANCY BRIDGES, MD**
Chief, Clinical Transplantation Immunology Branch
Division of Allergy, Immunology, and Transplantation
National Institute of Allergy and Infectious Diseases
6610 Rockledge Dr.; Room 6325
Bethesda, MD 20892
Phone: 301-451-4406
Fax: 301-402-2571
E-mail: nbridges@niaid.nih.gov

**BIOSTATISTICIAN**

**WILLIAM CLARKE, PHD**
Department of Biostatistics; CTSDMC
University of Iowa
2400 UCC
Iowa City, Iowa 52242
Phone: 319-384-2833
Fax: 319-335-6535
E-mail: William-clarke@uiowa.edu

**SENIOR REGULATORY OFFICER**

**Julia Goldstein, MD**
Senior Regulatory Officer
Division of Allergy, Immunology, and Transplantation
National Institute of Allergy and Infectious Diseases
6610 Rockledge Dr. Rm 6717
Bethesda, MD 20892
Phone: 301-451-3112
Fax: 301-480-1537
E-mail: goldsteinj@niaid.nih.gov

**PROJECT MANAGER**

**Allison Priore, BS**
Project Manager
Division of Allergy, Immunology, and Transplantation
National Institute of Allergy and Infectious Diseases
6610 Rockledge Dr.; Room 6304B
Bethesda, MD 20892
Phone: 301-560-4513
Fax: 301-402-2571
E-mail: priorea@niaid.nih.gov



| INVESTIGATOR SIGNATURE PAGE | |
|---|---|
| **Protocol Number:**<br>CIT-03 | **Version/Date:**<br>7.0 / (04 October 2012) |
| **IND:**<br>BB-IND 9336 | **CIT Principal Investigator:**<br>Bernhard Hering, MD, Xunrong Luo MD, PhD, Andrew Posselt, MD, PhD |
| **Short Title:**<br>Peritransplant deoxyspergualin in islet transplantation in type 1 diabetes | |
| **Study Sponsor(s):**<br>The National Institute of Allergy and Infectious Diseases (NIAID)<br>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 and Data Management Center**
**Department of Biostatistics**
**201 S Clinton St**
**Iowa City, IA 52240-4034**

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

As the Site Principal Investigator, I agree to conduct Protocol Number **CIT-03, "Peritransplant deoxyspergualin in islet transplantation in type 1 diabetes"**. 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.

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**Site Principal Investigator (Print)**

_____ _____

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

# Protocol Synopsis

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|---|---|
| **Short Title** | Peritransplant deoxyspergualin in islet transplantation in type 1 diabetes |
| **Clinical Phase** | Phase 2 |
| **IND Sponsor** | DAIT/NIAID/NIH |
| **IND Number** | BB-IND 9336 |
| **Activation Date** | October 2006 |
| **Accrual Objective** | 14 |
| **Accrual Period** | 24 months |
| **Follow-up Period** | 24 months after the final transplant |
| **Study Design** | A prospective, multi-center, single-arm, open-label trial assessing the safety and efficacy of DSG on post-transplant islet function in subjects with long-standing type 1 diabetes 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 ATG (1st transplant only), basiliximab (subsequent transplants only), etanercept, DSG, sirolimus and low-dose tacrolimus 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.<br><br>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<br><br>**Secondary Efficacy Endpoints:**<br>At 75 ± 5 days following the <u>first</u> islet transplant and following each <u>subsequent islet</u> transplant(s):<br>• The percent reduction in insulin requirements<br>• HbA1c<br>• MAGE[1]<br>• LI[2]<br>• Ryan hypoglycemia severity (HYPO) score[2]<br>• Basal (fasting) and 90-min glucose and C-peptide derived from the mixed-meal tolerance test (MMTT)<br>• β-score[3]<br>• C-peptide: (glucose · creatinine) ratio<br>• Acute insulin response to glucose ($AIR_{glu}$), insulin sensitivity, and disposition index (DI) derived from the insulin-modified frequently-sampled IV glucose tolerance (FSIGT)[4,5]<br>• Glucose variability[6] and hypoglycemia duration[7] derived from the CGMS®<br>• QOL measures |

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| | 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.<br><br>At 365 ± 14 days following the first and final islet transplant(s):<br>• The percent reduction in insulin requirements<br>• HbA1c<br>• MAGE<br>• LI<br>• Clarke score<br>• HYPO score<br>• Basal (fasting) and 90-min glucose and C-peptide (MMTT)<br>• β-score<br>• C-peptide: (glucose · creatinine) ratio<br>• Glucose variability[6] and hypoglycemia duration[7] derived from the CGMS®<br>• AIRglu, insulin sensitivity, and disposition index (DI) derived from the insulin-modified FSIGT[4, 5]<br>• QOL measures<br>• The proportion of subjects receiving a second islet transplant<br>• The proportion of subjects receiving a third islet transplant<br>• Rate of favorable outcome at each center preparing islets (rate of subjects with an Hb1Ac <7.0% and free of severe hypoglycemic events)<br><br>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±days following the final islet transplant.<br><br>At two years (730+14 days) following the final islet transplant:<br>• The percent change from baseline insulin requirements.<br>• The number of severe hypoglycemic events from 28 days to two years.<br>• HbA1c.<br>• Clarke score.<br>• Basal (fasting) and 90-min glucose and c-peptide (MMTT).<br>• β-score.<br>• C-peptide: (glucose• creatinine) ratio.<br>• CGMS.<br>• QOL<br><br>Secondary Safety Endpoints:<br>• Safety, including incidence of post-transplant infections, malignancies, morbidity, and other AES (e.g., increased body weight and hypertension) associated with conventional immunosuppression.<br>• Renal function as measured by serum creatinine, GFR and other relevant laboratory parameters. |

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| | • Lipid profiles (triglycerides, total cholesterol, LDL cholesterol, HDL cholesterol) over time.<br><br>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:<br>• The incidence and severity of AEs related to the islet transplant procedure including: bleeding (> 2 g/dL decrease in Hb concentration); segmental portal vein thrombosis; biliary puncture; wound complication (infection or subsequent hernia); and increased transaminase levels (> 5 times upper limit of normal [ULN])<br>• 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<br>• The incidence of a change in the immunosuppression drug regimen<br>• The incidence of immune sensitization defined by presence of anti-HLA antibodies absent prior to transplantation<br>• The incidence of discontinuation of immunosuppression<br><br>At 365 ± 14 days following the first islet transplant:<br>• The incidence of worsening retinopathy as assessed by change in retinal photography. If pupil dilation is not possible, then a manual ophthalmologic exam can be substituted. |
| **Inclusion Criteria** | Patients who meet all of the following criteria are eligible for participation in the study:<br><br>1. Male and female patients age 18 to 65 years of age.<br>2. Ability to provide written informed consent.<br>3. Mentally stable and able to comply with the procedures of the study protocol.<br>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.<br>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.<br>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. |

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| | 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.<br>7. At least one episode of severe hypoglycemiain the 12 months prior to study enrollment.<br>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;<br>**OR**<br>Marked glycemic lability characterized by wide swings in blood glucose despite optimal diabetes therapy and defined by a glycemic lability index (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;<br>**OR**<br>A composite of a Clarke score of 4 or more and a HYPO score greater than or equal to the 75th percentile (423) and a LI greater than or equal to the 75th percentile (329) during the screening period and within the last 6 months prior to randomization. |
| **Exclusion Criteria** | Patients who meet any of these criteria are not eligible for participation in the study:<br>1. Body mass index (BMI) >30 $kg/m^2$ or patient weight ≤ 50kg.<br>2. Insulin requirement of >1.0 IU/kg/day or <15 U/day.<br>3. HbA1c >10%.<br>4. Untreated proliferative diabetic retinopathy.<br>5. Blood Pressure: SBP >160 mmHg or DBP >100 mmHg.<br>6. Measured glomerular filtration rate (using iohexol) of <80 $mL/min/1.73m^2$ (or for subjects with an iodine allergy, calculated using the subject's measured serum creatinine and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[8]Strict vegetarians (vegans) with a calculated GFR <70 $mL/min/1.73m^2$ are excluded. The absolute (raw) GFR value will be used for subjects with body surface areas >1.73 $m^2$.<br>7. Presence or history of macroalbuminuria (>300mg/g creatinine).<br>8. Presence or history of panel-reactive anti-HLA antibodies above background by flow cytometry.<br>9. For female subjects: Positive pregnancy test, presently breast-feeding, or unwillingness to use effective contraceptive measures for the duration of the study and 4 months after discontinuation. For male subjects: intent to procreate during the duration of the study or within 4 months after discontinuation or unwillingness to use effective measures of contraception. Oral contraceptives, Norplant®, Depo-Provera®, and barrier devices with spermicide are acceptable contraceptive methods; condoms used alone are not acceptable. |

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| | 10. Presence or history of active infection including hepatitis B, hepatitis C, HIV, or tuberculosis (TB). ). Subjects with laboratory evidence of active infection are excluded even in the absence of clinical evidence of active infection.<br>11. Negative screen for Epstein-Barr Virus (EBV) by IgG determination.<br>12. Invasive aspergillus, histoplasmosis, or coccidioidomycosis infection within one year prior to study enrollment.<br>13. Any history of malignancy except for completely resected squamous or basal cell carcinoma of the skin.<br>14. Known active alcohol or substance abuse.<br>15. Baseline Hb below the lower limits of normal at the local laboratory; lymphopenia (<1,000/μL), neutropenia (<1,500/μL), or thrombocytopenia (platelets <100,000/μL). Participants with lymphopenia are allowed if the investigator determines there is no additional risk and obtains clearance from an independent hematologist.<br>16. A history of Factor V deficiency.<br>17. Any coagulopathy or medical condition requiring long-term anticoagulant therapy (*e.g.*, warfarin) after islet transplantation (low-dose aspirin treatment is allowed) or patients with an international normalized ratio (INR) >1.5. The use of Plavix is allowed only when portal vein access is obtained using a mini-laparotomy procedure at the time of islet transplant.<br>18. Severe co-existing cardiac disease, characterized by any one of these conditions:<br>a) recent myocardial infarction (within past 6 months).<br>b) evidence of ischemia on functional cardiac exam within the last year.<br>c) left ventricular ejection fraction <30%.<br>19. Persistent elevation of liver function tests at the time of study entry. Persistent serum glutamic-oxaloacetic transaminase (SGOT [AST]), serum glutamate pyruvate transaminase (SGPT [ALT]), Alk Phos or total bilirubin, with values >1.5 times normal upper limits will exclude a patient.<br>20. Symptomatic cholecystolithiasis.<br>21. Acute or chronic pancreatitis.<br>22. Symptomatic peptic ulcer disease.<br>23. Severe unremitting diarrhea, vomiting or other gastrointestinal disorders potentially interfering with the ability to absorb oral medications.<br>24. Hyperlipidemia despite medical therapy (fasting low-density lipoprotein [LDL] cholesterol >130 mg/dL, treated or untreated; and/or fasting triglycerides >200 mg/dL).<br>25. Receiving treatment for a medical condition requiring chronic use of systemic steroids, except for the use of ≤ 5 mg prednisone daily, or an equivalent dose of hydrocortisone, for physiological replacement, only. |

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| | 26. Treatment with any anti-diabetic medication other than insulin within 4 weeks of enrollment.<br>27. Use of any investigational agents within 4 weeks of enrollment.<br>28. Administration of live attenuated vaccine(s) within 2 months of enrollment.<br>29. Any medical condition that, in the opinion of the investigator, will interfere with the safe participation in the trial.<br>30. Treatment with any immunosuppressive regimen at the time of enrollment.<br>31. A previous islet transplant.<br>32. A previous pancreas transplant, unless the graft failed within the first week due to thrombosis, followed by pancreatectomy and the transplant occurred more than 6 months prior to enrollment. |

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# Glossary of Abbreviations

| | |
|---|---|
| AE | Adverse Event |
| AIDS | Acquired Immunodeficiency Syndrome |
| $AIR_{glu}$ | Acute Insulin Response to Glucose |
| ALS | Antilymphocyte Serum |
| APC | Antigen Presenting Cell |
| ATG | Anti-thymocyte Globulin |
| BG | Blood glucose |
| BMI | Body Mass Index |
| BW | Body Weight |
| CBC | Complete Blood Count |
| CFR | Code of Federal Regulations |
| cGCP | Current Good Clinical Practice |
| cGMP | Current Good Manufacturing Practice |
| CGMS | Continuous Glucose Monitoring System® |
| CIT | Clinical Islet Transplantation |
| CITR | Collaborative Islet Transplant Registry |
| CMV | Cytomegalovirus |
| CNI | Calcineurin-inhibitor |
| CRO | Clinical Research Organization |
| CsA | Cyclosporine A |
| CT | Computed Tomography |
| CTCAE | Common Terminology Criteria for Adverse Events |
| DAIT | Division of Allergy, Immunology, and Transplantation |
| DCC | Data Coordinating Center |
| DCCT | Diabetes Control and Complications Trial |
| DI | Disposition Index |
| DIC | Disseminated Intravascular Coagulation |
| DSG | deoxyspergualin |
| DSMB | Data Safety Monitoring Board |
| EBV | Epstein Barr Virus |
| EC | Ethics Committee |
| eCRF | Electronic Case Report Form |
| EDTA | Ethylenediaminetetraacetic Acid |

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| ELISA | Enzyme-linked immunosorbent assay |
| FDA | Food and Drug Administration |
| FSIGT | Frequently Sampled Intravenous Glucose Tolerance |
| GCRC | General Clinical Research Center |
| G-CSF | Granulocyte Colony Stimulating Factor |
| GFR | Glomerular Filtration Rate |
| Hb | Hemoglobin |
| HbA1c | Glycosylated hemoglobin |
| HFS | Hypoglycemic Fear Survey |
| HIPAA | Health Insurance Portability and Accountability Act |
| HIV | Human Immunodeficiency Virus |
| HLA | Histocompatability Antigen |
| HSA | Human Serum Albumin |
| HSV | Herpes Simplex Virus |
| ICH | International Conference on Harmonization |
| IEQ | Islet Equivalents |
| IITR | International Islet Transplant Registry |
| IND | Investigational New Drug |
| INR | International Normalized Ratio |
| IRB | Institutional Review Board |
| ITN | Immune Tolerance Network |
| IV | Intravenous |
| LDL | Low-density Lipoprotein |
| LFTs | Liver Function Tests |
| LI | Lability Index |
| MAGE | Mean Amplitude of Glycemic Excursions |
| MMF | Mycophenolate Mofetil |
| MMTT | Mixed-Meal Tolerance Test |
| MRI | Magnetic Resonance Imaging |
| NCI | National Cancer Institute |
| NIAID | National Institute of Allergy and Infectious Disease |
| NIDDK | National Institute of Diabetes & Digestive & Kidney Diseases |
| NIH | National Institutes of Health |
| NOD | Non-obese Diabetic |
| PAID | Problem Areas in Diabetes |

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| PBMC | Peripheral Blood Mononuclear Cell |
| PCR | Polymerase Chain Reactions |
| PI | Principal Investigator |
| pit-hGH | Pituitary Growth Hormone |
| PLT | Platelet Count |
| PML | Progressive Multifocal Leukoencephalopathy |
| PNF | Primary Non-Function |
| PRA | Panel Reactive Antibodies |
| PRES | Posterior Reversible Encephalopathy Syndrome |
| PTLD | Post-transplant Lymphoproliferative Disorder |
| PT | Prothrombin Time |
| PTT | Partial Thromboplastin Time |
| QOL | Quality of Life |
| RNA | Ribonucleic Acid |
| SAE | Serious Adverse Event |
| SAP | Statistical Analysis Plan |
| SC | Subcutaneous |
| SGOT | Serum Glutamic-oxaloacetic Transaminase |
| SGPT | Serum glutamate Pyruvate Transaminase |
| SOP | Standard Operating Procedure |
| sTNFR-Fc | Soluble Receptor for Tumor Necrosis Factor |
| T1D | Type 1 diabetes |
| TAT | Thrombin-antithrombin |
| TB | Tuberculosis |
| TCAE | Terminology Criteria for Adverse Events |
| TNF | Tumor Necrosis Factor |
| TNFR | Tumor Necrosis Factor Receptor |
| ULN | Upper Limit of Normal |
| UNOS | United Network for Organ Sharing Disorders |
| WHO | World Health Organization |

# Study Definitions

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

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

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

- One HbA1c level, one fasting serum glucose level, and a Mixed Meal Tolerance Test are documented within the visit window (e.g. 70-80 days at Day 75) and 7 consecutive days of blood sugar and insulin readings are documented within +/- 7 days of the visit window (e.g. 63-87 days at Day 75);
- HbA1c <7.0% or a ≥2.5% decrease from baseline;
- Fasting capillary glucose level should not exceed 140 mg/dL (7.8 mmol/L) more than three times in the 7 consecutive days (fasting is defined as 1st blood sugar reading of the day not noted as post-prandial or bedtime);
- Post-prandial serum glucose ≤ 180 mg/dl (10.0 mmol/L) at 90 minutes during the MMTT;
- Fasting serum glucose level ≤126 mg/dL (7.0 mmol/L); if the fasting serum glucose level is >126 mg/dL (7.0 mmol/L), it must be confirmed in an additional one out of two measurements;

At least one MMTT fasting or stimulated c-peptide ≥ 0.5 ng/ml.

