CLINICAL ISLET TRANSPLANTATION (CIT)

PROTOCOL CIT-01

Open Randomized Multi-Center Study to Evaluate Safety and Efficacy of Low Molecular Weight Sulfated Dextran in Islet Transplantation

Version 7.0 / September 2, 2010

This clinical study is sponsored by the National Institute of Allergy and Infectious Diseases (NIAID) and the National Institute of Diabetes & Digestive & Kidney Diseases (NIDDK).

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Protocol	**Version/Date:**
CIT-01	7.0/ September 2, 2010
IND:	**CIT Principal Investigator:**
N/A	Olle Korsgren, M.D.

Short Title:

Safety and Efficacy of Low Molecular Weight Dextran Sulfate (LMW-DS) in Islet Transplantation

Study Sponsor:

National Institute of Allergy and Infectious Diseases (NIAID)
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 Street
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 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 Principal Investigator, I agree to conduct **"CIT-01: Open Randomized Multi-Center Study to Evaluate Safety and Efficacy of Low Molecular Weight Sulfated Dextran in Islet Transplantation "** I agree to carry out the study by the criteria written in the protocol and understand that no changes can be made to this protocol without written permission of the NIAID.

_____
Site Principal Investigator (Print)

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

Glossary of Abbreviations

Ab	Antibody
AE	Adverse Event
AIRglu	Acute Insulin Response to Glucose
ALT (SGPT)	Alanine Aminotransferase (serum glutamatepyruvate transaminase)
APC-R	Activated Protein C Resistance
APTT	Activated Partial Thromboplastin Time
AST (SGOT)	Aspartate Aminotransferase (serum glutamicoxaloacetic transaminase)
ATG	Rabbit Anti-Thymocyte Globulin
BG	Blood Glucose
BID	Twice Daily
BMI	Body Mass Index
Boost ®	Liquid Meal Replacement Drink
BW	Body Weight
C3a	Complement Activation Fragment
CBC	Complete Blood Count
CFR	Code of Federal Regulations
CGMS	Continuous Glucose Monitoring System ®
COIMS	Council for International Organization of Medical Sciences
CIT	Clinical Islet Transplantation Consortium
CITR	Collaborative Islet Transplant Registry
CIT-TCAE	CIT Terminology Criteria for Adverse Events
CMV	Cytomegalovirus
CNI	Calcineurin Inhibitor
CPGCR	C-Peptide to Glucose, Creatinine Ratio
CRF	Case Report Form
CTL	Cytotoxic T Lymphocyte
CXR	Chest x-ray
DAIT	Division of Allergy, Immunology, and Transplantation
DCC	Data Coordinating Center
DCCT	Diabetes Control and Complications Trial
DIC	Disseminated Intravascular Coagulation
DMSO	Dimethylsulfoxide
DSMB	Data Safety Monitoring Board

EBV	Epstein-Barr Virus
EC	Ethics Committee
ECG or EKG	Electrocardiogram
EDTA	Ethylenediaminetetraacetric Acid
EMEA	European Medicines Agency
EU-GMP	European Union Good Manufacturing Practices
FDA	Food and Drug Administration
FSIGT	Insulin Modified Frequently Sampled Intravenous Glucose Tolerance Test
GCP	Good Clinical Practice
G-CSF	Granulocyte Colony Stimulating Factor
GFR	Glomerular Filtration Rate
GMP	Good Manufacturing Practice
Hb	Hemoglobin
HbA_{1C}	Glycosylated Hemoglobin
HBsAg	Hepatitis B Surface Antigen
HCV Ab or anti-HCV	Hepatitis C Antibody
HDL	High Density Lipoprotein
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HR	Heart Rate
HYPO	Ryan Hypoglycemia Severity Score
IBMIR	Instant Blood Mediated Inflammatory Reaction
ICH	International Conference on Harmonization
IEQ	Islet Equivalent
Ig	Immunoglobulin
IL-2	Interleukin 2
INR	International Normalized Ratio
IRB	Institutional Review Board (also Ethics Committee)
IRI	Immunoreactive Insulin
ITN	Immune Tolerance Network
ITT	Intent-to-Treat
IU	International Units
IV	Intravenous

JDFI	Juvenile Diabetes Foundation International
kPa	Kilo-Pascal (pressure)
LDL	Low Density Lipoprotein
LFTs	Liver Function Tests
LI	Lability Index
LMW-DS	Low Molecular Weight Sulfated Dextran
MAGE	Mean Amplitude Glycemic Excursion
MHC	Major Histocompatibility Complex
MMF	Mycophenolate Mofetil
MMTT	Mixed-Meal Meal Tolerance Test
MPA-AUC	Mycophenolic Acid- Area Under the Curve
NCI-CTCAE	National Cancer Institute- Common Toxicity Criteria for Reporting Adverse Events
NIAID	National Institute of Allergy and Infectious Diseases (United States)
NIDDK	National Institute of Diabetes & Digestive & Kidney Diseases (United States)
NIH	National Institutes of Health (United States)
PBMC	Peripheral Blood Mononuclear Cells
PCR	Polymerase Chain Reaction
PET	Positron Emissions Tomography
PI	Principal Investigator
pit-hGH	Pituitary Growth Hormone
PML	Progressive Multifocal Leukoencephalopathy
PNF	Primary Non-Function
PO	Orally
PRA	Panel Reactive Antibodies
PT	Prothrombin Time
PTT	Partial Thromboplastin Time
PT-INR	Prothrombin- International Normalized Ratio
PTLD	Post Transplant Lymphoproliferative Disease
QD	Every Day
QOL	Quality of Life
RNA	Ribonucleic Acid
SAE	Serious Adverse Event
SAEC	Safety Adverse Event Coordinator