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

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

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

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

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

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

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

# 1. BACKGROUND AND RATIONALE

## 1.1 Background

Type 1 diabetes (T1D) remains a therapeutic challenge. Failure to prevent hypoglycemia and hyperglycemia results in acute and chronic diabetes complications, leading to poor quality of life (QOL), premature death, and considerable health care costs in 30% to 50% of patients[9]. Therefore, establishing safe and effective methods of achieving and maintaining normoglycemia will have substantial implications for the well-being of individuals with diabetes. Intensive insulin has been shown to reduce the risk of chronic complications in patients who achieve near normalization of glycemia[10]. However, this therapy is labor intensive, difficult to implement for many patients, and limited by the accompanying increased frequency of severe hypoglycemia[11]. Currently, the only way to reliably restore normoglycemia is a pancreas or islet transplant. The success rate of islet transplants recently increased markedly by transplanting a higher islet mass prepared from 2 to 4 donor pancreases and using a glucocorticoid-free immunosuppressive protocol[12]. However, for islet transplants to become a clinical reality, diabetes reversal must be achieved on a consistent basis with a single donor pancreas as with pancreas transplants to reduce the costs and to justify prioritization of donor pancreases to islet recipients. We have presented preliminary evidence of the ability to reverse T1D with islets prepared from 1 donor pancreas[13, 14].

The proposed clinical protocol will build on these preliminary findings and is designed to examine the effect of peri-transplant administration of deoxyspergualin (DSG) on single-donor islet allograft function on day +75 post-transplant in type 1 diabetic recipients. The rationale for DSG administration in the peri-transplant period is detailed in sections 1.2 and 1.4.1.2 below. Improving efficacy and efficiency of islet transplants and achieving insulin independence after single-donor islet transplantation in a substantial proportion of type 1 diabetic islet allograft recipients across centers is likely to contribute to the transition of islet transplants from clinical research to clinical care.

## 1.2 Preclinical and Clinical Experience

### 1.2.1 Preclinical Studies

DSG has been extensively studied in experimental models of islet transplantation by us and others[15-27]. Walter *et al*. showed prolonged rat islet allograft survival from a mean of 5.2±0.6 days in untreated controls to 55.6±18.6 days ($p<0.02$) in recipients treated with DSG (2.5 mg/kg/day for 10 days)[15]. These findings were confirmed in a subsequent study by Kaufman *et al*. in which DSG (0.625 mg/kg/day administered as daily maintenance therapy for 100 days) abolished primary non-function (PNF) and significantly delayed the onset of classic rejection of islet allografts[16]. Recipients of a marginal mass of isogeneic islets receiving DSG 0.625 mg/kg/day exhibited a significantly shorter duration of temporary post-transplant hyperglycemia[17]. Furthermore, islet allograft survival in DSG-treated C57/BL6 strain recipient mice transplanted with a marginal islet mass (150 B10.BR strain islets) was prolonged from a mean of 17.0±1.5 days in controls to >100 days in DSG-treated recipients[17]. Kenmochi *et al*. found that mouse islet isografts were protected from nonspecific inflammatory damage by recipient treatment

with DSG and nicotinamide[18]. Gores *et al*. tested the combined effect of DSG and cyclosporine A (CsA) in a rat islet allograft model and showed synergistic interaction in prolonging functional allograft survival[19]. Low-dose DSG (0.5 mg/kg/d intravenous [IV] for 10 days) was added to a regimen of CsA, azathioprine, and goat antidog ALG to outbred canines undergoing total pancreatectomy and purified islet allotransplantation [20], and a significant improvement in functional graft survival from a mean of 10.8 days to a mean of 32.4 days was observed in recipients receiving DSG. The importance of peri-transplant treatment with DSG for the prolongation of pig islet xenograft survival has also been demonstrated[21-27].

### 1.2.2 Clinical Studies

Based on studies in Japan on the effects of DSG on acute renal allograft rejection in 436 patients[28-30], licensure was granted in 1994 for the treatment of renal allograft rejection. It is marketed by Nippon Kayaku Co, Ltd., under the trade name "Spanidin". DSG doses of 3-5 mg/kg/d were judged to be recommendable and the duration of treatment of 7 days was evaluated as a suitable duration[30]. DSG has also been effective in reversing acute rejection in steroid-resistant kidney transplant rejections[29]. Later reports have confirmed the efficacy of DSG in reversing rejection episodes, with over 2,250 cases treated with 73% efficacy; the major side-effect observed has been transient leukopenia[31, 32]. DSG has also been evaluated for refractory acute renal transplant rejection at the University of Minnesota[33]. The efficacy of prophylactic DSG treatment in improving long-term graft survival has been documented in living-related renal-transplant recipients transfused with donor-specific blood[34].

A University of Minnesota pilot study evaluated the utility of DSG in the setting of single-donor, unpurified islet and simultaneous kidney transplantation[35]. Subjects received 4 mg/kg/day DSG IV for the first 10 days in addition to prednisone, CsA, and either Minnesota ALG or ATGAM. Azathioprine was begun upon completion of the course of DSG. The two subjects who received DSG in combination with Minnesota ALG were insulin independent for 11 and 33 months after single donor islet transplant and also maintained renal transplant function. DSG has been used in 3 subjects receiving intraportal injections of fetal porcine islet-like cell clusters after previously undergoing successful renal transplantation. In this trial, DSG (4 mg/kg/d IV for 5 days) was added to their maintenance immunosuppression. A small amount of the transplanted porcine tissue escaped rejection as evidenced by low, but measurable amounts of porcine C-peptide in the urine several weeks to months post-transplant[36]. Pilot clinical trials evaluating DSG (2 mg/kg/day IV for 14 days) in T1D islet allograft recipients are currently underway at Northwestern University (Principal Ivestigator [PI]: Dixon B. Kaufman, M.D., Ph.D.) and at Carolinas Medical Center (PI: Paul F. Gores, M.D.). We believe that the extensive and compelling experimental data detailing safety and efficacy of DSG warrants extension of pilot clinical trials into a prospective, controlled, randomized clinical trial.

Pilot clinical trials evaluating DSG (2 mg/kg/day IV for 14 days) in type 1 diabetes (T1D) islet allograft recipients are currently underway at Northwestern University (PI: Dixon B. Kaufman, M.D., Ph.D.) and at Carolinas Medical Center (PI: Paul F. Gores, M.D.). As of July 25, 2005, there have been 5 recipients (Northwestern - 4; Carolina- 1) who have been enrolled in the DSG pilot study and completed the islet transplants. Three recipients are insulin independent -- 2 subjects receiving 2 islet transplants and 1 subject receiving 3 transplants. One recipient administers 5U/d of insulin and is 6 weeks post-islet transplant #2, and one recipient lost C-

peptide production over a 6 week time interval and returned to full-dose insulin requirements at a time 10 months following her only islet transplant. Loss of islet function was consistent with an immunologic event. For the 4 recipients with successful transplants, the mean (±S.D.) total #IEq/kg received was 14,220±3573. The mean time post-transplant from the first transplant was 12.5±5.7 months. The most recent mean stimulated C-peptide level was 4.24 ±1.02. The mean pre- and post-transplant HgbA1c levels were 7.08±1.07 and 5.62±0.25, respectively. Two recipients have experienced grade 4 SAEs associated with the study (no grade 5 SAE reported). We believe that the experimental data detailing safety and efficacy of DSG supports the study proposed here.

## 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[37]. Iatrogenic hypoglycemia is a consequence of 3 compromised defense mechanisms, whose pathophysiology was thoroughly reviewed by Cryer[37-40].

First and perhaps most important, glucose-regulated insulin levels are not present in C-peptide-negative type 1 diabetic patients. The second defense mechanism, glucagon secretion in response to developing hypoglycemia, is lost in virtually all patients with T1D within 5 to 10 years after its onset[41]. Third, epinephrine response to falling glucose levels is compromised, in terms of the magnitude of the response and the threshold for the response[42], in a subgroup of patients with T1D. Epinephrine is not normally critical, but becomes so when the insulin and glucagon responses are deficient or absent. Those type 1 diabetic patients with an absent insulin response and combined deficiencies of their glucagon and epinephrine responses to falling plasma glucose levels have the clinical syndrome of defective glucose counterregulation; their risk of severe hypoglycemia is 25-fold or more higher than that of those with absent glucagon but intact epinephrine responses[43, 44]. Type 1 diabetic patients with impaired epinephrine responses also have the clinical syndrome of hypoglycemia unawareness, which refers to the absence of adequate autonomic warning symptoms of developing hypoglycemia.

Hypoglycemia unawareness and the associated inability to respond adequately to falling glucose levels explain the frequent episodes of neuroglycopenia in such patients. Moderate hypoglycemia refers to a hypoglycemic episode complicated by neuroglycopenia in which the patient is still able to overcome the situation without assistance. Severe hypoglycemia refers to a situation in which neurologic impairment is severe enough to prevent self-treatment, placing patients at risk for injury to themselves or others. Accordingly, the Diabetes Control and Complications Trial (DCCT) Research Group defined severe hypoglycemia as an event with symptoms consistent with hypoglycemia in which the patient requires the assistance of another person; it is associated with a blood glucose (BG) level below 50 mg/dL and with prompt recovery after oral carbohydrate, IV glucose, or glucagon administration[11]. The DCCT Research Group definition replaced the more stringent 1980s definition of severe hypoglycemia based on loss of consciousness[45-47].

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[43, 48, 49]. 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[39, 50], 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[51]. 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[43, 44]. The patient characteristic that most strongly predicted severe hypoglycemia in the DCCT was a history of prior severe hypoglycemic events[52].

Hypoglycemia is said to be a major concern of prospective employers[53]. Neuroglycopenia can cause social embarrassment, and even lead to ostracism or be mistaken for disorderly or unlawful behavior[37]. The more distressing the severe hypoglycemic episode, the greater the psychological fear of hypoglycemia[54]. 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[37, 55]. 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[56].

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)[57-61]. 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[62]. Thus, it appears that, while hypoglycemia unawareness is reversible by meticulous prevention of hypoglycemia, defective glucose counterregulation may not be reversible[63].

A successful pancreas transplant restores epinephrine responses and symptom recognition during hypoglycemia in patients with longstanding T1D and autonomic neuropathy[64]. In type 1 diabetic islet transplant recipients with documented pre-transplant hypoglycemia unawareness and defective hormonal counterregulatory responses during hypoglycemia, Meyer *et al*. demonstrated, at 1 month post-transplant, improved glycemic thresholds and/or peak incremental responses of epinephrine, norepinephrine, and cortisol, as well as restoration of autonomic warning symptoms during hypoglycemia[65]. In a more recent study by Paty *et al*., intrahepatic islet transplantation did not restore hypoglycemic hormonal counterregulation or symptom recognition in type 1 diabetic recipients (Paty et al, Diabetes 2002). Ryan *et al*. documented the absence of episodes of severe hypoglycemia in 12 successful islet transplant

recipients (median follow-up, 10.2 months)[66] whose diabetes was complicated by recurrent episodes of severe hypoglycemia pre-transplant. This would suggest that hypoglycemia associated autonomic failure associated with defective counterregulation and impaired sympathodrenal responses is not just due to recurrent hypoglycemia. After a sustained period without any hypoglycemia, most patients post islet transplant still had defective responses to hypoglycemia. The absence of clinically significant hypoglycemia post islet transplant despite the persistent defect in counterregulation in most subjects demonstrates the dominance of the absence of glucose regulated insulin secretion in the pathogenesis of severe hypoglycemia. Correction of this can only currently be attained with transplantation of beta cell tissue.

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

### 1.3.1 Glycemic Lability

Defining labile diabetes is a challenge but a working definition of labile diabetes may be: "Very variable glucose control associated with unpredictable responses to insulin". Labile diabetes is akin to the more extreme brittle diabetes which has been defined as describing the patient "whose life is constantly being disrupted by episodes of hypo- or hyperglycemia whatever their cause"[67, 68]. Brittle diabetes in addition to lability has the added connotation that there may be associated frequent admissions to hospital[69, 70]. 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 subcutaneous (SC) insulin degradation[71], but now the most severe cases are recognized to usually have a factitious origin[72]. 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[69, 70], but these are subject to the vagaries of local practice. More quantitative measures have been the mean amplitude of glycemic excursions (MAGE)[1] and the M value of Schlichtkrull[73]. he 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[73], but this logarithmic expression of the glucose deviation from a standard glucose level has not been validated. More recently[6], the advent of continuous glucose monitoring system® (CGMS) has allowed insight into the patterns of glucose. The CGMS® profiles give exquisite details that have been quantified in terms of mean and standard deviation. Determining lability with this process has been more difficult, and the suggested method has been the determination of the absolute value of measured glucose minus 5.5 mmol/L. This has the drawback that sustained high glucose levels will result in a high value but the profile may not necessarily be labile. In addition, the technique is currently limited to three days of monitoring and may be less accurate at low glucose levels[74].

Any measure needs to be robust enough to handle a variety of glucose monitoring patterns used in day-to-day diabetes practice, intuitive in that it measured glucose swings, mathematically rigorous and finally easy to use. A newer measure of lability based on the change in glucose over time has been the Lability Index (LI)[2]. A typical range for a diabetes population was calculated in 100 subjects with T1D who were not selected because of any particular problems. Most subjects have scores under 300 $mmol/L^2/h \cdot wk^{-1}$ with a median of 223 (25 – 75th percentiles 130 – 329 $mmol/l^2/h \cdot wk^{-1}$). An LI ≥ 433 $mmol/L^2/h \cdot wk^{-1}$ ($90^{th}$ percentile) indicated serious problems with glycemic lability. The LI correlated well with a clinical scoring of lability by diabetologist 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 who experience it, and it 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[37]. However, this degree of control can be impossible to achieve in many patients despite modern insulin analogs and delivery systems[75] and also leads to life threatening episodes of insulin-induced hypoglycemia[76].

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[66]. 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[12]. The immunosuppression regimen consisted of a combination of novel T lymphocyte directed induction therapy with the interleukin-2 receptor monoclonal antibody daclizumab, and maintenance therapy with the potent calcineurin-inhibitor (CNI) tacrolimus and the more recently developed agent rapamycin. The efficacy of the Edmonton approach has now been confirmed by several other centers, including reports where single donor transplant recipients enjoyed a high rate of initial insulin-independence[77]. Unfortunately, loss of graft function occurs over time, and insulin-independence rates at Edmonton have declined from 72% at one year to 28% by three years[78]. Similarly insulin-independence rates at Miami have declined from 79% at one year to 20% by three years[79]. Recent data demonstrate a functionally low engrafted β-cell mass in insulin-independent transplant recipients under Edmonton immunosuppression that likely declines over time[80], suggesting that the eventual recurrence of diabetes and return to insulin therapy may result from both early (engraftment) and late (survival) immunologic graft loss. Novel strategies aimed at promoting the engraftment or survival of transplanted islets may lead to improved long-term graft function and more sustained insulin-independence for T1D patients.

#### 1.4.1.2 DEOXYSPERGUALIN

Isolated islets, hampered by low levels of antioxidants[81, 82], an exquisite susceptibility to the cytotoxic actions of free radicals[83], disrupted cell-matrix interactions[84] after enzymatic pancreas dissociation, and a finite potential to repair injury, face non-specific innate immunity immediately after transplantation[85]. The majority of transplanted tissue is promptly destroyed by this early non-specific cell-mediated injury response, primarily via macrophages and their by-products, before classic T-cell mediated rejection may ensue[85]. We hypothesize that the magnitude of alloantigen-independent inflammatory responses and the associated generation of oxidative radicals and expression of cellular adhesion molecules are greatly augmented in recipients with persistent autoimmunity. Thus, prevention of this early, non-specific inflammatory response coupled with suppression of adaptive immunity may diminish

alloantigen-independent cell loss, lessen the incidence and severity of acute cellular rejection, and foster the environment necessary for the survival of islet allografts with steroid-free and CI-sparing immunosuppression. We believe that among the many candidate strategies for targeting innate immunity peri-transplant, administration of DSG[86, 87] is supported by a wealth of experimental evidence and promising clinical observations.

DSG has potent immunosuppressive properties in experimental models of transplantation and autoimmunity[8, 88-91]. DSG is a potent anti-inflammatory agent and blocks proinflammatory cytokine production by specific inhibition of NF-κB-dependent signaling pathways[92-94]. Besides these inhibitory effects on innate immunity, DSG also interferes with adaptive immunity by inhibiting T cell[95, 96], B cell[97-99], and antigen presenting cell (APC) differentiation, maturation, and effector function[92-94, 100-110]. DSG also mediates decreased generation of reactive oxygen species and hydrolytic enzymes[100], diminishes expression of MHC class II antigens[101], as well as inhibits proliferation of monocytes/macrophages[102] and antigen processing/presentation[103]. At the molecular level[111, 112], DSG binds to hsp 70[113], which has chaperone activity within the MHC class II presentation pathway[106]. DSG also reduces the presence of CD83+ and CD86+ dendritic cells in lymph nodes, suggesting an inhibitory effect on dendritic cell maturation[92, 94].

DSG has been extensively studied in experimental models of islet transplantation by us and others[15-27]. Please see section 1.2 for details.

### 1.4.2 Immunosuppressive Medications for Initial Transplant

#### 1.4.2.1 ANTI-THYMOCYTE GLOBULIN

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

Two polyclonal anti-thymocyte antibody preparations have been marketed in the United States, Thymoglobulin® and ATGAM®. Two randomized double-blind clinical trials indicated that Thymoglobulin® is more efficacious than ATGAM® for induction immunosuppressive therapy and for the treatment of acute graft rejection episodes in adult renal transplant recipients [114, 115] Thymoglobulin® induction therapy achieved rejection-free allograft survival in 96% of the patients. The incidence of CMV disease in the first year was 12.5%, and no patient developed post-transplant lymphoproliferative disease (PTLD). ATG is known to contain a variety of anti-adhesion molecule antibodies[116]. It interferes with leukocyte responses to chemotactic signals and inhibits the expression of integrins required for firm cellular adhesion. Such mechanisms of action may account for the effect of ATG on nonspecific inflammation and reperfusion injury and may explain the 1% incidence of delayed graft function in kidney recipients[114, 116-119]. Recent studies have shown that early administration of a variety of antibodies directed at adhesion molecules reduces graft dysfunction, and acute and chronic rejection associated with ischemia-reperfusion injury and brain death[120].

The resistance of islet-directed autoimmune responses to conventional immunosuppressive drugs[121-124] and the immediate exposure of intraportally transplanted islets to primed

autoreactive, islet beta cell-directed T cells provide a strong rationale for pre-transplant initiation of ATG, which is known to cause selective depletion of activated T cells and dose-dependent depletion of resting T cells[118]. Experimental data suggest that the protection of whole pancreas transplants from recurrent autoimmunity is functionally related to the inclusion of a significant quantity of lymphoid tissue (possibly containing an immunoregulatory T cell subset) as part of the pancreas graft and not to immunosuppression alone[125, 126]. Clinical evidence also indicates that destructive anti-islet autoimmunity persists for decades after manifestation of T1D[122, 127, 128] and that type 1 diabetic individuals with long disease duration do not spontaneously anergize their autoreactive effector Th1 cells and/or restore Th2 or other regulatory T cell function. Accordingly, reprogramming the recipient's immune system seems to be of paramount importance if autoimmune recurrence in transplanted islets is to be prevented.

Maki *et al*. demonstrated that immunotherapy of non-obese diabetic (NOD) mice with antilymphocyte serum (ALS) after development of overt autoimmune diabetes leads to long-lasting abrogation of autoimmunity[129]. ALS given within 14 days of disease onset gradually reversed hyperglycemia with a 76% cumulative incidence of remission. Diabetic NOD mice that failed to respond to ALS treatment accepted subsequent islet isografts for a prolonged period (mostly >100 days), indicating that autoimmunity was abrogated in the latter animals in which extensive irreversible beta cell destruction had already occurred by the time of ALS treatment. These experimental findings are corroborated by clinical observations reported by the Brussels group[130]. Of 7 islet-after-kidney recipients treated at Brussels, only the 3 patients who had received ATG as induction immunosuppressive therapy during the first 10 days following their previous kidney transplant showed long-term islet graft survival. Furthermore, according to an analysis performed by the International Islet Transplant Registry (IITR) on all 50 insulin-independent, type 1 diabetic islet allograft recipients transplanted through 1999, 23 had received single-donor islet transplants, and 19 of those 23 had received anti-thymocyte or anti-lymphocyte globulin for induction immunosuppression and one had received ATG at the time of a previous pancreas transplant[131]. It is conceivable that the need for 2-3 donor pancreata as a source of islets in the Edmonton experience reflects the inability of the induction immunotherapy to completely abrogate the anti-islet autoimmune response. Even a low level of persistent autoimmunity may interfere with the function of transplanted islets via pro-inflammatory cytokine mediated inhibition of insulin secretion. The ATG immunotherapy as proposed in this trial may be advantageous due to the deletion/inhibition of anti-islet directed autoreactive T cells.

There are two published reports of steroid-free transplantation with Thymoglobulin®. Birkeland *et al*. reported on 68 kidney transplant recipients treated with steroid-free immunosuppression using an initial 10-day ATG induction and maintenance therapy with cyclosporine and mycophenolate mofetil. No steroids were given at any time. After an observation for up to 2.5 years (median 488 days, range 127-945 days), 66 patients (one died from sepsis after six months and one died from peritonitis after returning to dialysis) were alive and well, 64 grafts were functioning well, hemolytic-uremic syndrome recurred in one graft, one graft had to be removed for non-compliance, and two patients returned to dialysis after chronic rejection. These investigators observed only 10 acute rejections (15%)[132]. Cantarovich reported on 28 consecutive type 1 diabetic patients who underwent simultaneous kidney-pancreas transplantation. All patients received ATG, cyclosporine, and mycophenolate mofetil. Steroids were not administered at any time. Only two patients required anti-rejection

treatment. Patient, kidney, and pancreas survival has been reported to be 96.4%, 96.4% and 75%, respectively. Cytomegalovirus infection (CMV) was diagnosed in eight patients. All but one patient tolerated the ATG course well[133]. These two studies indicate that ATG can be used safely and effectively without concomitant steroid administration.

The total ATG dose to be administered is 6 mg/kg. This dose is based on studies performed by Daniel Brennan at Washington University in St. Louis[134]. This reduced total dose of ATG has been found to be equally effective for induction immunosuppression in kidney transplantation when compared to historical controls that had received 1.5 mg/kg per day for at least seven days[114]. The proposed ATG dose escalation strategy has been pioneered by James Russell in Calgary, Alberta, in more than 70 bone marrow transplant recipients (presented at the European Bone Marrow Transplant Meeting in Innsbruck, Austria, April, 2000). The University of Minnesota has reported their preliminary experience with this regimen of ATG administration in 8 type 1 diabetic islet transplant recipients[131]. ATG was found to be effective in preventing rejection and autoimmune recurrence. All eight recipients have achieved insulin independence. The medication was well tolerated in all subjects, unexpected acute complications were not encountered. Serious adverse events (SAEs) were not encountered secondary to ATG.

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

Induction immunotherapy with anti-interleukin-2 receptor antibody is a critical component of the steroid-free immunosuppressive protocol recently developed for islet transplantation by the Edmonton group[12]. The safety and efficacy of daclizumab and basiliximab have previously been documented in multi-center trials in renal transplantation. When added to therapy with cyclosporine, azathioprine, and prednisone, daclizumab reduced the frequency of acute rejection and improved short-term graft survival in renal transplant recipients, and basiliximab reduced the frequency of acute rejection and did not affect graft or patient survival. At six months, there were no significant differences between the daclizumab or basiliximab and the placebo group with respect to infectious complications or cancers[135].

#### 1.4.2.2 MAINTENANCE IMMUNOSUPPRESSION WITH SIROLIMUS AND LOW DOSE TACROLIMUS

Diabetogenic side effects of immunosuppressive therapy are particularly deleterious in the situation of a reduced beta cell mass (like in islet transplantation), contributing to the historically poor success rate of human islet allografts. The combination of CNIs and prednisone is associated with the development of an insulin-dependent diabetic state in up to 25% of non-diabetic kidney transplant recipients[136]. To maintain normoglycemia, immunosuppressed non-diabetic kidney transplant recipients must increase insulin secretion 2.5 times[137]. Even when systemic drug levels are carefully controlled, intraportally transplanted islets bathed in portal blood are exposed to higher and probably toxic local concentrations of orally administered immunosuppressive drugs[138]. This may not matter when there is a normal beta cell mass, as with a whole pancreas transplant. The limited mass of engrafted islet beta cells however, is inadequate to restore insulin independence in the presence of impaired insulin secretion and action mediated by CNIs in combination with steroids[12, 137, 139].

The Edmonton group therefore developed a steroid-free maintenance immunosuppressive protocol based on the combination of sirolimus and low-dose tacrolimus[12]. This strategy was designed to provide potent synergistic immunosuppression, thus avoiding diabetogenic impact on a limited beta cell transplant reserve[138, 139].

Sirolimus is a promising agent for maintenance immunosuppression of islet allograft recipients, mainly because of its efficacy in the absence of diabetogenic side effects[140]. Sirolimus is as effective as CsA in preventing renal graft loss due to rejection while maintaining superior graft function[141]. Sirolimus combined with the concentration-controlled regimen of cyclosporine presents a promising synergistic regimen which reduces the incidence of acute rejection episodes among recipients of kidney grafts markedly, permits profound cyclosporine dose reduction, and facilitates corticosteroid avoidance or withdrawal[142]. A recent pilot study in 32 organ transplant recipients (liver, kidney, and pancreas) demonstrated the safety and efficacy of a regimen combining sirolimus with a low dose (33% of the recommenation), of tacrolimus and steroids[143]. The almost complete absence of renal dysfunction, hypertension, and diabetes in these patients is explained by the low blood levels of tacrolimus (5.7 ± 3.2 ng/mL). Extremely low rejection rates are an essential prerequisite for islet transplantation, since without access to reliable diagnostic markers or early rejection, irreversible islet destruction may occur before the onset of hyperglycemia. The low rate of opportunistic infections suggests that the patients were not excessively immunosuppressed. The data from the Edmonton group suggest that sirolimus combined with low-dose tacrolimus without the addition of steroids may represent a safe and very effective maintenance immunosuppressive regimen[12].

### 1.4.3 Induction Immunosuppression for Subsequent Islet Transplants

The immunosuppressive regimen for subsequent islet transplants will be identical to the regimen for the initial islet transplant with the exception of Thymoglobulin®. Basiliximab will be used instead of Thymoglobulin® for all subsequent islet transplants.

### 1.4.4 Immunosuppressive / Anti-inflammatory Therapy: Etanercept

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

The basic premise behind the proposed amendment is that peri-transplant administration of etanercept will interfere with the biological activity of TNF-α released early post-transplant as part of the activation of the innate immune response. Blockade of TNF-α in the early post-transplant period is expected to lessen early islet loss and promote a milieu favoring the induction of immunologic tolerance. It is well recognized that TNF-α and TNF-β play multiple roles in the development and function of the immune system and have pleomorphic regulatory effects on the development and expression of autoimmunity[144]. Blockade of TNF in the neonatal period results in a dramatic increase in the levels of $CD4^+CD25^+$ regulatory T cells in NOD mice[144, 145]. Such an effect of etanercept on $CD4^+CD25^+$ regulatory T cells in islet transplant recipients could prove critical for protection of transplanted islets from alloimmunity and recurrent autoimmunity.

In a clinical trial at the University of Minnesota, insulin independence and normoglycemia were restored in 8 of 8 recipients of 7,271±1,035 islet equivalents (IEQ)/kg from a single-donor pancreas[14]. These subjects received antithymocyte globulin and etanercept for induction immunotherapy. The available information suggests that restoration of insulin independence with a lower islet mass prepared from a single-donor pancreas can in part be ascribed to the administration of etanercept in the peri-transplant period. The following sections therefore describe in more detail the experimental findings and clinical observations that form the basis for administering etanercept in the peri-transplant period.

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

TNF-α is known to be cytotoxic to human islet beta cells[146]. In murine models, selective inhibition of TNF-α in the peri-transplant period has promoted reversal of diabetes after marginal-mass islet isografts[8] . Peri-transplant administration of etanercept has subsequently been studied in a mouse islet allograft model by Farney *et al*. (unpublished). Streptozotocin-diabetic C57BL/6 mice received 150 allogeneic B10.BR islets and either etanercept (100 μg at –24hrs, 50 μg at +24, +72, +120, and +168 hrs post-transplant) or saline. The proportion of euglycemic recipient animals was significantly higher in the etanercept group (4/7 versus 0/11). These findings demonstrate that specific TNF-α inhibition improves the functional outcome of a marginal mass islet allograft, again confirming that islets are sensitive to nonspecific inflammation in the peri-transplant period.

*Clinical observations.* Temporary etanercept administration has previously been studied in globally immunosuppressed kidney[147-150] and bone marrow transplant recipients[151, 152]. In renal transplant recipients, etanercept was combined with depleting T cell antibodies (OKT3 or ATG). These studies demonstrated that etanercept is well tolerated and may limit the severity of the acute cytokine release syndrome associated with OKT3 and ATG administration. The most significant observation of one study[147] was a more rapid improvement in renal function in the etanercept-treated patients. Another study in renal transplant recipients found a higher incidence of infection in treated patients compared to controls in the 3 months after transplant. The etiology of this difference was unclear and the overall conclusion of this study was that etanercept is well tolerated by renal transplant patients receiving OKT3 induction therapy. Recent studies in bone marrow transplant recipients[151, 152] provide preliminary evidence of the safety and efficacy of etanercept administration for the treatment of chronic graft-versus-host disease. In summary, in renal and bone marrow transplant recipients, SAEs related to the administration of etanercept were not communicated, suggesting that transient etanercept administration does not pose significant risks to globally immunosuppressed patients.

Nineteen islet transplant recipients have received etanercept in the peri-transplant period for the purpose of enhancing engraftment and functional survival of transplanted human islets at the University of Minnesota, University of California San Francisco, and the University of Miami. Etanercept was administered as follows: 50 mg IV at 1 hr prior to transplant, 25 mg SC on days +3, +7, and +10 post-transplant. The treatment schedule (an intravenous loading dose of 50 mg followed by three subcutaneous injections of 25 mg) is based on the results of a safety

trial in healthy volunteers[153], a bioavailability study in healthy volunteers[154] and a toxicity and dose finding trial in refractory rheumatoid arthritis[155]. The time to maximum concentration after subcutaneous and intravenous etanercept administration were found to be 66 and 0.8 hrs, respectively[154]. An IV loading dose administered 1 hr prior to transplant is given to ensure therapeutic etanercept levels at the time of islet infusion. In 10 patients transplanted at the University of Minnesota, etanercept was combined with ATG, and in 2 patients at the University of California San Francisco, etanercept was combined with hOKT3γ1 (Ala-Ala) for induction immunotherapy. At the University of Miami, etanercept was combined with daclizumab (n=4) or Campath® (n=3) induction immunotherapy. No adverse events (AEs) related to etanercept were encountered in these 12 patients.

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

Compared with the hOKT3γ1 (Ala-Ala) trial[140] at the University of Minnesota, in which 4 of 6 single-donor islet recipients achieved and maintained insulin independence, the 8 single-donor islet allograft recipients given peri-transplant ATG plus etanercept trial had a significantly higher acute C-peptide response to arginine (ACRArg) on days ≥180 post-transplant: 1.07±0.15 ng/mL (vs. 0.74±0.21 ng/mL in hOKT3γ1 (Ala-Ala) trial[140]; p=0.028). This improvement occurred despite transplantation of fewer islets: 7,271±1,035 (vs. 10,302±2,594 IE/kg in our previous trial[140]; p=0.01). To facilitate comparison of the proportion of engrafted islets between studies, the ACRArg was corrected for implanted IE/kg, and expressed as the engraftment index. The engraftment index in the ATG plus etanercept islet transplant trial was 150±29 $x10^{-6}$ ng•kg/mL, as compared with 73±23 $x10^{-6}$ ng•kg/mL in the hOKT3γ1 (Ala-Ala) trial[140]. Since pancreas procurement, preservation, islet processing, and culture protocols in the 2 studies were all identical, it is assumed that the islet potency was the same and therefore interpret the high efficacy of single-donor, marginal-dose islet transplants in the ATG plus etanercept trial as preliminary evidence of improved engraftment. Many of the effects of ATG are shared with the anti-CD3 monoclonal antibody, hOKT3γ1 (Ala-Ala). Thus, they may not sufficiently explain the ability of the ATG plus etanercept protocol to facilitate diabetes reversal after single-donor, marginal-dose islet transplants. Therefore, the results are most likely related to the peri-transplant administration of etanercept.

# 1.5 Known and Potential Risks and Benefits to Human Subjects

## 1.5.1 Risks of Use of Investigational Agents

### 1.5.1.1 TRANSPLANT OF ALLOGENEIC ISLETS

Transplantation of islets is associated with the several potential risks. These risks may be categorized in terms of: a) transmission of disease from donor to recipient, b) risk of microbial contamination of islet preparations, c) sensitization of the recipient to donor antigens, d) acceleration of retinopathy with acute correction in glycemic control, and e) psychological

impact of successful or failed islet transplantation. Other risks including portal thrombosis, portal hypertension, bleeding or hepatic steatosis are discussed separately in Section 1.5.4.

**Transmission of disease from donor to recipient**

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

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

Donors are excluded if: a) there is known pre-existing metabolic disease including Type 1 or Type 2 diabetes, or if the HbA1c is elevated above 6.1% in the absence of transfusions in the week prior to death, b) if there is malignancy other than 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 (TB), 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 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 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 that became CMV positive and one did develop CMV disease occurring late, after discontinuation of anti-viral prophylactic therapy.

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

With respect to EBV transmission, only recipients who are EBV positive are acceptable for the current trial. EBV polymerase chain reaction (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%.

**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 diminish further the subjects' risk of infection.

In 152 islet preparations transplanted consecutively at the University of Alberta since 1999, there have been no cases of transmission of bacterial or fungal disease through islet transplantation, when islets are prepared under cGMP conditions. One recipient of an islet autograft received an infected islet preparation as the autograft pancreas contained a chronic embedded pancreatic stent that likely led to bacterial colonization and contamination. This recipient developed transient complete thrombosis of the portal vein with subsequent recanalization.

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

There have been previous reports of two cases of islet transplantation-related septicemia (Enterobacter cloacae) due to transplantation of contaminated cryopreserved pancreatic islets[156]. Additionally, the University of Minnesota investigators have previously reported on the incidence and significance of contaminated islet preparations in clinical islet auto- and allotransplantation[157]. Positive cultures from islet tissue preparations were identified in 11 of 29 patients (38%) receiving autologous islets. The occurrence of serious infection morbidity (as defined as positive blood cultures, abscesses, or intra-abdominal infections) did not differ significantly between the positive and negative culture groups (p=0.99). In the allogeneic islet transplant group, 7 of 33 patients (21%) received tissue that retrospectively were determined to be contaminated. None of these patients developed serious infectious complications (despite broad-spectrum immunosuppression). Despite the occurrence of contaminated grafts, there was no serious increase in infectious morbidity. Presumably the inocula were kept low by the multiple washing steps allowing the recipients to clear the organisms without serious sequelae.