SAP	Statistical Analysis Plan
SC	Subcutaneous
SGOT (AST)	Serum Glutamic-oxaloacetic Transaminase
SGPT (ALT)	Serum Glutamate Pyruvate Transaminase
SMT	Standard Medical Treatment
SOP	Standard Operating Procedure
SPC	Summary of Product Characteristics
SSL	Secure Sockets Layer
SUSARs	Suspected Unexpected Serious Adverse Reactions
T1D	Type 1 Diabetes
TAT	Thrombin-Antithrombin
TB	Tuberculosis
TCAE	Terminology Criteria for Adverse Events
TF	Tissue Factor
ULN	Upper Limit of Normal
UNOS	United Network for Organ Sharing
WHO	World Health Organization

Study Definitions Page

Control Arm- State of the Art	The study treatment group randomized to receive protocol specified immunosuppression without LMW-DS, including anticoagulative treatment with Heparin with the islet infusion.
Experimental Arm- Low Molecular Weight Sulfated Dextran	The study treatment group randomized to receive protocol specified immunosuppression with Low Molecular Weight Sulfated Dextran (LMW-DS), and excluding Heparin treatment.
Full Islet Graft Function	Islet transplant recipients are considered to have full islet graft function if all of the following criteria are met: o Titrated off insulin therapy for at least 1 week (7 consecutive days) with the last day within the day 75 and day 365 windows; o One HbA1c level, one fasting serum glucose level, and 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); o HbA1c <7.0% or a ≥2.5% decreased from baseline; o 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 capillary glucose should not exceed 180 mg/dl (10.0 mmol/L) at 90 minutes during the MMTT; o Fasting serum glucose level ≤ 126 mg/dL (≤7.0 mmol/L) from central lab results; 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; o At least one MMTT fasting or stimulated c-peptide ≥0.5 ng/ml.
Graft Failure	Islet allograft failure will be defined as absence of insulin production by transplanted islets, as evidenced by absence of c-peptide <0.1nmol/L (<0.3 ng/mL). This will be determined by: (1) c-peptide <0.1nmol/L (<0.3 ng/mL) on random testing, followed by (2) c-peptide <0.1nmol/L (<0.3 ng/mL) at baseline, and at 60 and 90 minutes after MMTT. C-peptide levels obtained in the course of the MMTT levels will be run at the core laboratory in Seattle, WA. Subjects with graft failure do not need to complete the day 75 metabolic assessment.
Immune Sensitization	Defined by detecting anti-HLA antibodies not present prior to transplantation
Insulin Dependent	Islet transplant recipients who do not meet the criteria for full islet graft function 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 full islet graft function but have either a basal or stimulated C-peptide level ≥0.1nmol/L (≥0.3ng/L).
Primary Non-Function	Graft failure that occurs between 3-7 days post-transplant. Participants with graft failure do not need to complete the day 75 metabolic assessments.
Progressive Renal Dysfunction	A creatinine rising above 2.0 mg/dL (177 μmol/L) with calcineurin inhibitor trough levels within maintenance levels.

Protocol Eligible	After completion of the screening assessments required to confirm eligibility, the subject is considered "protocol eligible".
Severe Hypoglycemic Event	A severe hypoglycemic event is defined as an event with one of the following symptoms: memory loss; confusion; uncontrollable behavior; irrational behavior; unusual difficulty in awakening; suspected seizure; seizure; loss of consciousness; or visual symptoms, in which the subject was unable to treat him/herself and which was associated with either a blood glucose level < 54 mg/dL (3.0 mmol/L) or prompt recovery after oral carbohydrate, IV glucose, or glucagon administration.
Wait List	Protocol eligible subjects who have been listed for islet transplant on the Nordic islet transplant wait list.