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

Additional steps have been taken to decrease the incidence of contamination. First, since 2000, pancreatectomy specimens for clinical islet allotransplantation have exclusively been processed under current Good Manufacturing Practices (cGMP) regulations. Overall, the risk of islet

transplantation-related septicemia is considered very low in view of the precautions detailed in the islet manufacturing protocol.

**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[158-160]. In the ITN-sponsored trial of islet transplantation, 5 of 36 subjects had evidence of elevated panel reactive antibody (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[161-163]. 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[164].

**Acceleration of retinopathy with acute correction in glycemic control**

In the DCCT study[11], about 10% of patients with pre-existing retinopathy receiving intensive treatment experienced a transient worsening of their retinopathy during the first year, but nonetheless had a lower cumulative incidence of sustained progression when compared to the conventional group after the third year. A transient worsening of retinopathy has not been formally documented in islet transplantation trials, but it is assumed that a similar process might occur. Exclusion of patients with unstable retinopathy and careful post-transplant follow-up will help to minimize the incidence of such occurrences and their morbidity should they occur.

When type 1 diabetic recipients of successful and unsuccessful pancreas transplants were compared for the end point of an increase of two or more grades in the retinopathy score, they did not differ significantly in the rate of progression whether retinopathy was mild (Grade P0 to P5) or advanced (Grade P6 to P14) at baseline[38]. 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.

**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.

#### 1.5.1.2 DEOXYSPERGUALIN

Clinically, DSG has been used in several studies. DSG was initially used for patients with advanced malignancies as monotherapy[165]. Although no efficacy was observed, valuable information regarding toxicity was obtained. Doses ranged from 2 to 75 mg/kg/d and were delivered by 3 or 24 hours of transplant for 5 days. The toxicities encountered included transient mild hypotension and perioral numbness at the higher dose levels; gastrointestinal toxicity and mild, reversible myelosuppression were also observed. The drug insert label (December 1999 version) describes the pertinent adverse reactions in 910 patients that received the drug either during investigations or after approval. The frequent observed adverse reactions were: thrombocytopenia (38.9%), leukopenia (37.9%), decreased hemoglobin (Hb; 16%), fascial hot flashes (5.2%), and numbness in the face (4.2%). It is to be used with caution in patients with depressed bone marrow function, with hemorrhagic diathesis or in patients with marked hepatic or renal hypofunctions. The important precautions include blood dyscrasia. It has been demonstrated that the blood count nadir is reached approximately 2 weeks after the beginning of the administration of the drug. Notably, DSG was not diabetogenic, and there was no evidence of nephrotoxicity. Because of its favorable risk-benefit profile, DSG has also been evaluated in patients with autoimmune disorders[166, 167].

The most recent series of clinical events has been at Northwestern University where four islet transplant recipients received DSG infusions. The DSG was administered at a dose of 2 mg/kg for 14 daily doses beginning at the time of transplant. A review of the tolerability of the DSG in conjunction with Daclizumab induction and tacrolimus/sirolimus maintenance immunotherapy was retrospectively assessed. The 4 recipients received a combined total of 9 courses of DSG.

In this series, neutropenia was the cause for stopping the drug in 4 out of 9 courses of DSG. However, if only 7 doses per course had been required, then 3 out of 4 courses would have been considered a full course of DSG. Three out of 9 courses were held for the following reasons: one patient who received 2 courses of DSG experienced elevated LFTs, facial numbness/tingling, and epigastric discomfort. However, DSG was resumed when LFTs declined. If 7 doses had been required per course, one of these courses would have been completed. The other course of DSG not completed was not because of safety reasons.

The number of full courses of DSG would have increased from 2 out of 9 courses to 6 out of 9 courses, if only 7 doses of DSG had been required per course. One out of the nine courses would have been discontinued for neutropenia. It is on the basis of the experience from this series of patients receiving islet transplant that the proposed DSG administration is at a dose of 2 mg/kg IV daily starting on day 0, the day of transplant, and continuing through day +6.

### 1.5.2 Risk of Induction and Maintenance Immunosuppressive Therapies

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

#### 1.5.2.1 BASILIXIMAB (SIMULECT®)

Basiliximab is a chimeric (murine/human) monoclonal antibody (IgG1k) approved by the Food and Drug Administration (FDA) for prophylaxis against acute organ rejection in adult recipients of renal allografts. It is usually given at a dose of 20 mg IV on Days 0 and 4. Basiliximab is associated with constipation, nausea, abdominal pain, vomiting, diarrhea, dyspepsia, peripheral edema, fever, viral infections, hyperkalemia, hypokalemia, hyperglycemia, hypercholesterolemia, hypophosphatemia, hyperuricemia, urinary tract infections, upper respiratory infections, surgical wound complications, acne, hypertension, headache, tremor, insomnia, and anemia. In the four placebo-controlled studies, the pattern of adverse events in 590 patients treated with the recommended dose of basiliximab was similar to that in 594 patients treated with placebo (see product monograph for details). Basiliximab did not increase the incidence of serious adverse events observed compared with placebo. As with any protein product, anaphylaxis can occur, particularly with repeated administration, but this has been reported only rarely.

#### 1.5.2.2 RABBIT ANTI-THYMOCYTE GLOBULIN (THYMOGLOBULIN®)

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

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

Published results of the use of Thymoglobulin® in clinical and experimental islet transplantation are limited to relative small cohorts. Hirshberg *et al* described the successful role of rabbit ATG and sirolimus in reducing rejection of islet allografts in primates, with no evidence of direct islet toxicity from Thymoglobulin®[168]. Hering *et al.* described a beneficial role of Thymoglobulin® induction (6mg/kg) in 8 patients with T1D receiving single donor islet grafts, all of whom achieved insulin independence and were protected against recurrence of hypoglycemia[14]. Acute islet rejection was described in patients receiving calcineurin-free immunosuppression when sirolimus levels fell below 9ng/mL. The use of higher doses of sirolimus exacerbated the neutropenic side effects of Thymoglobulin®, but these could be managed safely without risk of opportunistic infections when appropriate dose reduction and/or administration of Granulocyte Colony Stimulating Factor (G-CSF; Neupogen®) if required[14].

#### 1.5.2.3 SIROLIMUS (RAPAMUNE®)

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

Of infections, only mucosal herpes simplex virus (HSV) occurred at a greater rate with sirolimus. There was no increase in rate of malignancy (3.4 vs. 3.1%). While sirolimus was originally proposed as a non-nephrotoxic agent, it is becoming apparent that sirolimus-associated nephrotoxicity does occur in clinical practice. Crew *et al.* described two patients with thrombotic microangiopathy secondary to sirolimus exposure[169]. Sirolimus alters the pharmacokinetic profiles of other CNIs (*e.g.*, tacrolimus) and may thereby potentiate nephrotoxicity[170]. Fervenza *et al.* described nephrotoxicity from sirolimus in patients with chronic glomerulopathies that was non-reversible on cessation of therapy[171]. 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[172, 173].

The majority of islet transplant recipients receiving sirolimus in conjunction with tacrolimus have experienced transient mouth ulceration, lower extremity edema[12, 173]; perinephric edema and a high incidence of benign ovarian cysts have also been described in islet recipients in association with sirolimus[174]. Pneumonitis and colitis have also occurred[175]. The most common (>30%) adverse reactions are: peripheral edema, hypertriglyceridemia, hypertension, hypercholesterolemia, creatinine increased, abdominal pain, diarrhea, headache, fever, urinary tract infection, anemia, nausea, arthralgia, pain, and thrombocytopenia.

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

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

#### 1.5.2.4 TACROLIMUS (PROGRAF®)

Tacrolimus (Prograf®, FK506) has been in wide clinical use for the prevention of allograft rejection since 1994 when the FDA approved it after several years of testing. Tacrolimus is a macrolide antibiotic which inhibits calcineurin after binding intracellularly to FKBP12 within T cells, inhibiting IL-2 transcription. Tacrolimus is usually 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 (in subjects who receive a renal transplant) , nausea, vomiting and diarrhea (see product monograph for details). In 205 kidney transplant recipients receiving tacrolimus, the principal AEs were neurologic (tremor [54%], headache [44%], insomnia [32%], paresthesia [23%]) and gastrointestinal (diarrhea [44%], nausea [38%], constipation [35%]) complaints, hypertension (50%), and kidney dysfunction (52%); hyperkalemia (31%) and hyperglycemia (22% in previous non-diabetics) also occurred. The severity of these events appears to be dose dependent, with very high plasma levels also producing delirium, seizures, and coma. Complications can be minimized with the relatively low dose long-term therapy typically used in islet transplant trials.

#### 1.5.2.5 CYCLOSPORINE (NEORAL®)

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

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

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

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

Etanercept is a dimeric soluble form of the p75 TNFR that blocks TNF binding and reduces inflammation[147-151]. It is FDA-approved for use in severe rheumatoid arthritis, juvenile arthritis, ankylosing spondylitis, and psoriatic arthritis. In controlled trials, approximately 37% of patients treated with Enbrel® developed injection site reactions (see package insert). All injection site reactions were described as mild to moderate (erythema and or itching, pain or swelling) and generally did not necessitate drug discontinuation. In placebo controlled trials, there was no increase in the incidence of serious infections. The observed rates and incidence of malignancies were similar to those expected for the population studied. However, the incidence of TB has been shown to be statistically higher in anti-TNF-alpha-treated patients[176-178], and based on post-marketing studies warnings have been issued about the following conditions, which have been reported with the use of Enbrel®:serious infections and sepsis, including fatalities, an increased risk of lymphoma and other malignancies in children and adolescents; and leukemia. Many of the serious infections occurred in patients on concomitant immunosuppressive therapy.

Experience with anti-TNF alpha therapies in clinical and experimental islet transplantation has been limited. Farney *et al.* described a beneficial role of etanercept in promoting engraftment of marginal mass islet grafts in mice[8]. Hering *et al.* used etanercept in a recent trial of 8 type 1 diabetic patients receiving single donor islet transplant, and all 8 achieved insulin independence suggesting a beneficial role for anti-TNF therapy in clinical islet transplantation[14].

### 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 canulation 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 Hb (n=1), right hemothorax (n=1), and subcapsular hematoma (n=1) of the liver[179]. Subcapsular hematoma of the liver following percutaneous transhepatic injection of islets into the portal vein in two cases has also been reported to the IITR. No

surgical intervention was necessary[180]. One instance of injury to hepatic artery leading to death during percutaneous transhepatic catheterization of the portal vein has been reported previously to CITR[180]. Reports on intra-abdominal (n=1) [175] and intrathoracic bleeding (n=1)[181] have been published. The risk of significant hemorrhage after percutaneous islet transplantation defined as a drop in Hb of more than 25 g/L or the need for transfusion or surgery was 9% in the Edmonton series[182]. 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[183]. The risk has since been ameliorated by avoidance of pre-transplant aspirin and more effective measures to seal the catheter tract in the liver[183]. 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[184]. 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[14].

**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[179]. 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 third infusion, and 318 days post 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[185].

**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[186-188]. 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[189, 190]. 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[179]. 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 (LFTs) usually resolved within 4 weeks[189]. No correlation between the increase in LFTs and graft characteristics or graft function was found. Periportal hepatic steatosis has been described following intraportal islet allotransplantation in 20% of the studied subjects[77, 191] 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[191], 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[180]. 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[192]. 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)[193]. The baseline pressures were normal in all cases, suggesting absence of chronic portal hypertension[193].

**Portal Vein Thrombosis**

Transplanted islets release tissue factor and exhibit prothrombotic properties when infused to an intravascular site such as the portal vein[194]. A partial portal vein thrombosis has been reported in one of six patients transplanted at the intramural National Institutes of Health (NIH) program[175]. In the Edmonton single-center experience, the risk of partial vein thrombosis was 3% in more than 100 intraportal islet transplants[172]. The management of partial vein thrombosis includes anticoagulation therapy which may lead to intra-abdominal hemorrhage requiring transfusion and surgical intervention [195]. 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[196]. 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 infusions 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[180]. 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[179]. Gall bladder hematoma (n=1) and gall bladder opacification (n=2) have been observed as well (Dr James Shapiro, personal communication).

#### 1.5.4.4 FOLLOW-UP PROCEDURES: GLOMBERULAR 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[197].

### 1.5.5 Benefits

#### 1.5.5.1 BENEFITS OF ALLOGENEIC ISLET TRANSPLANTATION

Successful islet transplantation alleviates T1D patients from life-threatening hypoglycemia and psychosocially debilitating glycemic lability[2]. While the long-term durability of these responses is at present uncertain, they persist for as long as some graft function is maintained, despite the eventual return to insulin therapy in the majority of recipients. This partial function, as indicated by continued C-peptide production, may be present in as many as 80% of recipients after 5 years[78]. Furthermore, as long as graft function is maintained, fear of hypoglycemia and anxiety are significantly lower after islet transplantation[198]. Indeed, T1D subjects in the DCCT who had persistent C-peptide production had a significantly reduced risk of severe hypoglycemia despite intensive insulin therapy[199]. 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

This clinical pilot trial will extend our previous experience in 8 T1D islet transplant recipients treated with the same induction immunosuppressive protocol except for the use of daclizumab

instead of DSG[14]. All 8 recipients achieved insulin independence after single-donor transplant, 3 recipients lost graft function after CNI withdrawal. DSG is a specific inhibitor of NF-κB-dependent signaling pathways in pro-inflammatory cytokine production and dendritic cell maturation[92-94]. The inhibition of an early innate immune response by DSG has been shown to promote a tolerogenic environment by downregulating NF-κB -mediated "danger/alarm" signals[92-94, 200]. The inhibitory effect of DSG on NFκB activation and consequently pro-inflammatory cytokine secretion is expected to translate into lower post-transplant islet death, improved engraftment and insulin secretory capacity of transplanted islets. We further hypothesize that inhibition of early innate immune responses by peri-transplant DSG will promote rejection-free, 1-yr survival of islet transplants in T1D. To further reduce the risk of immune-mediated islet allograft loss we will not replace CNIs with mycophenolate mofetil at 1 month post-transplant as in our previous trial. Thus, in summary, the proposed study regimen is expected to improve islet engraftment and immunoprotection, leading to stable insulin independence in a large proportion of T1D islet allograft recipients.

# 2. OBJECTIVES

## 2.1 Primary Objective

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 post-transplant islet function in subjects with T1D.

## 2.2 Secondary Objective

The secondary objective of the proposed protocol is to improve our mechanistic understanding of determinants of success and failure of islet transplants in T1D.

# 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 glycemic lability index (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 a LI greater than or equal to the 75th percentile (329) during the screening period and within the last 6 months prior to randomization.

## 3.2 Exclusion Criteria

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

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

2. Insulin requirement of >1.0 IU/kg/day or <15 U/day.
3. HbA1c >10%.
4. Untreated proliferative diabetic retinopathy.
5. Blood Pressure: SBP >160 mmHg or DBP >100 mmHg.
6. Measured glomerular filtration rate (using iohexol) of <80 mL/min/1.73m$^2$ (or for subjects with an iodine allergy, calculated using the subject's measured serum creatinine and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI)[8] equation. Strict vegetarians (vegans) with a calculated GFR <70 mL/min/1.73m$^2$ are excluded. The absolute (raw) GFR value will be used for subjects with body surface areas >1.73 m$^2$.
7. Presence or history of macroalbuminuria (>300mg/g creatinine).
8. Presence or history of panel-reactive anti-HLA antibodies above background by flow cytometry.
9. For female subjects: Positive pregnancy test, presently breast-feeding, or unwillingness to use effective contraceptive measures for the duration of the study and 4 months after discontinuation. For male subjects: intent to procreate during the duration of the study or within 4 months after discontinuation or unwillingness to use effective measures of contraception. Oral contraceptives, Norplant®, Depo-Provera®, and barrier devices with spermicide are acceptable contraceptive methods; condoms used alone are not acceptable.
10. Presence or history of active infection including hepatitis B, hepatitis C, HIV, or tuberculosis (TB). Subjects with laboratory evidence of active infection are excluded even in the absence of clinical evidence of active infection.
11. Negative screen for Epstein-Barr Virus (EBV) by IgG determination.
12. Invasive aspergillus, histoplasmosis, or coccidioidomycosis infection within one year prior to study enrollment.
13. Any history of malignancy except for completely resected squamous or basal cell carcinoma of the skin.
14. Known active alcohol or substance abuse.
15. Baseline Hb below the lower limits of normal at the local laboratory; lymphopenia (<1,000/μL), neutropenia (<1,500/μL), or thrombocytopenia (platelets <100,000/μL). Participants with lymphopenia are allowed if the investigator determines there is no additional risk and obtains clearance from an independent hematologist.
16. A history of Factor V deficiency.
17. Any coagulopathy or medical condition requiring long-term anticoagulant therapy (*e.g.*, warfarin) after islet transplantation (low-dose aspirin treatment is allowed) or patients with an international normalized ratio (INR) >1.5. The use of Plavix is allowed only when portal vein access is obtained using a mini-laparotomy procedure at the time of islet transplant.
18. Severe co-existing cardiac disease, characterized by any one of these conditions:

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

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

c) left ventricular ejection fraction <30%.

19. Persistent elevation of liver function tests at the time of study entry. Persistent serum glutamic-oxaloacetic transaminase (SGOT [AST]), serum glutamate pyruvate transaminase (SGPT [ALT]), Alk Phos or total bilirubin, with values >1.5 times normal upper limits will exclude a patient.
20. Symptomatic cholecystolithiasis.
21. Acute or chronic pancreatitis.
22. Symptomatic peptic ulcer disease.
23. Severe unremitting diarrhea, vomiting or other gastrointestinal disorders potentially interfering with the ability to absorb oral medications.
24. Hyperlipidemia despite medical therapy (fasting low-density lipoprotein [LDL] cholesterol >130 mg/dL, treated or untreated; and/or fasting triglycerides >200 mg/dL).
25. Receiving treatment for a medical condition requiring chronic use of systemic steroids, except for the use of ≤ 5 mg prednisone daily, or an equivalent dose of hydrocortisone, for physiological replacement, only.
26. Treatment with any anti-diabetic medication other than insulin within 4 weeks of enrollment.
27. Use of any investigational agents within 4 weeks of enrollment.
28. Administration of live attenuated vaccine(s) within 2 months of enrollment.
29. Any medical condition that, in the opinion of the investigator, will interfere with the safe participation in the trial.
30. Treatment with any immunosuppressive regimen at the time of enrollment.
31. A previous islet transplant.
32. A previous pancreas transplant, unless the graft failed within the first week due to thrombosis, followed by pancreatectomy and the transplant occurred more than 6 months prior to enrollment.

# 4. STUDY DESIGN

This is a prospective, multi-center, single-arm, open-label trial assessing the safety and efficacy of DSG on post-transplant islet function in subjects with long-standing T1D that is refractory to intensive insulin therapy. The centers participating in this phase 2 study will also undertake a separate, phase 3 study in islet transplantation, using a consensus manufacturing and immunosuppressive regimen. The phase 3 trial 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 at sites participating in both studies, 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. The participating centers will accrue subjects over a 24 month period and will treat a total of 14 study subjects.

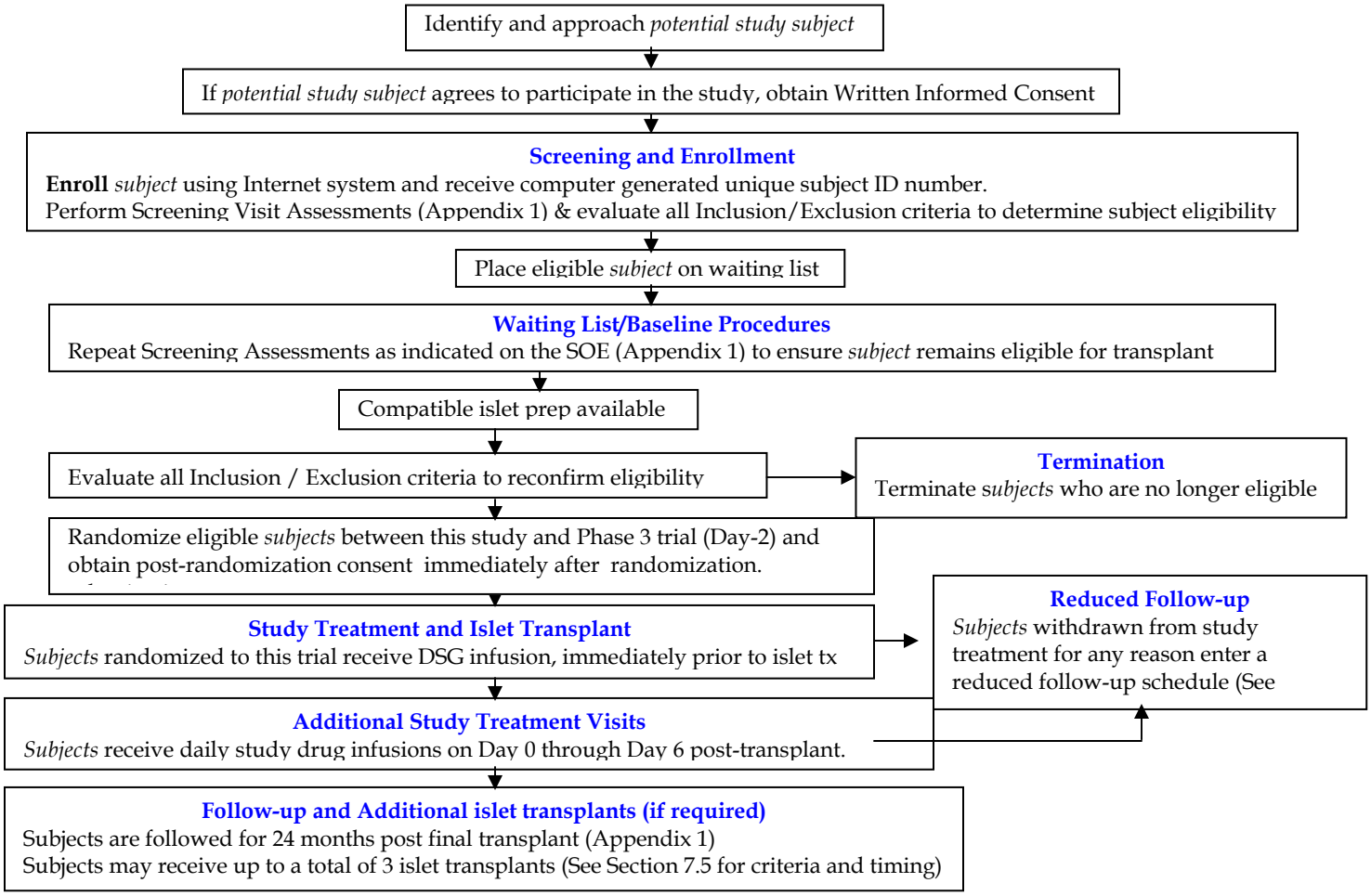


*Figure 1: Study design schema*

## 4.1 Study Endpoints

### 4.1.1 Primary Endpoint

The primary endpoint for the 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(s):

- The percent reduction in insulin requirements
- HbA1c
- MAGE[1]
- LI[2]
- Ryan hypoglycemia severity (HYPO) score[2]
- Basal (fasting) and 90-min glucose and C-peptide derived from the mixed-meal tolerance test (MMTT)
- β-score[3]
- C-peptide: (glucose · creatinine) ratio
- Acute insulin response to glucose ($AIR_{glu}$), insulin sensitivity, and disposition index (DI) derived from the insulin-modified frequently-sampled IV glucose tolerance (FSIGT)[5]
- Glucose variability[6] and hypoglycemia duration[7] derived from the CGMS®
- 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(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 · creatinine) ratio
- Glucose variability[6] and hypoglycemia duration[7] derived from the CGMS®
- AIRglu, insulin sensitivity, and DI derived from the insulin-modified FSIGT[4, 5]
- 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 Hb1Ac <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 Hb concentration); segmental portal vein thrombosis; biliary puncture; wound complication (infection or subsequent hernia); and increased transaminase levels (> 5 times upper limit of normal [ULN])
- The incidence and severity of AEs related to the immunosuppression including: allergy; reduction in GFR; increase in urinary albumin excretion; addition or intensification of anti-hypertensive therapy; addition or intensification of anti-hyperlipidemic therapy; oral ulcers;

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

At 365 ± 14 days following the first islet transplant:
- The incidence of worsening retinopathy as assessed by change in retinal photography. If pupil dilation is not possible, then a manual ophthalmologic exam can be substituted.

# 5. STUDY TREATMENT REGIMEN

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

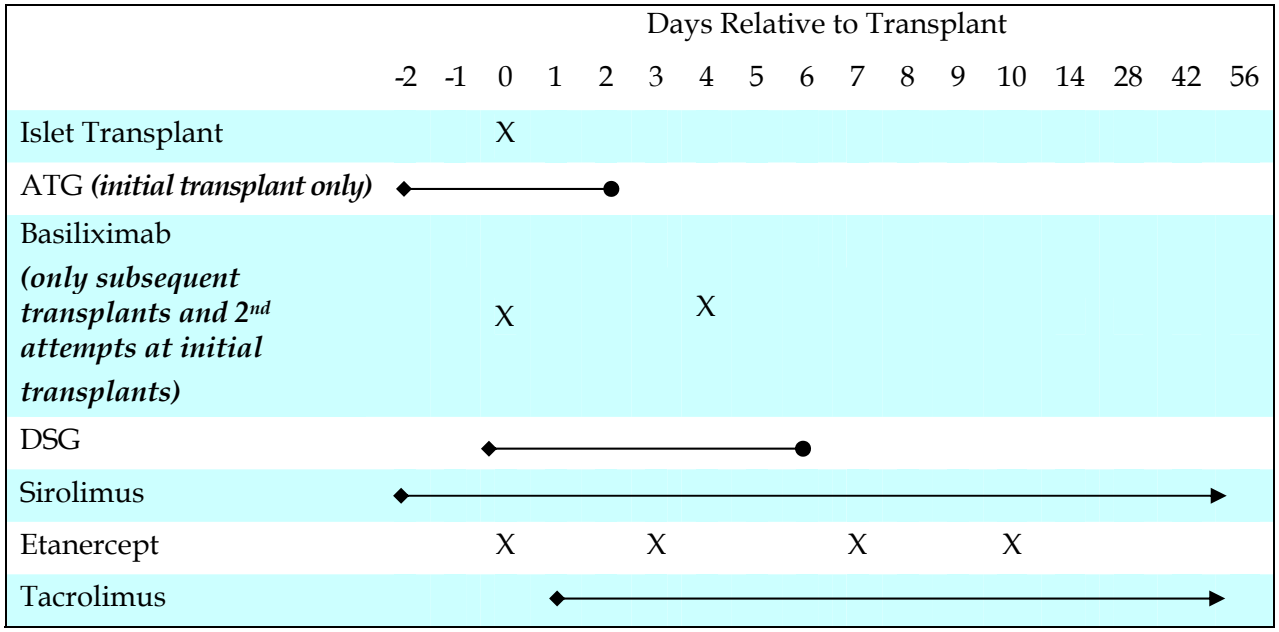


*Figure 2. Islet transplant and immunosuppression regimen.*

## 5.1 Investigational Agents

The investigational agents for this study include:

- Allogeneic islets: The allogeneic islets are considered the primary investigational agent being regulated by the FDA under BB-IND 9336.
- DSG: DSG is considered the secondary investigational agent in this protocol.

### 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 200mL Ricordi® bags, containing a dose of ≥ 5,000 IEQ/kg recipient body weight (BW) for the first transplant, and ≥ 4,000 IE/kg recipient BW for subsequent transplants.

*Table 1: Composition of Final Drug Product [Product Code: PHPI-A-01]:*

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

#### 5.1.2.1 FORMULATION, PACKAGING AND LABELING

Each clinical vial of DSG for injection contains 100 mg gusperimus (as hydrochloride salt) and 200 mg lactose as an inactive ingredient. The drug is packaged as a lyophilized powder in glass vial of 10 mL volume. The intact vials should be stored at 2-8°C and are stable for at least 4 years under these conditions.

#### 5.1.2.2 PREPARATION, ADMINISTRATION, AND DOSAGE

DSG 100 mg/vial should be reconstituted with 3.8 mL of Sterile Water for Injection, USP (or equivalent) or 0.9% Sodium Chloride Injection, USP (or equivalent) to a gusperimus concentration of 25 mg/mL. The drug reconstituted in this fashion is stable for 4 days at 25°C and 14 days at 5°C.

As the single lyophilized dosage form contains no antibacterial preservative, the reconstituted product must be administered within 12 hours of reconstitution. All doses are to be prepared in laminar flow hoods by trained technicians using aseptic technique. The prepared DSG dose should be refrigerated at 2-8°C in a drug refrigerator with access limited to authorized staff. The infusion must be completed within 12 hours after the DSG was reconstituted.

When DSG for injection is further diluted with 0.9% Sodium Chloride Injection, USP (or equivalent) or 5% Dextrose Injection, USP (or equivalent) to a final concentration of 0.2 mg/mL, the solution is stable for 4 days at 4 days at 25°C and 14 days at 5°C; however, the solution should be injected within 12 hours after dissolving.

DSG will be administered at a dose of 2 mg/kg IV daily starting on day 0, the day of transplant, and continuing through day +6. The initial DSG infusion will be given over 3 hours and will be started 3 hours prior to the planned islet transplant so that it is completed by the time the islet transplant is started. DSG will be administered over 3 hours with subsequent transplants following the same schedule.

### 5.1.3 Drug Accountability

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

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

All records regarding the disposition of the investigational product will be available for inspection by the clinical trial monitor. All vials for DSG (used, partially used, and unused) must be noted on the study provided Study Drug Accountability Form. It is the site investigator's responsibility to ensure proper study drug dispensing procedures and proper record keeping. All unused investigational products will be returned to the manufacturer. Expired drug will be destroyed appropriately at each site.

## 5.2 Immunosuppression Medications

### 5.2.1 Initial Allogeneic Islet Transplant

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

#### 5.2.1.1 RABBIT ANTI-THYMOCYTE GLOBULIN (ATG, THYMOGLOBULIN®)

A total of 6 mg/kg will be given as an IV infusion on days –2, -1, 0, +1, and +2. The dose will be 0.5 mg/kg on day –2, 1.0 mg/kg on day –1, and 1.5 mg/kg on days 0, +1, and +2. The doses will be administered as directed on the package insert and in the Manual of Procedures. Premedications will be used as follows:

#1: Acetaminophen (Tylenol®) 650 mg PO/PR ½ hr before and midway through ATG infusion

#2: Diphenhydramine (Benadryl®) 50 mg PO ½ hr before and midway through ATG infusion

#3: Methylprednisolone (Solu-Medrol®) 1 mg/kg IV one hour prior to and as needed during the first ATG infusion only (*i..e.*, on day –2)

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

If the subject is admitted when the vascular access team is not available or at a time when the placement of a Peripherally Inserted Central Catheter could delay the first Thymoglobulin® dose, it may be administered IV via a peripheral line as follows:

- Dilute the thymoglobulin® in 500 cc Normal Saline (not D5W)
- Combine with heparin 1000 units and hydrocortisone 20 mg.

#### 5.2.1.2 SIROLIMUS (RAPAMUNE®)

Sirolimus will be administered at an initial dose 0.05-0.2 mg/kg PO on day -2 relative to islet transplant, followed by 0.05- 0.1 mg/kg QD. The daily dose will be adjusted to the whole blood 24-hr trough to target, as tolerated, 10-15 ng/mL for the first 3 months and 8-12 ng/mL thereafter. If a subject develops intolerable or clinically undesirable side-effects related to sirolimus therapy, his/her therapy may be converted to a maintenance mycophenolate mofetil (MMF) or mycophenolate sodium (MYFORTIC®) therapy at the discretion of the principal investigator.

#### 5.2.1.3 TACROLIMUS (PROGRAF®)

Tacrolimus will be administered at an initial dose 0.015 mg/kg PO BID on day +1, whole blood 12-hr trough adjusted to 3-6 ng/mL. For subjects who have converted to MMF, tacrolimus will be administered to target whole blood trough levels of 10-12 ng/ml for the first 3 months post-transplant, 8-10 ng/ml from 3-6 months post-transplant, and 6-8 ng/ml thereafter. Should subjects experience a decrease in their GFR of ≥ 33% compared with baseline, a nephrology consult will be obtained, and tacrolimus target trough levels will be reduced by 25% should CNI toxicity be suspected as the primary cause for the decline in renal function.

#### 5.2.1.4 CYCLOSPORINE, USP (NEORAL®)

Cyclosporine may be used as a replacement for tacrolimus if clinically indicated. Cyclosporine will be administered at an initial dose of 6 mg/kg/d in 2 divided doses, with target levels of 150-200 ng/mL.

#### 5.2.1.5 MYCOPHENOLATE MOFETIL (CELLCEPT®)

Mycophenolate mofetil may be used at a dose of 500 to 1500 mg PO BID as a replacement for tacrolimus or sirolimus. Subjects must practice two methods of contraception while taking MMF. If a subject experiences severe neutropenia (absolute neutrophil count <1x10^9/L) while taking mycophenolate mofetil, mycophenolate mofetil exposure will be reviewed and mycophenolate mofetil administration will be adjusted as part of the study protocol's neutropenia management plan.

#### 5.2.1.6 MYCOPHENOLATE SODIUM (MYFORTIC®)

Mycophenolate sodium may be used as a replacement for tacrolimus, sirolimus, or mycophenolate mofetil. Mycophenolate sodium will be dosed at 360 to 720 mg PO BID. Subjects must practice two methods of contraception while taking Myfortic®.

### 5.2.2 Subsequent Allogeneic Islet Transplants and Second Attempts at Initial Transplants

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

#### 5.2.2.1 BASILIXIMAB (SIMULECT®)

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

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

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

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

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

## 5.3 Concomitant Medications

### 5.3.1 Immunosuppressive / Anti-Inflammatory Therapy

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

### 5.3.2 Antibacterial, Antifungal, and Antiviral Prophylaxis

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

#### 5.3.2.1 TRIMETHOPRIM/SULFAMETHOXAZOLE (SEPTRA SS®/BACTRIM®)

Trimethoprim/sulfamethoxazole will be administered at a dose of 80 mg/400 mg PO QD starting on Day +1 for the duration of study follow-up. In the event that a subject is unable to take trimethoprim/sulfamethoxazole, he/she will be treated on a case-by-case basis as is medically indicated.

#### 5.3.2.2 CLOTRIMAZOLE (MYCELEX TROCHE®)

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

#### 5.3.2.3 VALGANCICLOVIR (VALCYTE®)

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

#### 5.3.2.4 FUROSEMIDE (LASIX®)

Furosemide may be administered for fluid retention (pleural effusion, etc) as clinically indicated. Recommended administration will be 40 mg. IV QAM on days -2, -1, 0 and if needed day +1.