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Protocol Synopsis

Title	Open Randomized Multi-Center Study to Evaluate Safety and Efficacy of Low Molecular Weight Sulfated Dextran in Islet Transplantation
Short Title	Safety and Efficacy of Low Molecular Weight Dextran Sulfate (LMW-DS) in Islet Transplantation
Clinical Phase	Phase 2
Study Sponsors	National Institute of Allergy and Infectious Disease (NIAID) National Institute of Diabetes & Digestive & Kidney Disease (NIDDK)
Activation Date / Accrual Period	June 2008 (36 month accrual period)
Follow Up Period	12 months from time of the final islet transplantation
Accrual Objective	36 subjects; 2 Study Arms • 18 Subjects randomized to protocol immunosuppression without LMW-DS • 18 Subjects randomized to protocol immunosuppression and LMW-DS
Study Design	Open, Randomized (1:1), Multi-Center, Phase II study in islet transplantation recipients randomized to either Low Molecular Weight Sulfated Dextran (LMW-DS) or "State of the Art" therapy.
Treatment Description	Low Molecular Weight Sulfated Dextran (LMW-DS 20 mg/mL) is manufactured by Apoteket AB, Produktion & Laboratorier, Umeå, Sweden, and will be produced in compliance with EU-GMP. LMW-DS will be administered as a bolus of 4.5mg LMW-DS / kg to subjects randomized to protocol immunosuppression and LMW-DS. 1) One-third (1.5 mg/kg BW) prior to transplantation, intraportal. 2) Two-thirds (3.0 mg/kg BW) with the islet preparation, intraportal. There will be a continuous infusion of LMW-DS targeting an APTT of 150±10s directly after the islet infusion, and maintained for 5 hours. The infusion rate will be based on APTT immediately after the islet infusion. This infusion should be given intraportally. If technical problems occur, the remaining dose can be given through a peripheral vein. The APTT should be analyzed according to the Instructions for Administration of LMW-DS worksheet (available on the CIT website: www.isletstudy.org), or more often if problems to adjust the infusion are encountered. Patients randomized to protocol immunosuppression without LMW-DS will receive heparin 70 U/kg body weight of recipient, with the islet infusion, followed by a continuous intraportal infusion of heparin targeting an APTT of 50±10s for the next 5 hrs. The protocol includes the following induction drugs; Rabbit anti-thymocyte globulin (ATG, Thymoglobulin®) for the initial islet transplant, for a second or third transplantation a monoclonal IL-2 receptor blocker, Basiliximab (Simulect ®), replaces Rabbit anti-thymocyte globulin. In addition, the protocol contains one cell proliferation inhibitor (CellCept® *or* Rapamune®) and one calcineurin inhibitor (Prograf® *or* Sandimmune Neoral®). The protocol includes one Anti-Inflammatory agent, Etanercept (Enbrel®).
Primary Endpoint	The level of stimulated c-peptide at 90-minutes derived from the mixed-meal tolerance test (MMTT) at 75±5 days following the first islet infusion.
Secondary Endpoints	1. TAT complexes and C-peptide immediately prior to islet infusion, when 125 mL is left in infusion bag (before rinsing), and at 0, 15, 60, 180, 270, 360 minutes after completion of islet transplant, and 24h after completion of islet transplant; 2. Conduction Velocity and RR interval at screening, and month 12 after first and last islet transplant; 3. Portal pressure before and 15 minutes after completion of islet transplantation; 4. Liver enzymes (ALT, AST), one and seven days after all islet transplantation(s); 5. Quality of life Questionnaires (DTSQc, SF36), 1 year after the first and last islet transplantation, to be compared

with the same test done as a part of the screening prior to being put on the waiting list (DTSQs).

6. Percentage of administered radioactivity found in liver after the start of islet transplantation. Determined through the use of PET/CT (protocol section 9.1.1.11)

Efficacy Secondary Endpoints

At 75 ± 5 days following the <u>first</u> infusion:

- The percent reduction in insulin requirements
- HbA1c
- Mean amplitude of glycemic excursions (MAGE)
- Glycemic lability index (LI)
- Clarke hypoglycemia awareness score
- Ryan hypoglycemia severity (HYPO) score
- Basal (fasting) glucose and c-peptide and 90-min glucose derived from the mixed-meal tolerance test (MMTT)
- β-score
- C-peptide:glucose·creatinine ratio (CPGCR)
- Acute insulin response to glucose (AIR_{glu}), insulin sensitivity, and disposition index derived from the insulin-modified frequently-sampled intravenous glucose tolerance (FSIGT) test
- Glucose variability and hypoglycemia duration derived from the continuous glucose monitoring system® (CGMS)
- The proportion of subjects with full islet graft function.

At 365 ±14 days following the <u>first and final</u> islet infusion:

- The proportion of subjects with full graft function
- The proportion of subjects with an HbA1c <7.0% and free of severe hypoglycemic events from day 28 through day 365.
- 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
- Acute insulin response to glucose (AIR_{glu}), insulin sensitivity, and disposition index derived from the insulin-modified frequently-sampled intravenous glucose tolerance (FSIGT) test
- Glucose variability and hypoglycemia duration derived from the continuous glucose monitoring system® (CGMS)
- The proportion of subjects receiving a second islet infusion
- The proportion of subjects receiving a third islet infusion

Full Islet Graft Function Definition

Islet transplant recipients are considered to have full islet graft function if all of the following criteria are met:

- Titrate off insulin therapy for at least 1 week (7 consecutive days) with the last day within the day 75 and 365 day windows;
- One HbA1c level, one fasting serum glucose level, and 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 <u>≥</u>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 capillary glucose should not exceed 180 mg/dL (10.0 mmol/L) at 90 minutes during the MMTT;
- Fasting serum glucose level ≤ 126 mg/dL (<u>≤</u>7.0 mmol/L) from central lab results; 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 <u>≥</u> 0.5 ng/ml.

Safety Secondary Endpoints

At 75 ± 5 and 365 ± 14 days following the <u>first</u> islet infusion:

- The incidence and severity of adverse events related to the islet infusion procedure including: bleeding (>2 g/dl (20g/L) decrease in hemoglobin concentration); segmental portal vein thrombosis; biliary puncture; wound complication (infection or subsequent hernia); and increased transaminase levels (> 5 times ULN)
- The incidence and severity of adverse events 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.

Safety

- The incidence of immune sensitization defined by detecting anti-HLA antibodies not present prior to transplant.
- The incidence of a change in the immunosuppression drug regimen.

Secondary Endpoints Continue…

At 365 ± 14 days following the first islet infusion:

- The incidence of worsening retinopathy as assessed by change in retinal photography from pre-transplant to 365 ± 14 days following the first islet infusion. If pupil dilation is not possible, then ophthalmologic exams can be substituted.

Inclusion Criteria For Study Enrollment

Subjects must meet all of the following criteria to be considered eligible for participation in the study:

1. Patients between 18 to 65 years of age.
2. Subjects who are able to provide written informed consent and comply with the procedures of the study protocol.
3. Clinical history compatible with type 1 diabetes with onset of disease at < 40 years of age and insulin-dependence for ≥ 5 years at the time of enrollment, and a sum of patient age and insulin dependent diabetes duration of ≥28.
4. Absent stimulated C-peptide <0.3ng/ml [0.099 nmol/L] 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 90min after the start of consumption.
5. 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 previous 12 months prior to enrollment.
6. At least one episode of severe hypoglycemia, 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, intravenous glucose, or glucagon administration, in the 12 months prior to study enrollment.
7. At least one of the following:
   a. 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 12 months prior to randomization;
   b. 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 $mmol/L^2/hr \cdot wk^{-1}$) during the screening period and within the last 6 months prior to randomization;
   c. A composite of a Clarke score of 3 or more or a HYPO score greater than or equal to the 75th percentile (423) in combination with a LI greater than or equal to the 75th percentile (329) during the screening period and within the last 12 months prior to randomization.

Exclusion Criteria For Study Enrollment

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

1. Known IgE mediated allergy to antibiotics and antifungal medications (ciprofloxacin, gentamycin, and amfotericin B) used in the culture medium.
2. Known hypersensitivity to dextran.
3. Body mass index (BMI) >30 kg/m^2
4. Insulin requirement of > 1.0 U/kg/day.
5. HbA1c >10%.
6. Untreated proliferative diabetic retinopathy.
7. Blood Pressure SBP >160mmHg or DBP >100mmHg.
8. Measured glomerular filtration rate (GFR) using 51Cr-EDTA, 99technetium-DPTA, or iohexol <80 ml/min/1.73 m^2. The absolute (raw) GFR value will be used for subjects with body surface areas >1.73 m^2.
9. Presence or history of macroalbuminuria (>300mg/g creatinine).
10. Presence or history of panel-reactive anti-HLA antibodies >80% by flow cytometry. Subjects with panel reactive anti-HLA antibodies above background but ≤ 80%, can be included if the antigen specificity of the antibodies can be determined for future avoidance; however, if the antigen specificity of the antibodies cannot be determined they will be excluded.
11. 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.

12. Active infection including hepatitis B, hepatitis C, or HIV .
13. Negative screen for Epstein - Barr Virus (EBV) by IgG determination.
14. Any history of malignancy except for completely resected squamous or basal cell carcinoma of the skin.
15. Known active alcohol or substance abuse.
16. Baseline Hgb below the lower limits of normal at the local laboratory; lymphopenia (<1,000/μL), neutropenia (<1,500/μL), or thrombocytopenia (platelets <100,000/μL).
17. Homocygotic Activated Protein C Resistance (APC-R).
18. History of hypercoagulability disorder or coagulopathy or international normalized ratio (INR) > 1.5.
19. Known history of severe co-existing cardiac disease, characterized by ***any one*** of the following 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%.
20. Consistently abnormal liver function tests at the time of study entry. SGOT (AST), SGPT (ALT), Alk Phos or total bilirubin, with values >1.5 times normal upper limits on two consecutive measurements > 2weeks apart.
21. Acute or chronic pancreatitis.
22. Patients with active peptic ulcer disease, symptomatic gallstones or a history of portal hypertension.
23. Severe unremitting diarrhea, vomiting or other gastrointestinal disorders potentially interfering with the ability to absorb oral medications.
24. Receiving treatment for a medical condition requiring chronic use of systemic steroids, except for the use of ≤ 5mg prednisone daily, or an equivalent dose of hydrocortisone, only for physiological replacement.
25. Treatment with any anti-diabetic medication, other than insulin, within 4 weeks of enrollment.
26. Use of any investigational agents within 4 weeks of enrollment.
27. Administration of live attenuated vaccine(s) within 2 months of enrollment.
28. Patients with any condition or any circumstance that in the opinion of the investigator would make it unsafe to undergo an islet transplant.
29. Treatment with any immunosuppressive regimen at the time of enrollment.
30. A previous islet transplant.
31. 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.

1. BACKGROUND AND RATIONALE

1.1. Background

1.1.1. Diabetes and Transplantation

Type 1 Diabetes (T1D) is an autoimmune disease where destruction of the insulin producing pancreatic β cells occurs, leading to severely dysregulated glucose homeostasis. Despite the effectiveness of insulin therapy in allowing these patients to survive, the imperfect control of blood glucose excursions common with insulin injections eventually results in vascular complications in many. The Diabetes Control and Complications Trial (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.(Shamoon, Duffy et al. 1993) However, this degree of control can be impossible to achieve in many patients despite modern insulin analogs and delivery systems,(Hirsch 2005) and also leads to life threatening episodes of insulin-induced hypoglycemia.(Cryer, Davis et al. 2003)

Kidney transplantation has been performed in patients with diabetes as their underlying illness since the 1970s. The long-term results of these transplantations are inferior compared to transplantations in patients with other causes of uremia. This is thought to be due to different diabetes complications in the transplanted kidney and other organs.

Transplantation of combined kidney-pancreas is being performed at most of the participating centers within the Nordic Network. The first transplantations were done in the 1970s, then in the late 1980s about 40 procedures were performed per year in Sweden and Norway. When successful this combined transplantation normalizes the patient's glucose metabolism without need for exogenous insulin therapy. The major drawback with the procedure is postoperative complications caused, ironically enough, by the exocrine tissue transplanted. Infections and pancreatitis occur frequently. Results of pancreas transplantation alone in diabetics without uremia have been inferior compared to the combined procedure.