### 5.3.3 Anticoagulation Prophylaxis / Hematological Agents

#### 5.3.3.1 HEPARIN

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

#### 5.3.3.2 ENOXAPARIN (LOVENOX®)

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

#### 5.3.3.3 ASPIRIN

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

#### 5.3.3.4 PENTOXIFYLLINE

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

### 5.3.4 Updated Vaccinations

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

### 5.3.5 Insulin Therapy

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

### 5.3.6 Other Standard Therapies

Anti-hypertensive, anti-hyperlipidemia and other approved therapies for pre-existing and new medical conditions will be provided per standard of care. Pre- and post-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 ≤ 5 mg daily, or an equivalent dose of hydrocortisone, for physiological replacement, only)
- any medications in the macrolide antibiotic class other than Zithromax
- other investigational products
- other immunosuppressive therapies
- immunomodulatory agents
- other anti-diabetic agents
- Dapsone

## 5.6 Assessment of Compliance with Study Treatment

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

## 5.7 Modification or Discontinuation of Study Treatment

### 5.7.1 Modification of Consensus Immunosuppression

#### 5.7.1.1 ISLETS ARE UNSUITABLE

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

#### 5.7.1.2 GRAFT FAILURE

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

#### 5.7.1.3 ALLERGIC REACTION TO ATG

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

#### 5.7.1.4 INTOLERANCE OF PROTOCOL MEDICATIONS

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

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

#### 5.7.1.5 RABBIT ANTI-THYMOCITE GLOBULIN-INDUCED ANAPHYLAXIS

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

#### 5.7.1.6 RABBIT ANTI-THYMOCITE GLOBULIN-INDUCED CYTOKINE RELEASE

Thymoglobulin® infusion may cause cytokine release-related fever and chills. To minimize these, the first dose should be infused over a minimum of 6 hours into a high-flow vein. Also, premedication with corticosteroids, pentoxifylline, acetaminophen, and/or an antihistamine will be provided in order to minimize the reaction incidence and/or intensity. At any sign of the above reaction, slowing the infusion rate by 50% will also occur.

#### 5.7.1.7 NEUTROPENIA

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

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

- Hold DSG (do not reinstate until the subject is tolerating standard doses of all other immunosuppressive agents and the absolute neutrophil count has risen above 1000 cells/µL)
- Reduce rabbit ATG by 50%.
- Reduce the prophylactic use of valganciclovir from 900 mg per day to 450 mg per day or hold valganciclovir.
- Reduce trimethoprim/sulfamethoxazole to 80/400 mg on Monday, Wednesday, and Friday or hold trimethoprim/sulfamethoxazole.
- Review and obtain current sirolimus trough levels and consider dosage adjustment if trough level are >12ng/mL.
- If subject is using mycophenolate mofetil or mycophenolate sodium in lieu of sirolimus consider dose reduction.
- Consider administration of G-CSF.
- Monitor temperature BID.
- Follow up within 48-72 hours to obtain: repeat complete blood count (CBC) with differential, 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:**

- Hold DSG (do not reinstate until the subject is tolerating standard doses of all other immunosuppressive agents and the absolute neutrophil count has risen above 1000 cells/µL)
- Obtain Infectious Disease Consult.
- Hold rabbit ATG.
- Hold valganciclovir and trimethoprim/sulfamethoxazole.
- Review and obtain current sirolimus trough levels and consider dosage adjustment if trough level are >12ng/mL.
- If subject is using mycophenolate mofetil or mycophenolate sodium in lieu of sirolimus consider dose reduction.
- Administer G-CSF.
- Monitor temperature BID.
- Follow up within 48-72 hours to obtain: repeat CBC with differential, patient 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 DSG (do not reinstate until the subject is tolerating standard doses of all other immunosuppressive agents and the absolute neutrophil count has risen above 1000 cells/μL)
- Hold rabbit ATG.
- Hold administration of trimethoprim/sulfamethoxazole and/or valganciclovir.
- Review and obtain current sirolimus trough levels and hold dose if trough level are >12ng/mL.
- If subject is using mycophenolate mofetil or mycophenolate sodium in lieu of sirolimus consider holding dose.
- Obtain CMV antigenemia or PCR for CMV.
- Consider fluoroquinolones in afebrile patients.
- 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 DSG (do not reinstate until the subject is tolerating standard doses of all other immunosuppressive agents and the absolute neutrophil count has risen above 1000 cells/μL)
- Hold rabbit ATG.
- Hold administration of trimethoprim/sulfamethoxazole and/or ganciclovir.
- Review and obtain current sirolimus trough levels and hold dose if trough level are >12ng/mL.
- If subject is using mycophenolate mofetil or mycophenolate sodium in lieu of sirolimus consider holding dose.
- Obtain CMV antigenemia or PCR for CMV.
- Administer G-CSF.
- Follow up within 24 hours with admitting physician.

#### 5.7.1.8 THROMBOCYTOPENIA

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

#### 5.7.1.9 NEPHROTOXICITY

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

*Table 2: Response to nephrotoxicity*

| Allowable therapeutic responses to CNI-induced nephrotoxicity | Rationale |
|---|---|
| Discontinue sirolimus, and replace it with mycophenolate mofetil or mycophenolate sodium | The nephrotoxic effect of CNIs is increased by concomitant administration of sirolimus[170, 201] |
| If the trough sirolimus level is maintained at > 10 ng/mL without adverse effects, discontinue the CNI and replace it with mycophenolate mofetil or mycophenolate sodium. | CNI should be discontinued only if the subject can tolerate a trough level of sirolimus that will result in adequate immunosuppression. |
| Decrease the target CNI trough level by 25% | CNI toxicity is dose-related |

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

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

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

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

1. The subject is unwilling or unable to comply with the protocol.
2. The investigator believes that the study treatment is no longer in the best interest of the subject.
3. Graft Failure: (see Study Definitions and section 5.7.1.2).
4. An unexpected related SAE. The agent(s) to which the event is attributed will be discontinued.

Subjects who prematurely discontinue study treatment will remain in the study until normal termination, for the purpose of monitoring safety and efficacy parameters and will enter the reduced follow-up scheduled outlined in Appendix 2. Data from these subjects will be used in the intent-to-treat analysis. These subjects are permitted to simultaneously enroll in a CIT or site-specific graft failure follow-up protocol, if available.

# 6. CRITERIA FOR PREMATURE TERMINATION OF THE STUDY

## 6.1 Subject Withdrawal Criteria

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

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

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

## 6.2 Study Stopping Rules

### 6.2.1 Protocol Suspension and Review

Study enrollment at all participating clinical sites will be suspended pending expedited review of all pertinent data by the institutional review board (IRB), the National Institute of Allergic 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. The Medical Monitor finds any unexpected fatal or life-threatening AE possibly related to the use of the test therapy;
2. **Primary Non Function** occurs in 3 or more consecutive subjects at 2 or more participating clinical sites.
3. There are 6 consecutive study subjects with a c-peptide less than 0.3ng/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. Statistical guidelines for terminating the study based on monitoring guidelines are provided in section 10.

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

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

### 6.2.2 Site Suspension and Review

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

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

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

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

# 7. STUDY PROCEDURES

## 7.1 Enrollment and Screening

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

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

*Table 3: Timeframes for screening assessments*

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

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

In addition to the protocol related 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. At sites with an actively recruiting site specific Phase 2 trial, eligible subjects will be randomized on Day -2 relative to transplant, between this Phase 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 (Phase 3 trial) for every subject assigned to the site-specific CIT03 (Phase 2 trial). Subjects in this Phase 2 trial will receive immunosuppressive therapy beginning on Day -2 (See Section 5 for full description of Study Treatment Regimen).

On Day 0, subjects will receive their initial DSG infusion over 3 hours, immediately followed by the islet transplant. Subjects will receive daily DSG infusions, each administered over 3 hours, on Day 1 through Day 6 post-transplant and will continue the immunosuppression regimen detailed in Section 5.

## 7.4 Follow-up Visits

Subject will undergo a 24-month follow-up period following their last islet transplant. Please refer to the Schedule of Events (Appendices 1 & 4), for the clinical time points of specific follow-up study procedures. The timing of all follow-up assessments will "reset" with additional transplants; *i.e.*, the day of the 2nd transplant becomes day 0 and subsequent assessments are conducted in relation to this day.

Subjects who have completed their 365 visit following their initial transplant (primary endpoint assessment) and are thus unable to obtain a subsequent transplant in CIT are allowed to concurrently enroll in a non-CIT islet transplant study. Subjects will be followed for adverse events only until 24 months after their final CIT islet transplant.

## 7.5 Criteria and Timing for Subsequent Islet Transplants

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

### 7.5.1 Second Islet Transplant

Islet transplant recipients with **partial islet graft function** (see Study Definitions) will be considered for a second transplant in the interim between the 75± 5 days/metabolic assessment visit and 8 months post-initial infusion.

Islet transplant recipients with **graft failure** will be considered for a second islet transplant before 8 months post-initial infusion. Islet transplant recipients with graft failure can receive a second islet transplant before 75 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. Absence of any medical condition that, in the opinion of the investigator, will interfere with a safe and successful islet transplant.
4. Subject has no unresolved SAEs.
5. No evidence of progressive renal dysfunction, with blood creatinine rising above 2.0 mg/dL (177 μmol/L).
6. No evidence of hypersensitization, allergic responses, or other potentially serious drug reactions to medications required by the protocol.
7. 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).

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

### 7.5.2 Third Islet Transplant

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

1. The subject received greater than 4,000 IE/kg with the second transplant, but remains dependent on insulin for longer than one month after the second transplant.
2. There is evidence of **partial graft function.**
3. The CIT PIs, Site PIs, and the Steering Committee have determined that there were no relevant protocol deviations at the site.
4. The subject has been compliant with study monitoring and prescribed immunosuppressive therapy.
5. No evidence of a serious and life-threatening infection, AE, or other condition that precludes attempting an intraportal injection or continuation of the post-transplant treatment regimen.
6. No evidence of PTLD.

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

## 7.6 Visit Windows

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

# 8. SAFETY MONITORING

AEs that are classified as serious according to the definition set forth by the health authorities must be reported promptly to NIAID/NIDDK, Clinical Research Organization (CRO)/Data Coordinating Center (DCC), health authorities, PIs, and IRBs. This section defines the types of AEs and outlines the procedures for appropriately collecting, grading, recording, and reporting them. Information in this section complies with *International Conference on Harmonization (ICH) Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting* and *ICH E6: Guideline for Good Clinical Practice,* and applies the standards set forth in the *Terminology Criteria for Adverse Events in Trials of Adult Pancreatic Islet Transplantation (CIT-TCAE)*. This document, created by the CIT, modifies the National Cancer Institute (NCI), *Common Terminology Criteria for Adverse Events (CTCAE)* version 4.0 (May 2, 2007), to ensure applicability in the setting of Islet Transplantation.

## 8.1 Definitions

### 8.1.1 Adverse Event

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

### 8.1.2 Serious Adverse Event

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

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

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

7. Other conditions specified in the protocol.

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

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

### 8.1.3 Unexpected Adverse Event

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

## 8.2 Adverse Events

### 8.2.1 Collecting Procedure

AEs that are associated with a protocol mandated procedure, which is not part of the normal standard of care for the participant, and **severe hypoglycemic events** (see study definitions) will be collected beginning immediately after enrollment consent has been obtained. All other AEs will be collected beginning immediately after randomization. All AEs will continue to be collected until study completion, or for 30 days after the subject prematurely withdraws from the study. If a subject enrolls in a non-cit islet transplant study, adverse events will no longer be collected in CIT starting at the time of the non-CIT study intervention. All adverse event reporting from that point on will be done through the non-CIT study.

AEs will be followed until the time the event is resolved, stabilized, or the subject completes or withdraws from the study, whichever comes first.

AEs may be discovered through any of these methods:

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

### 8.2.2 Recording Procedure

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

### 8.2.3 Grading and Attribution

#### 8.2.3.1 GRADING CRITERIA

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

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

Grade 1 = Mild AE.

Grade 2 = Moderate AE.

Grade 3 = Severe and undesirable AE.

Grade 4 = Life-threatening or disabling AE.

Grade 5 = Death.

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 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. |

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

#### 8.2.3.2 DEFINITION OF ATTRIBUTION

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

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

*Table 5: Attribution of adverse event*

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

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

## 8.3 Serious Adverse Events

### 8.3.1 Collecting Procedure

SAEs will be collected following the subject's signing of the enrollment consent until 30 days after the subject completes or withdraws from the study. SAEs will be followed until the time the event is resolved, stabilized, or until 30 days after the subject completes or withdraws from the study, whichever comes first.

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

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

### 8.3.2 Recording Procedure

SAEs will be recorded on the AE eCRF.

### 8.3.3 Reporting Procedure

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

#### 8.3.3.1 REPORTING CRITERIA FROM THE SPONSOR TO HEALTH AUTHORITIES

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

- **No reporting.** This requirement applies if the AE is deemed not serious by the DCC medical reviewer and the NIAID/NIDDK medical monitor.
- **Standard reporting** (*i.e.*, should be included in the 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 SAEs will be reported to the DCC by the sites on at least a monthly basis.

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

The DCC is responsible for notifying the sponsor within 2 business days of receiving the report by the clinical site. The sponsor is responsible for disseminating reports to the health authorities, all investigators in the study, and the manufacturer of the secondary study drug. 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.

#### 8.3.3.5 NOTIFYING THE INSTITUTIONAL REVIEW BOARD AND ETHICS COMMITTEE

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

#### 8.3.3.6 REPORTING PREGNANCY AS A SERIOUS ADVERSE EVENT

Any pregnancy that occurs during a clinical study that is using an investigational drug must be reported to the DCC utilizing the SAE report form. This report is *for tracking purposes only*. All pregnancies that are identified during the study must be followed to conclusion and the outcome of each must be reported. The investigator should report all pregnancies within 24 hours (as described in section 8.3.3.2) using the SAE report form. The investigator should counsel the subject and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. A woman who becomes pregnant or wishes to while on the study will be counseled as to her choices and will be encouraged to discuss those choices with her

obstetrician. Monitoring of the subject should continue until the conclusion of the pregnancy, and a follow-up SAE report form detailing the outcome of the pregnancy should be submitted.

### 8.3.4 Updating Source Documentation

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

# 9. MECHANISTIC ASSAYS

## 9.1 Metabolic Testing

### 9.1.1 Study Endpoints

The primary analysis will be to provide an estimate of the proportion of subjects with insulin independence and a corresponding 95% confidence interval. The secondary analyses will compare the primary and secondary endpoints in this study to the corresponding outcomes in the CIT07 Phase 3 study. Estimates and 95% confidence intervals will also be calculated. 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 Appendix 1.

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

#### 9.1.2.1 INSULIN REQUIREMENTS

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

#### 9.1.2.2 GLYCEMIC CONTROL

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

#### 9.1.2.3 GLYCEMIC LABILITY

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

The MAGE requires 14 – 16 capillary BG measurements over two consecutive days taken before and 2-hours after breakfast, lunch, and dinner, and at bedtime with an optional measurement at 3 AM. A glycemic excursion is calculated as the absolute difference in peak and subsequent nadir (or vice versa) glucose values, with the direction (peak to nadir versus nadir to peak) determined by the first quantifiable excursion in the two day period. All excursions > 1 S.D. of the 7 – 8 glucose readings for the day in which they occurred qualify for the analysis, where they are summed and divided by the number of qualified excursions to give the MAGE in mmol/L (or mg/dL) glucose. A MAGE >11.1 mmol/L (200 mg/dL) is indicative of marked glycemic lability.

The LI requires 4 or more daily capillary BG measurements over a 4 week period and is calculated as the sum of all the squared differences in consecutive glucose readings divided by the hours apart the readings were determined (range 1 to 12 hours) in $mmol/L^2/h \cdot wk^{-1}$. A LI greater than or equal to the 90th percentile (433 $mmol/L^2/hr \cdot wk^{-1}$) 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 BG level < 54 mg/dL (3.0 mmol/L) or prompt recovery after oral carbohydrate, IV glucose, or glucagon administration[11].

In addition, composite indices of hypoglycemia frequency, severity, and symptom recognition will be assessed by both the Clarke survey[202] and the HYPO score[2].

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

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

#### 9.1.2.5 MIXED-MEAL TOLERANCE TEST

Basal (fasting) and stimulated glucose and C-peptide levels will be determined every 3 months following transplantation, including 365 days post-initial transplant, 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 = 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) standard operating procedure (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 basal or stimulated C-peptide as developed by Ryan *et al*[3]. The score may range from 0 (no graft function) to 8, with all subjects reported with a score of 8 also having 90-minute glucose levels during a MMTT that are ≤ 10.0 mmol/L (180 mg/dL), indicative of excellent graft function.

#### 9.1.2.7 THE C-PEPTIDE: (GLUCOSE X CREATININE) RATIO

The C-peptide: (glucose X creatinine) ratio (CPGCR) will be determined from the fasting (basal) glucose and C-peptide, and a simultaneous serum creatinine. This measure accounts for both the dependence of C-peptide secretion on the ambient glucose concentration and the dependence of C-peptide clearance on kidney function[203, 204]. The CPGCR is calculated as [C-peptide (ng/ml) * 100]/[glucose (mg/dL) * creatinine (mg/dL)]. An index of islet graft function, this measure correlates well with both the 90-minute glucose levels during a MMTT and the β-score.