Islet transplantation as a cure for diabetes has been explored for 35 years. Three hundred and five islet transplantations were performed worldwide from 1974-1996. Thirteen (2.3%) of these patients became independent of insulin and stayed so more than 12 months(Brendel, Hering et al. 2001).

In July 2000 Shapiro et al (Shapiro, Lakey et al. 2000) reported seven consecutive cases where the patients had become insulin independent. Later reports have shown that 80% of their patients were still off insulin after twelve months and 50% after 36 months (Shapiro 2004). These transplantations were performed in diabetics with brittle diabetes but without uremia. The procedure was repeated up to four times until the patient became insulin independent. A total of more than 12,000 Islet Equivalents (IEQ)/ kg BW was needed to achieve insulin independence. An immunosuppressive protocol without corticosteroids was used.

A "Nordic Network for Clinical Islet Transplantation" was formed in 2000. In the first study we transplanted Type 1 diabetics who had previously received a kidney graft. Forty-four patients have been transplanted at six centers (Lundgren, Korsgren et al. 2005). The patient with the longest "full islet graft function" has been free of exogenous insulin with perfect metabolic function for more than four years following his last transplantation.

The fact that there is a need for islets from more than one pancreas and that treated patients off insulin in several cases have had to be reinstalled with exogenous insulin suggests that the grafted functional islet mass is inadequate.

New methods to assess the islet graft in the immediate post transplantation period are urgently needed. We have used dynamic Positron Emissions Tomography (PET) examination to visualize the peritransplant phase of clinical pancreatic islet transplantation (Eich et al. New England Journal of Medicine, June 28, 2007). Isolated islets were allowed to internalize [^{18}F] FDG just prior to transplantation. Preliminary results show that labeled islets could readily be visualized after intraportal infusion, with a heterogeneous distribution in the liver. The PET scan also indicated that almost half of the transplanted islets were lost within the first few minutes after transplantation. The PET/CT technology is readily available and allows real-time quantitative and qualitative measurement of islet survival and distribution after transplantation.

1.1.2 Background of the Planned Study in Type 1 Diabetes

A thrombotic/inflammatory reaction is elicited when islets come in direct contact with ABO compatible blood. This is characterized by a rapid binding, and activation of platelets to the islet surface and activation of the coagulation and complement systems. Within 15 minutes leukocytes are found infiltrating the islets. After an hour most of the islets are infiltrated by numerous leukocytes, consisting of both monocytes and granulocytes, resulting in disruption of islet integrity and islet loss. This innate immune response has been named the instant blood-mediated inflammatory reaction (IBMIR).

The occurrence of IBMIR was further established in clinical islet transplantation. Fifteen minutes after the islet infusion there was a peak in thrombin-antithrombin complex levels reflecting an ongoing clotting process. Slightly displaced, the C-peptide increased indicating that the islet cells were damaged (fig 1). Also, FVIIa-AT complexes were generated soon after infusion. These complexes peaked after 60 minutes, underscoring the involvement of the tissue factor (TF) pathway in the IBMIR. Tissue factor (TF) expressed by the islet cells seems to be the main trigger of IBMIR.

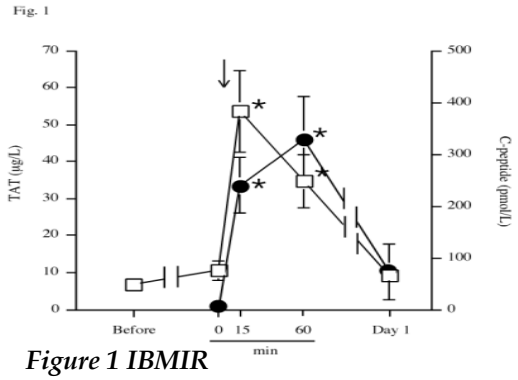
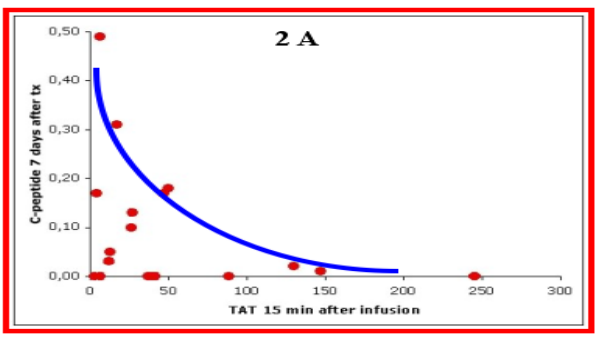
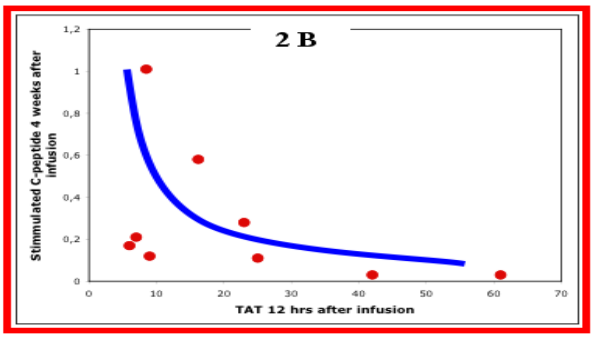


Figure 1 IBMIR

IBMIR has a large impact on the outcome of clinical islet transplantation. The detrimental effects of IBMIR provide an explanation for the relatively low success rate of clinical islet transplantation and an explanation for the need of islets from several donors in order to obtain normoglycemia(Shapiro, Lakey et al. 2000; Ryan, Lakey et al. 2001). The C-peptide levels obtained in patients after transplantation reflect the function of the transplanted islets in the liver. Figures 2A and B show the thrombin-antithrombin (TAT) values plotted against the C-peptide values after the transplantation in patients transplanted within the Nordic Network (fig 2A) as well as results obtained in patient samples from the Shapiro group (Brendel, Hering et al. 2001) (fig 2B). The results show that high TAT levels were never combined with good islet graft function, and vice versa. This relationship suggested that an immediate, strong IBMIR was destructive to the transplanted islets. There are several possible explanations for this result:





One is that large clot formation in the wide branches of the portal tree may prevent the islets from reaching the smaller vessels causing the islets to succumb as a result of poor nutrition and hypoxia in the clot. Another is that the IBMIR has a direct destructive effect on the islets.

1.2 Rationale

1.2.1 Rationale for the Trial

Our current goal is to come to a situation where islets from one pancreas is routinely enough to give a long lasting cure to one diabetic patient. According to our notion, the problem with IBMIR must be overcome to achieve this goal mainly due to two reasons:

1. The loss of islets due to IBMIR and as a consequence, the need of islets from several donors to achieve normoglycemia.

2. The strong signal (cf. the adjuvant effect in immunization / vaccination) IBMIR sends to the specific immune response (i.e. allogenic rejection and autoimmunity).

The mechanisms triggering the IBMIR in clinical islet transplantation have been defined and clinically applicable inhibitors have been identified. The present proposal aims to translate these findings to clinical islet transplantation. The clinical study proposal builds on the following approach:

- The identification of low molecular weight dextran sulfate (LMW-DS) as an extremely powerful inhibitor of the IBMIR. LMW-DS effectively inhibited platelet activation and activation of both the complement and coagulation cascades as well as the infiltration of leukocytes into the islets.
- The proposed clinical study aims to develop a clinically applicable protocol that will enable us to "cure" patients with Type 1 diabetes using islets from only one human pancreas.

In summary, isolated islets will be dissolved in LMW-DS at the time of transplantation in an attempt to minimize the adverse effects of the IBMIR on the clinical outcome of islet transplantation.

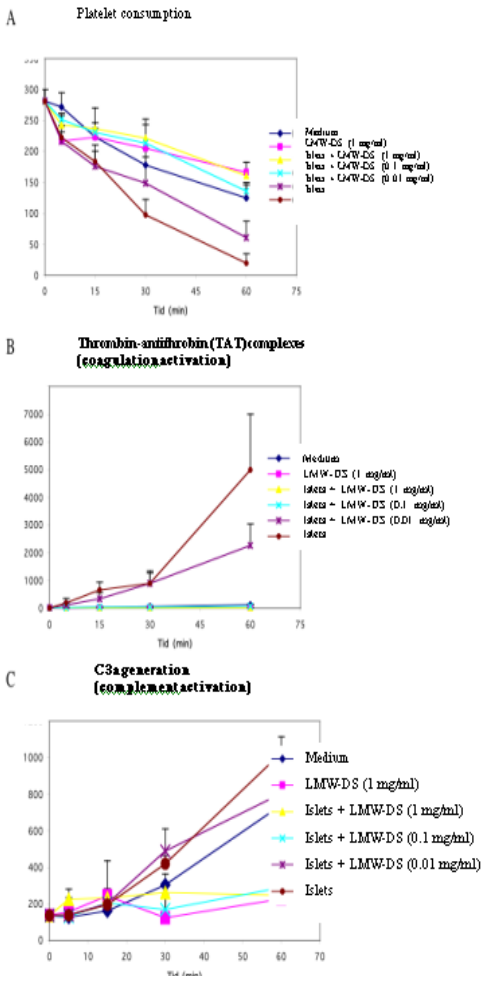


Figure 3: Efficacy of LMW-DS

1.2.2 Rationale for Selection of Study Regimen

Low Molecular Weight Dextran Sulfate

It has already been shown that low molecular weight dextran sulfate (LMW-DS, 5 kDa) inhibits activation of the complement cascade and contact activation of the coagulation system(Wuillemin, te Velthuis et al. 1997; Fiorante, Banz et al. 2001). The substance has also been shown to have direct effects on cell interactions, such as inhibition of E-selectin-mediated adhesion of neutrophils to endothelial cells(Matsumiya, Yamaguchi et al. 1999). We have demonstrated the efficacy of LMW-DS in preventing the IBMIR (Goto, Groth et al. 2004) (Fig. 3). Already at a dose of

0.1 mg/mL LMW-DS totally prevented the IBMIR in human blood. Apart from IBMIR, LMW-DS has also been shown to exert a protective effect on intrahepatic islet grafts *in vivo*. Culturing islets in LMW-DS did not adversely affect islet function at concentrations ranging from 0.01 to 1 mg/mL. Moreover, LMW-DS has already been used in several clinical studies. LMW-DS is a potent inhibitor of both coagulation and complement activation. Unlike high molecular weight DS it does not activate the fibrinolytic system (Goto, Groth et al. 2004). LMW-DS has been tried clinically both as an anti-coagulant combination with kallikrein in stroke patients and as an anti-viral agent for treatment of HIV(Fujishima, Omae et al. 1986; Flexner, Barditch-Crovo et al. 1991; Hiebert, Wice et al. 1999).