#### 9.1.2.8 INSULIN-MODIFIED FREQUENTLY-SAMPLED INTRAVENOUS GLUCOSE TOLERANCE TEST

The $AIR_{glu}$, insulin sensitivity, and DI will be determined using the FSIGT test. This assessment provides a composite measure of β-cell function, the DI, which relates the effect of insulin sensitivity on first-phase insulin secretion ($AIR_{glu}$). Understanding the effect of insulin sensitivity on insulin secretory dynamics post-transplant is important because insulin resistance imposes an increased demand on β-cell function to maintain the same level of glycemia. Whether insulin resistance, possibly attributable to immunosuppressive drugs, is an important problem post-transplant is not known. Preliminary data indicate that insulin sensitivity may actually be improved post-transplant, despite immunosuppression, possibly due to the improved glycemia that occurs with transplantation[4]. These results require confirmation by longitudinal analysis.

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

injection of glucose at t = -30 seconds with an injection of insulin at t = 20 min. Each pre-transplant blood sample will be used for insulin, and glucose determination. Each post-transplant blood sample will be used for insulin and glucose determination; in addition, the baseline (t = -10, -5, and -1 min) and t = 1, 2, 3, 4, 5, 7, and 10 minutes post-glucose injection samples will be used for c-peptide determination.

All samples will be drawn according to University of Washington (Seattle, WA) SOP and will be shipped frozen to U of W for measurement in the core laboratory. The $AIR_{glu}$ is calculated as the incremental area-under-the-curve for insulin between 0 and 10 minutes post-injection (the same calculation can be performed for C-peptide). Glucose effectiveness (SG), a measure of insulin-independent glucose disposal, and insulin sensitivity (SI), 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 DI (DI = $AIR_{glu}$ • SI).

#### 9.1.2.9 CONTINUOUS GLUCOSE MONITORING SYSTEM®

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[6, 7].

#### 9.1.2.10 QUALITY OF LIFE

Generic and disease-specific measures will be used to assess QOL.

**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.

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

### 9.2.1 Immune Assays

#### 9.2.1.1 HLA TYPING OF DONORS AND RECIPIENTS, CROSSMATCHING

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

#### 9.2.1.2 ALLOANTIBODY

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

#### 9.2.1.3 AUTOANTIBODY

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

#### 9.2.1.4 MEASURES OF INNATE IMMUNITY

In order to correlate expression of proinflammatory or procoagulant markers on islets with recipient response in the early post-transplant period, ethylenediaminetetraacetic acid (EDTA) anti-coagulated blood will be collected for assessment of thrombin-antithrombin (TAT), C3a, and c-peptide levels.

#### 9.2.1.5 ARCHIVED SAMPLES

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

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

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

# 10. STATISTICAL CONSIDERATIONS AND ANALYSIS PLAN

## 10.1 Statistical Analyses

The details of the analyses will be provided in the SAP. All primary and secondary analyses will be done using the intention to treat principle. Every subject who receives study drug will be accounted for in the analysis.

## 10.2 Study Endpoint Assessment

### 10.2.1 Primary Endpoint

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 be to provide an estimate of the proportion of subjects with insulin independence and a corresponding 95% confidence interval. The analysis will compute an exact binomial estimate and a 95% confidence interval for the true rate.

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

### 10.2.2 Secondary Endpoints

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 sensitivity analyses to determine the potential magnitude of the contributions of the missing values. Details of the sensitivity analysis will be included in the SAP.

Initial analysis of secondary endpoints will use methods similar to those defined for the primary outcome. When the endpoint is a proportion such as subjects 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.

Additional secondary analyses will compare the primary and secondary endpoints in this study to the corresponding outcomes in the CIT07 Phase III study. The first secondary analysis will compare the rates of insulin independence at 75 days. This analysis will use a logistic random effects model (i.e. a random effects model with a logit link function) with a fixed effect for the DSG group versus the comparison group and random effects for the four contributing centers to account for the correlation of subjects within centers. The odds ratio for the effect of treatment effect will be used as the measure of effect and will be reported with the appropriate 95% confidence interval.

Similar methods will be used for the other secondary variables. Dichotomous secondary outcome variables will be analyzed using logistic random effects models. If the normality assumptions are valid, continuous outcomes such as insulin and C-peptide levels will be analyzed using similar models but assuming a normal link function. Differences in means will be reported as measures of effect. Appropriate 95% confidence intervals will be reported for the differences in the means.

## 10.3 Subject and Demographic Data

Summary descriptive statistics for baseline and demographic characteristics will be provided for all randomized and transplanted subjects. 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.

### 10.3.1 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.

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.

### 10.3.2 Study Completion

The percent of subjects who complete the study, losses to follow-up, times to lost to follow-up, and reasons for discontinuation (*e.g.*, AEs) will be presented. Statistical presentation of study completion may be further defined in the 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 20 subjects. The point estimate of the true insulin independence rate will be the proportion of the 20 subjects 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 10 of the 20 subjects achieve insulin independence, then the estimated rate will be 0.5, and a 95% confidence interval will be 0.272 to 0.729. That is, we are 95% confident that the true rate is at least 27.2% and no more than 72.9%. The confidence interval rules out any rate less than 27.2% or greater than 72.9%. If 6 subjects (30%) achieve insulin independence then the confidence interval will rule out any rate less than 11.9% or greater than 54.3%.

*Table 6: Exact 95% confidence intervals for all possible outcomes*

| Number of Subjects insulin Independent at 75 Days | Estimated Rate | Exact 95% Confidence Interval | |
|---|---|---|---|
| | | Lower Bound | Upper Bound |
| 0 | 0.00 | 0.000 | 0.069 |
| 1 | 0.05 | 0.001 | 0.249 |
| 2 | 0.10 | 0.012 | 0.317 |
| 3 | 0.15 | 0.032 | 0.379 |
| 4 | 0.20 | 0.057 | 0.437 |
| 5 | 0.25 | 0.087 | 0.492 |
| 6 | 0.30 | 0.119 | 0.543 |
| 7 | 0.35 | 0.154 | 0.593 |
| 8 | 0.40 | 0.191 | 0.640 |
| 9 | 0.45 | 0.230 | 0.685 |
| 10 | 0.50 | 0.272 | 0.729 |
| 11 | 0.55 | 0.315 | 0.770 |
| 12 | 0.60 | 0.360 | 0.809 |
| 13 | 0.65 | 0.408 | 0.847 |
| 14 | 0.70 | 0.457 | 0.882 |
| 15 | 0.75 | 0.509 | 0.914 |
| 16 | 0.80 | 0.563 | 0.943 |
| 17 | 0.85 | 0.621 | 0.968 |
| 18 | 0.90 | 0.683 | 0.988 |
| 19 | 0.95 | 0.751 | 0.999 |
| 20 | 1.00 | 0.831 | 1.000 |

## 10.5 Interim Analyses to Ensure Subject Safety

The DSMB will be convened to review safety and efficacy data following NIH policy. Formal interim analyses will include distributions of endpoints, biomarkers and AEs.

The monitoring plan will be developed with input from the DSMB. Details of this plan will be included in the SAP. Because this is a small study and it is important to collect as much safety data as possible, it is not likely that the DSMB or the investigators will recommend stopping early for evidence of efficacy. Therefore, the monitoring plan will recommend not enrolling the entire 20 subjects only if there is sufficient evidence to conclude that treatment with DSG is harmful. Should the monitoring boundaries be crossed then the DSMB will be provided with an analysis for the primary endpoint, all secondary endpoints (excluding those comparing to the Phase III study), biomarkers, and adverse experiences. The DSMB will make recommendations to NIH.

The following provides information on a potential strategy for stopping when there is evidence that the insulin independence rate is too low. This strategy is based on ruling out rates below which clinicians would recommend that treatment with DSG was likely to achieve an unacceptably low rate of insulin independence at 75 days. The following table describes boundaries for selected minimal rates of insulin independence. These recommendations are based on calculate 95% exact binomial confidence intervals for the true rate and recommending stopping if the upper bound of the computed confidence interval does not contain the minimal rate.

For example, if 50% is the targeted minimal rate then the rule would recommend stopping enrollment if any of the following occurred: 0 successes in the first 5 subjects entered, no more than 1 success in the first 7 subjects, no more than 2 successes in the first 9 subjects, no more than 3 success in the first 11 subject, no more than 4 success in the first 16 subjects, or no more than 5 successes in the first 19 subjects. Note that these confidence intervals were not adjusted for the multiplicity of the calculated confidence intervals.

*Table 7: Stopping rules for selected minimal rates of insulin independence*

| Level | Minimal Rate of Insulin Independence | | | |
|---|---|---|---|---|
| | 50% | 40% | 30% | 20% |
| 1 | 0 in first 5 | 0 in first 6 | 0 in first 10 | 0 in first 14 |
| 2 | 1 in first 7 | 1 in first 10 | 1 in first 14 | |
| 3 | 2 in first 9 | 2 in first 14 | 2 in first 19 | |
| 4 | 3 in first 11 | 3 in first 17 | | |
| 5 | 4 in first 16 | | | |
| 6 | 5 in first 19 | | | |

The investigators have suggested that there would be a concern if the rate of insulin independence were less than 30%. We plan to use the rule that uses 30% as the targeted minimal rate. The rule would recommend stopping enrollment if any of the following occurred:

0 successes in the first 10 subjects, no more than 1 success in the first 14 subjects or no more than 2 successes in the first 19 subjects.

## 10.6 Reporting Deviations from Original Statistical Plan

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

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# 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 eCRFs.

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

## 11.2 Permitting Access to Source Data

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

# 12. QUALITY CONTROL AND QUALITY ASSURANCE

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

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

## 12.1 Compliance, Access, Entry and Handling of Study Data

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

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

All data will be entered, stored, and managed in a relational database supported by database servers at the DCC. The results of all clinical and laboratory evaluations will be maintained in the subjects medical records and the data will be transferred from these source documents directly to the study eCRFs. 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* [205], 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.

In accordance to the Health Insurance Portability and Accountability Act (HIPAA) of 1996, a federal law related to privacy of health information, potential study subjects will be approached to authorize the researcher, the researcher's study staff, and the clinical personnel of the General Clinical Research Center (GCRC) to use and disclose their individual health information for the purpose of conducting the research project.

Individual health information to be used or disclosed to conduct this research includes: (1) Demographic information (*i.e.*, name, address, phone, social security, date of birth, marital status, race, ethnicity, etc.); (2) past medical history and previous medical records; (3) physical

exam results; and (4) results of diagnostic procedures (*i.e.*, laboratory, x-ray, magnetic resonance imaging [MRI], computed tomography [CT], etc.)

Parties who may disclose individual health information to the researcher, the researcher's study staff, and the clinical personnel of the GCRC include previous health care providers, medical clinics and hospitals.

During the subject's study participation all specimens sent to outside laboratories will be sent anonymously. Also, if the subject receives compensation for participating in this study, identifying information about the subject may be used or disclosed as necessary to provide compensation.

Subjects do not have to sign the authorization to use and disclose their individual health information. If the subject decides not to sign the authorization, the subject will not be allowed to participate in clinical research studies or receive any research related treatment that is provided through the study. However, the subject's decision not to sign this authorization will not affect any other treatment, payment, or enrollment in health plans or eligibility for benefits.

The subject can change his/her mind and withdraw this authorization at any time by sending a written notice to the researcher or the researcher's study staff to inform the researcher or the researcher's study staff of his/her decision. If the subject withdraws this authorization, the researcher may only use and disclose the protected health information already collected for this research study. No further health information about the subject will be collected by or disclosed to the researcher for this study.

The subject's individual health information disclosed under this authorization may be subject to re-disclosure outside the research study and no longer protected. Examples include potential disclosures for law enforcement purposes, mandated reporting of abuse or neglect, judicial proceedings, health oversight activities and public health measures.

The researcher and the researcher's study staff will maintain absolute confidentiality of the name of the donor. Copies of hospital records of donors are maintained in the Organ Procurement Office and will be reviewed by the researcher and the researcher's study staff only, to determine whether conditions existed to exclude the pancreas from consideration as a source of islets for transplantation.

# 14. PUBLICATION POLICY

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

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# Appendix 1: Schedule of Events

| Time points (specified in Days relative to transplant) | SCR | WL/BL[1] | 0[2] | 1 | 2 | 3[3] | 5[3] | 7 | 10 | 14 | 21 | 28 | 42 | 56 | 75 | 120 | 150 | 180 | 270 | 365 | 365 post initial tx |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Visit Number** | **01** | **02** | **03** | **04** | **05** | **06** | **07** | **08** | **09** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **1Y** |
| **Visit Windows (in days)** | N/A | N/A | N/A | N/A | N/A | +/-2 | +/-2 | +/-3 | +/-3 | +/-3 | +/-3 | +/-3 | +/-3 | +/-7 | +/-5 | +/-7 | +/-7 | +/-7 | +/-14 | +/-14 | +/-14 |
| **Equivalent Week/Month** | N/A | N/A | N/A | N/A | N/A | N/A | N/A | W1 | W1.5 | W2 | W3 | W4 | W6 | M2 | M2.5 | M4 | M5 | M6 | M9 | M12 | Varies |
| **GENERAL ASSESSMENTS** | | | | | | | | | | | | | | | | | | | | | |
| Informed Consent | X[4] | X[5] | | | | | | | | | | | | | | | | | | | |
| Med/Diabetes Hx & Demographics | X | | | | | | | | | | | | | | | | | | | | |
| Evaluation of Inclusion / Exclusion | X | X | | | | | | | | | | | | | | | | | | | |
| Retinopathy Evaluation[6] | X | X-yrly[7] | | | | | | | | | | | | | | | | | | | X |
| Physical Exam | X | X-yrly | X | X | X | | | X | | X | | X | | X | X | X | X | X | X | X | |
| Telephone Consult | | | | | | | | | | | X | | | | | | | | | | |
| Vital Signs | X | X | X | X | X | X | X | 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 (including Pelvis/Liver) | X | X-yrly | | X | | | | X | | | | | | | | | | | | X | |
| ECG | X | X-yrly | | | | | | | | | | | | | | | | | | X | |
| Cardiac Stress Test or Angiogram | X | | | | | | | | | | | | | | | | | | | | |
| PPD | X | X-yrly | | | | | | | | | | | | | | | | | | X | |
| AE/Hypo Events/Toxicity Assess | | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | |
| **LOCAL LABORATORY ASSESSMENTS[8]** | | | | | | | | | | | | | | | | | | | | | |
| CBC (WBC + Diff & Plat) | X | X-q6mo | X | X | X | X | X | X | X | X | X[9] | X | X | X | X | X | X | X | X | X | |
| Coagulation (PT, PTT, INR) | X | X-yrly | X | | | | | | | | | | | | | | | | | | |
| Chemistry[9] | X | X-q6mo | X | X | X | X | X | X | X | X | X[9] | 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 (females) | X[10] | X[11] | | | | | | | | | | | | | | | | | | | |
| Serology[12] (Hep B, Hep C, HIV) | X | X-yrly | | | | | | | | | | | | | | | | | | | X |
| EBV IgG | X | | | | | | | | | | | | | | | | | | | | |
| CMV IgG, CMV IgM | | X-yrly[13] | | | | | | | | | | | | | | | | | | | X[13] |

| **Time points (specified in Days relative to transplant)** | **SCR** | **WL/ BL[1]** | **0[2]** | **1** | **2** | **3[3]** | **5[3]** | **7** | **10** | **14** | **21** | **28** | **42** | **56** | **75** | **120** | **150** | **180** | **270** | **365** | 365 post initial tx |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Visit Number** | **01** | **02** | **03** | **04** | **05** | **06** | **07** | **08** | **09** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **1Y** |
| **Visit Windows (in days)** | N/A | N/A | N/A | N/A | N/A | +/-2 | +/-2 | +/-3 | +/-3 | +/-3 | +/-3 | +/-3 | +/-3 | +/-7 | +/-5 | +/-7 | +/-7 | +/-7 | +/-14 | +/-14 | +/-14 |
| **Equivalent Week/Month** | N/A | N/A | N/A | N/A | N/A | N/A | N/A | W1 | W1.5 | W2 | W3 | W4 | W6 | M2 | M2.5 | M4 | M5 | M6 | M9 | M12 | Varies |
| Blood Type | | X[14] | | | | | | | | | | | | | | | | | | | |
| HLA | | X | | | | | | | | | | | | | | | | | | | |
| Crossmatch | | X[15] | | | | | | | | | | | | | | | | | | | |
| Fasting & post-prandial c-pep[16] | | | | | | X | | X | | | | | | | | | | | | | |
| Glucose (immediately post tx) | | | X[17] | | | | | | | | | | | | | | | | | | |
| PRA by flow cytometry | | X[18] | | | | | | | | | | | | | | | | | | | |
| Urine dipstick - proteinuria | | | | | | | | | | | | X | | | X | | | | | | |
| CMV by PCR | | X | | | | | | | | | | | | | X | | | X | | | |
| EBV by PCR[19] | | X | | | | | | | | | | | | | | | | | | | |
| **CENTRAL METABOLIC ASSESSMENTS[20]** | | | | | | | | | | | | | | | | | | | | | |
| First morning spot urine[21] | X | X | | | | | | | | | | X | | | X | | | | | X | X |
| GFR | X | X-yrly | | | | | | | | | | X | | | X | | | | | X | X |
| HbA1c | X | X-q3mo | | | | | | | | | | | | | X | | | X | X | X | X |
| Fasting serum glucose and c-peptide | X | X | | | | | | | | | | X | | X | X[22] | X | X | X[23] | X[24] | X | X |
| 90 min[25] c-pep/gluc (MMMT) | X | | | | | | | | | | | | | | X[23] | | | X | X | X | X |
| Insulin modified FSIGT | | X-yrly[6] | | | | | | | | | | | | | X[23] | | | | | X | X |
| Atherogenic Profile[26] | | X | | | | | | | | | | | | | | | | | | | X |
| **LOCAL METABOLIC ASSESSMENTS** | | | | | | | | | | | | | | | | | | | | | |
| Glycemic Stability (CGMS®) | | X-yrly[6] | | | | | | | | | | | | | X[23] | | | | | X | X |
| BSR eCRF[27] | X | X-q6mo | | | | | | | | | | | | | X[23] | | | X | X | X | X |
| **CALCULATED METABOLIC ASSESSMENTS** | | | | | | | | | | | | | | | | | | | | | |
| MAGE | | X-q6mo | | | | | | | | | | | | | X | | | X | X | X | X |
| LI | X | X-q6mo | | | | | | | | | | | | | X | | | X | X | X | X |
| Clarke Score | X | X-q6mo | | | | | | | | | | | | | | | | X | | X | X |