In our *in vitro* studies we have seen that blood concentrations between 10 and 100 µg/mL yield a substantial reduction in the IBMIR. In order to minimize the risk of bleeding while producing the optimal effect on the IBMIR, islets, dissolved in LMW-DS, will be infused just after an infusion of a bolus dose of LMW-DS. The islets will be followed by an intraportal infusion of LMW-DS over 5 hours. In a recently performed phase I study on normal subjects (see IB) we confirmed the results of a study made by Lorentsen and coworkers (Lorentsen, Hendrix et al. 1989) in which they demonstrated that APTT can be used as a surrogate parameter of LMW-DS concentration. The phase I study was also performed to gather pharmacokinetics data, to monitor safety and tolerability, and to optimize the proposed administration protocol for clinical islet transplantation. The highest systemic concentration of LMW-DS during the 5-hours continuous infusion that was tested was 30µg/ml is our target level. As a control 5000IU of heparin, which is routinely given intraportally during clinical islet transplantation, was administered IV to some normal subjects. The APTT increased far above the levels reached by LMW-DS. Our experience is that LMW-DS increases the risk of bleeding to a lesser degree than the routinely used concentration of heparin used in clinical islet transplantation. (Flexner, Barditch-Crovo et al. 1991).

1.2.3 Rational for Selection of Induction Therapy

The rationale for anti-thymocyte globulin (ATG) induction immunosuppression includes prevention of autoimmune recurrence in transplanted islets via deletion of autoreactive memory cells, prophylaxis of islet allorejection, avoidance of the use of calcineurin inhibitors (CNIs) in the immediate post-transplant period, induction of regulatory T cells with reduced requirements for maintenance immunosuppression, and attenuation of non-specific 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 (58, 59). Thymoglobulin® induction therapy achieved rejection-free allograft survival in 96% of the patients. The incidence of cytomegalovirus (CMV) disease in the first year was 12.5%, and no patient developed post-transplant lymphoproliferative disease (PTLD). ATG is known to contain a variety of anti-adhesion molecule antibodies (60). 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 non-specific inflammation and reperfusion injury and may explain the 1% incidence of delayed graft function in kidney recipients (58, 60-63). 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 (64).

The resistance of islet-directed autoimmune responses to conventional immunosuppressive drugs (65-69) 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 (70). 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 (71, 72). Clinical evidence also indicates that destructive anti-islet autoimmunity persists for decades after manifestation of T1D (66, 73, 74) and that type 1 diabetic individuals with long disease duration do not spontaneously anergize their autoreactive effector Th1 cells and/or restore Th2 or other regulatory T cell function. Accordingly, reprogramming the recipient's immune system seems to be of paramount importance if autoimmune recurrence in transplanted islets is to be prevented.

Maki *et al*. demonstrated that immunotherapy of non-obese diabetic (NOD) mice with ALS after development of overt autoimmune diabetes leads to long-lasting abrogation of autoimmunity(75). 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 (76). Of 7 islet-after kidney recipients treated in Brussels, only the 3 patients who had received ATG as induction immunosuppressive therapy during the first 10 days following their previous kidney transplant showed long-term islet graft survival. Furthermore, according to an analysis performed by the International Islet Transplant Registry on all 50 insulin-independent, type 1 diabetic islet allograft recipients transplanted through 1999, 23 had received single-donor islet transplants, and 19 of those 23 had received anti-thymocyte or anti-lymphocyte globulin for induction immunosuppression and 1 had received ATG at the time of a previous pancreas transplant (77). It is conceivable that the need for 2-3 donor pancreata as a source of islets in the Edmonton experience reflects the inability of the induction immunotherapy to completely abrogate the anti-islet autoimmune response. Even a low level of persistent autoimmunity may interfere with the function of transplanted islets via pro-inflammatory cytokine mediated inhibition of insulin secretion. The ATG immunotherapy as proposed in this trial may be advantageous due to the deletion/inhibition of anti-islet directed autoreactive T cells.

There are two published reports of steroid-free transplantation with Thymoglobulin®. Birkeland reported on 68 kidney transplant recipients treated with steroid-free immunosuppression using an initial 10-day ATG induction and maintenance therapy with cyclosporine and mycophenolate mofetil. No steroids were given at any time. After an observation for up to 2.5 years (median 488 days, range 127-945 days), 66 patients (one died from sepsis after six months and one died from peritonitis after returning to dialysis) were alive and well, 64 grafts were functioning well, hemolytic-uremic syndrome recurred in one graft, one graft had to be removed for non-compliance, and two patients returned to dialysis after chronic rejection. These investigators observed only 10 acute rejections (15%) (78). 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. CMV infection was diagnosed in eight patients. All but one patient tolerated the ATG course well (79). These two studies indicate that ATG can be used safely and effectively without concomitant steroid administration.

The total ATG dose to be administered is 6 mg/kg. This dose is based on studies performed at Washington University in St. Louis (80, 81). 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 (58). 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 (77). 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, the 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 (5).

The immunosuppression regimen for the subsequent islet transplants will be identical to the regimen for the initial islet transplant with the exception of the Thymoglobulin®. Basiliximab will be used instead of Thymoglobulin® for all subsequent islet transplants. The safety and efficacy of basiliximab has previously been documented in multi-center trials in renal transplantation. When added to therapy with cyclosporine, Azathioprine, and prednisone, 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 basiliximab and the placebo group with respect to infectious complications or cancers[82]

1.2.3 Rational 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 (Cryer, Fisher et al. 1994).
Neuroglycopenia can cause social embarrassment, and even lead to ostracism or be mistaken for disorderly or unlawful behavior(Cryer, Fisher et al. 1994). The more distressing the severe hypoglycemic episode, the greater the psychological fear of hypoglycemia(Irvine, Cox et al. 1992). The threat and fear of severe hypoglycemia can significantly discourage patients and health care providers from pursuing intensive insulin therapy and can therefore be a major but unrecognized impediment to achieving euglycemia(Irvine, Cox et al. 1991; Cryer, Fisher et al. 1994).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 (Pramming, Thorsteinsson et al. 1991).