| Time points (specified in Days relative to transplant) | SCR | WL/BL[1] | 0[2] | 1 | 2 | 3[3] | 5[3] | 7 | 10 | 14 | 21 | 28 | 42 | 56 | 75 | 120 | 150 | 180 | 270 | 365 | 365 post initial tx |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Visit Number** | **01** | **02** | **03** | **04** | **05** | **06** | **07** | **08** | **09** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **1Y** |
| **Visit Windows (in days)** | N/A | N/A | N/A | N/A | N/A | +/-2 | +/-2 | +/-3 | +/-3 | +/-3 | +/-3 | +/-3 | +/-3 | +/-7 | +/-5 | +/-7 | +/-7 | +/-7 | +/-14 | +/-14 | +/-14 |
| **Equivalent Week/Month** | N/A | N/A | N/A | N/A | N/A | N/A | N/A | W1 | W1.5 | W2 | W3 | W4 | W6 | M2 | M2.5 | M4 | M5 | M6 | M9 | M12 | Varies |
| **CALCULATED METABOLIC ASSESSMENTS (Con't)** | | | | | | | | | | | | | | | | | | | | | |
| HYPO | X | X-q6mo | | | | | | | | | | | | | X | | | X | X | X | X |
| Beta Score | | X | | | | | | | | | | | | | X | | | X | X | X | X |
| C-pep: (glucoseXcreatinine) ratio | X | X | | | | | | | | | | X | | X | X | X | X | X[28] | X[28] | X | X |
| **IMMUNOSUPPRESSION LEVELS** | | | | | | | | | | | | | | | | | | | | | |
| Sirolimus 24-hr trough levels | | | X | X | X | X | X | X | X | X | X[9] | X | X | X | X | X | X | X | X | X | X |
| Tacrolimus 12-hr trough levels | | | | | | X | X | X | X | X | X[9] | X | X | X | X | X | X | X | X | X | X |
| **MECHANISTIC ASSAYS** | | | | | | | | | | | | | | | | | | | | | |
| Alloantibody | X | X-q6mo[29] | | | | | | | | | | | | | X | | | X | X | X | X |
| Autoantibody (GAD, IA-2, IAA) | | X | | | | | | | | | | | | | X | | | X | X | X | X |
| **ARCHIVED SAMPLES** | | | | | | | | | | | | | | | | | | | | | |
| Serum | | X | | | | | | | | | | | | | X | | | X | X | X | X |
| Plasma | | X | | | | | | | | | | | | | X | | | X | X | X | X |

[1] WL=Waiting List. BL=Baseline. Repeat assessments as indicated (i.e. yrly, q3mo), while subject is on the WL. All one-time WL/BL assessments should be completed on Day -2 whenever possible, but always prior to start of immunosuppression (IS). For repeat WL/BL assessments, results from test done closest to the start of IS will be used as the baseline value.

[2] Day 0 = the day of transplant. This SOE applies to the 1st, 2nd, and 3rd transplant as applicable. The SOE is restarted at Day 0 for each subsequent transplant.

[3] For day 4 and day 6 of DSG infusion, these will be unscheduled visits.

[4] *Informed consent #1* includes information on CIT03 and multi-center Phase 3 protocol (CIT07).

[5] *Informed Consent #2* includes information specific to CIT03. IC#2 must be signed immediately after randomization.

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

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

[8] Local labs can only be performed at an outside lab; proper regulatory documentation must be maintained.

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

[10] Serum β-HCG

[11] Urine HCG. Complete pregnancy test within 72 hours prior to initiation of study medication.
[12]Serology includes: HBc Ab, HBs Ab, HBs Ag, HCV Ab, HIV, and HTLV-I/II. Do not repeat Hepatitis B tests if HBs Ab was previously positive.
[13] Repeat only if previous test was negative.
[14] Repeat for subsequent transplant(s).
[15] 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.
[16] 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.
[17] Finger stick glucose should be done locally and drawn every hour for the first 6 hours immediately post-transplant.
[18] Subsequent transplants only. Local result used to determine eligibility for subsequent transplants only.
[19] EBV by PCR should only be done post-randomization if reactivation is suspected.
[20] Do not collect for participants with graft failure. Results of tests performed at the time of graft failure will be used for day 75 endpoint calculations.
[21] First morning spot urine includes: albumin, protein, and creatinine
[22] 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.
[23]If blood drawn locally at Months 7 & 8 (Visits 18a, 18b respectively), sample should be sent from local lab to study site and then shipped to the central laboratory (Univ of Washington for fasting glucose/c-peptide and serum creatinine). Visit window is ± 7 days.
[24] If blood drawn locally at Months 10 & 11 (Visits 19a, 19b respectively), sample should be sent from local lab to study site and then shipped to the central laboratory (Univ of Washington for fasting glucose/c-peptide and serum creatinine). Visit window is ± 7 days.
[25] MMTT should include 60 and 90 minute c-peptide and glucose measurements for the screening visit and as necessary when determining graft failure.
[26] 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).
[27] Blood Sugar Record (BSR) eCRF is completed using information gathered from subject diary logs, glucometer download data, and insulin requirements.
[28] C-peptide glucose creatinine ratio calculated monthly.
[29] For each transplant, complete alloantibody assessment every 6 months and again on Day -2, regardless of the most recent draw. Central PRA result, current within 6 months, is used to determine subject eligibility for first transplant.

# Appendix 2: Reduced Follow-up Schedule of Events

Subjects withdrawn from study therapy should be followed according to the reduced follow-up schedule provided below. All reduced follow-up assessments should be scheduled relative to the day on which the study treatment is discontinued. The last follow-up visit will vary depending on when the subject discontinues study therapy and should be done at 1 and 2 years post the subject's **last** transplant.

**REDUCED FOLLOW-UP SCHEDULE**

Complete the following assessments at the intervals (+/- 7 days) indicated below relative to the day the subject discontinued study treatment. Continue conducting these assessments at the defined intervals until the subject reaches 2 years post **last** transplant.

- Assess SAEs and hypoglycemic events: q1 month. If subject does not come to the study site for the visit, attempt to obtain information via a phone contact.
- Alloantibody (central lab): q 1 month for the first 3 months and q 3 months thereafter.

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

- Assess SAEs and hypoglycemic events
- Alloantibody (central lab)
- HbA1c (central lab)
- 90 minute c-peptide post MMTT (central lab)[1]
- Serum creatinine (central lab)
- QOL questionnaire (via mail or in-person)

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

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

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

# Appendix 3. Study Contacts

**SITE PRINCIPAL INVESTIGATOR**

**Bernhard Hering, MD**
Director Islet Transplantation
University of Minnesota
Department of Surgery
420 Delaware St SE MMC 280
Minneapolis, MN 55455
Phone: 612-626-5735
Fax: 612-626-5855
E-mail: bhering@umn.edu

**SITE PRINCIPAL INVESTIGATOR**

**Xunrong Luo, MD, PhD,**
Surgery, Microbiology and Immunology,
Divisions of Nephrology and Organ
Transplantation, Northwestern U. Feinbe
School of Medicine
303 East Chicago Avenue
Tarry Building 4-751
Chicago, IL 60611
Phone: 312-908-8147
Fax: 312-503-0622
Email: xunrongluo@northwestern.edu

**SITE PRINCIPAL INVESTIGATOR**

**Andrew M. Posselt, MD, PhD**
Associate Professor in Residence
Division of Transplantation
Department of Surgery
University of California, San Francisco
505 Parnassus Ave., Room M-896
San Francisco, CA 94143-0780
Tel: 415-353-1473
Fax: 415-353-8709
Email: andrew.posselt@ucsfmedctr.org

# Appendix 4. Schedule of Events for 2-Year Additional Follow-up

| Time Point (months [M] relative to final islet transplant; years [Y] relative to initial transplant) | M15 | M18 | M21 | M24 | 730 post initial tx Y2 |
|---|---|---|---|---|---|
| **Visit Number (relative to final islet transplant)** | **21** | **22** | **23** | **24** | **25** |
| **Visit Window (specified in days)** | **± 14** | **± 14** | **± 14** | **± 14** | **± 90** |
| **GENERAL ASSESSMENTS** | | | | | |
| Physical Exam | | X | | X | X |
| Telephone Consult | X | | X | | |
| QOL | | | | X | X |
| AE /Hypoglycemic Events/Toxicity Assessment | X | X | X | X | X |
| **LOCAL LABORATORY ASSESSMENTS** | | | | | |
| CBC (WBC + Diff & Plat) | X[1] | X | X[1] | X | |
| Chemistry | X[1] | X | X[1] | X | |
| Lipids | | X | | X | |
| **CENTRAL LABORATORY/METABOLIC ASSESSMENTS** | | | | | |
| First morning spot urine[2] | | X | | X | |
| GFR | | | | X | |
| HbA1c | X[3] | X | X[4] | X | X |
| 90-min c-pep/glucose (MMTT)[5] | | X | | X | X |
| Atherogenic Profile[6] | | | | X | |
| **LOCAL METABOLIC ASSESSMENTS** | | | | | |
| Glycemic Stability (CGMS) | | | | X | X |
| 7-Day BSR eCRFs[7] | | | | X | X |
| **CALCULATED METABOLIC ASSESSMENTS** | | | | | |
| Clarke Score | | X | | X | |
| **IMMUNOSUPPRESSION LEVELS** | | | | | |
| Sirolimus Levels | X[1] | X | X[1] | X | |
| Tacrolimus Levels | X[1] | X | X[1] | X | |
| **MECHANISTIC ASSAYS** | | | | | |
| Autoantibody | | X | | X | |
| Alloantibody | | X | | X | |

[1] Can be performed at a local laboratory.
[2] Central laboratory assessment. First morning spot urine contains albumin, protein, and creatinine.
[3] Can be performed at a local laboratory.
[4] Can be performed at a local laboratory.
[5] Also collect as necessary to confirm 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 for 7 consecutive days. See MOP for guidance.

# 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)

**Bernhard Hering, MD** – University of Minnesota
**Xunrong Luo, MD, PhD**– Northwestern University
**Olle Korsgren, MD, PhD** – Uppsala Univ. Hospital
**Nicole Turgeon, MD** – Emory University
**Ali Naji, MD, PhD** – University of Pennsylvania
**Andrew Posselt, MD, PhD** – University of California, San Francisco
**Camillo Ricordi, MD** – University of Miami
**James Shapiro, MD, PhD** – University of Alberta
**Dixon Kaufman, MD, PhD, FACS** – University of Wisconsin
**James Markmann, MD, PhD** – Massachusetts General Hospital

### BIOSTATISTICIAN

**William Clarke, PhD; CTSDMC**
Department of Biostatistics
University of Iowa
2400 UCC
Iowa City, Iowa 52242
Phone: 319-384-2833
Fax: 319-335-6535
E-mail: William-clarke@uiowa.edu

### PROJECT MANAGER

**Allison Priore, BS**
Project Manager
Division of Allergy, Immunology, and Transplantation
National Institute of Allergy and Infectious Diseases
5601 Fishers Lane, Room 6B24
Rockville, MD 20852
Phone: 240-627-3550
E-mail: priorea@niaid.nih.gov

### MEDICAL MONITORS

**Nancy Bridges, MD**
Chief, Transplantation Branch
Division of Allergy, Immunology, and Transplantation
National Institute of Allergy and Infectious Diseases
5601 Fishers Lane, Room 6B31
Rockville, MD 20892
Phone: 240-627-3535
E-mail: nbridges@niaid.nih.gov

**Thomas L. Eggerman MD, PhD**
Director Islet Transplantation Program
Division of Diabetes, Endocrinology and Metabolic Diseases
National Institute of Diabetes and Digestive and Kidney Diseases
6707 Democracy Blvd. Rm 697 MSC5460
Bethesda, MD 20892 (overnight delivery 20817)
Phone: 301-594-8813
Fax: 301-480-3503
E-mail: eggermant@extra.niddk.nih.gov

### SENIOR REGULATORY OFFICER

**Julia Goldstein, MD**
Senior Regulatory Affairs Officer
Division of Allergy, Immunology, and Transplantation
National Institute of Allergy and Infectious Diseases
5601 Fishers Lane, Room 7B29
Rockville, MD 20852
Phone: 240-627-3509
E-mail: goldsteinj@niaid.nih.gov

**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:**<br>CIT-08 | **Version/Date:**<br>Version 6.0 / April 25, 2017 |
| **IND:**<br>Exempt | **CIT Principal Investigators:**<br>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:**<br>*Extended Follow-Up after Islet Transplantation in Type 1 Diabetes* | |
| **Study Sponsors:**<br>The National Institute of Allergy and Infectious Diseases (NIAID)<br>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:<br>• Serum creatinine and calculated eGFR at each annual study visit<br>• Incidence of serious adverse events during the 12-month period preceding each annual study visit<br>• Insulin requirements during a one-week period preceding each annual study visit<br>• Incidence of severe hypoglycemic events during the 12-month period preceding each annual study visit<br>• HbA1c levels at each annual study visit<br>• All causes of mortality |
| **Inclusion Criteria** | 1. Participation in any of the following CIT parent studies: CIT02, CIT03, CIT04, CIT05, CIT06, and CIT07.<br>2. Willingness of participants to continue to use an approved method of contraception during and 4 months after study participation.<br>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.

# Table of Contents

## List of Tables



## List of Appendices

# 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.

# 15. REFERENCES

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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

**SITE PRINCIPAL INVESTIGATOR**

**Bernhard Hering, MD**
Director Islet Transplantation
University of Minnesota
Department of Surgery
420 Delaware St SE MMC 280
Minneapolis, MN 55455
Phone: 612-626-5735
Fax: 612-626-5855
E-mail: bhering@umn.edu

**SITE PRINCIPAL INVESTIGATOR**

**Ali Naji, MD, PhD**
J. William White Professor of Surgery
University of Pennsylvania Medical Center
4th Floor Silverstein Building
3400 Spruce Street
Philadelphia, PA 19104-4283
Phone: (215) 662-2066
Fax: (215) 662-7476
E-mail: Ali.Naji@uphs.upenn.edu

**SITE PRINCIPAL INVESTIGATOR**

**Camillo Ricordi, MD**
Professor of Surgery
Department of Surgery
University of Miami Miller School of
Diabetes Research Institute
1450 NW 10th Ave (R-134)0
Miami, FL, 33136
Phone: 305-243-6913
Fax: 305-243-4404
E-mail: cricordi@med.miami.edu

**SITE PRINCIPAL INVESTIGATOR**

**Nicole Turgeon, MD**
Department of Surgery
Division of Transplantation
Emory University
101 Woodruff Circle, Suite 5105-WMB
Atlanta, GA 30322
Phone: 404-727-3257
Fax: 404-712-4348
Email: nturgeo@emory.edu

**SITE PRINCIPAL INVESTIGATOR**

**Xunrong Luo, MD, PhD**
Assistant Professor of Medicine, Surgery, Microbiology and Immunology, Divisions of Nephrology and Organ Transplantation, Northwestern University Feinberg School of Medicine
303 East Chicago Avenue
Tarry Building 4-751
Chicago, IL 60611
Phone: 312-908-8147
Fax: 312-503-0622
Email: xunrongluo@northwestern.edu

**SITE PRINCIPAL INVESTIGATOR**

**Andrew Posselt, MD, PhD**
Associate Professor in Residence
University of California San Francisco
Department of Surgery
505 Parnassus Ave. Room M-896
San Francisco, CA 94143-0780
Phone: 415-353-1473
Fax: 415-353-8709
E-mail:
andrew.posselt@ucsfmedctr.org

**SITE PRINCIPAL INVESTIGATOR**

**Jose Oberholzer, MD**
Transplant Surgeon
Division of Transplantation, M/C 958
840 S. Wood Street, Suite 402
Chicago, IL 60612
Phone: 312-996-6771
Cell : 312-848-9749
Page: 877-5675240
Fax: 312-413-3483
Email: jober@uic.edu

**SITE PRINCIPAL INVESTIGATOR**

**Dixon Kaufman, MD**
Professor of Surgery
Chairman of Transplantation
University of Wisconsin –Madison
600 Highland Avenue
Madison, WI 53792
Phone: 608-265-6471
Fax: 608-262-6280
Email: kaufman@surgery.wisc.edu

**SITE PRINCIPAL INVESTIGATOR**

**James F. Markmann, MD, PhD**
Massachusetts General Hospital
Department of Surgery
55 Fruit St.
White Room 517
Boston, MA 02493
Tel: 617-643-4533
Fax: 617-643-4579
Email: jmarkmann@partners.org