Ryan et al. documented the absence of episodes of severe hypoglycemia in 12 successful islet transplant recipients (median follow-up, 10.2 months) (Ryan, Lakey et al. 2001)whose diabetes was complicated by recurrent episodes of severe hypoglycemia pretransplant. This would suggest that hypoglycemia associated autonomic failure associated with defective counterregulation and impaired sympathodrenal responses is not just due to recurrent hypoglycemia. After a sustained period without any hypoglycemia most patients post islet transplant still had defective responses to hypoglycemia. The absence of clinically significant hypoglycemia post islet transplant despite the persistent defect in counterregulation in most subjects demonstrates the dominance of the absence of glucose regulated insulin secretion in the pathogenesis of severe hypoglycemia. Correction of this can only currently be attained with transplantation of beta cell tissue.

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 or with 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 Investigational Product

The manufacturer of the drug substance LWM-DS (Mw 5000Da) is pK Chemicals A/S (Köge, Denmark). The final product of Low Molecular Weight Sulfatated Dextran (LMW-DS 20 mg/mL) is manufactured by Apoteket AB, Produktion & Laboratorier (Umeå, Sweden) with an average molecular mass 5000 Da. LMW-DS for the clinical trial will be produced in compliance with EU-GMP.

1.4 Concomitant Immunosuppressive Medications

The protocol includes the following induction drugs; Rabbit anti-thymocyte globulin (ATG, Thymoglobulin®) for the initial islet transplant, for a second or third transplantation a monoclonal IL-2 receptor blocker, Basiliximab (Simulect®), replaces Rabbit anti-thymocyte globulin. In addition, the protocol contains one cell proliferation inhibitor (CellCept® *or* Rapamune®) and one calcineurin inhibitor (Prograf® *or* Sandimmune Neoral®). The protocol includes one Anti-Inflammatory agent, Etanercept (Enbrel®).

If there are no contraindications the patient should receive maintenance immunosuppression of full dose CellCept and low dose Prograf. The immunosuppressive treatment should be adapted to side effects and tolerability. A clinically motivated switch of immunosuppressive medications, among the drugs listed above, is not to be seen as a protocol deviation.

1.5 Known and Potential Risks to Human Participants

1.5.1 Low Molecular Weight Sulfated Dextran

Prolonged oral administration and infusion of LMW-DS (molecular mass 8 kDa) have been associated with thrombocytopenia after 3-7 days. The thrombocytopenia led to bleeding complications (e.g. epitaxis) in some cases. In none of these studies, however, were serious bleeding events reported. Reversible alopecia was seen in 50% of the patients who received the LMW-DS for more than 8 days. In the present study, LMW-DS will not be used for more than 6 hours and side effects of the kind reported above are therefore not expected. This notion has been confirmed in a recently performed phase I study with a treatment protocol, targeting an APTT of 150±10s was tested. This concentration did not affect platelet count or function and did not increase the risk of bleeding. No other adverse events were reported. Immobilized dextran sulfate has been used to treat patients with hyperlipidemia by plasmapheresis (Thompson 2003). Anaphylactoid reactions with hypotension have been reported in patients treated with ACE-inhibitors due to activation of the contact system, therefore on the morning of the islet transplant, ACE inhibitors should not be administered to the participant. Unlike high molecular weight dextran sulfate, however, soluble LMW-DS does not activate the contact system and is therefore not anticipated to produce this side effect.

1.5.2 Immunosuppression Medications

The risks of immunosuppression treatment are well understood and apply to subjects in this clinical trial. These risks can be mitigated by dose adjustments, antihypertensives, and prophylactic antibiotics. All agents listed below are considered standard of care in islet transplantation.

1.5.2.1 RABBIT ANTITHYMOCYTE GLOBULIN (THYMOGLOBULIN®)

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

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

Published results of the use of Thymoglobulin® in clinical and experimental islet transplantation are limited to relative small cohorts. Hirshberg *et al.* described the successful role of rabbit ATG and sirolimus in reducing rejection of islet allografts in primates, with no evidence of direct islet toxicity from Thymoglobulin®(Hirshberg, Preston et al. 2003). 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(Hering, Kandaswamy et al. 2005). 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[12]. Studies comparing risk profiles between Thymoglobulin® and Basiliximab® have shown relatively small differences

1.5.2.2 BASILIXIMAB (SIMULECT®)

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. As with any protein product, anaphylaxis can occur, particularly with repeated administration, but this has been reported only rarely.

1.5.2.3 Mycophenolate Mofetil (CellCept®)

Mycophenolate Mofetil (Roche Laboratories) is associated with diarrhea, leukopenia, vomiting, and evidence of higher frequency of certain types of infections, in particular BKV infection.
CellCept® may increase the risk of developing lymphoproliferative disease, lymphomas and other malignancies, particularly of the skin. Lymphoproliferative disease or lymphoma developed in 0.4% to 1% of patients receiving CellCept® at 1-1.5mg BID.

Severe neutropenia developed in up to 2% of renal transplant recipients receiving CellCept® at a dose of 1.5mg BID. Gastrointestinal bleeding (requiring hospitalization) has been observed in approximately 3% of renal, in 1.7% of cardiac, and in 5.4% of hepatic transplant patients treated with CellCept® at a dose of 1.5mg BID.

CellCept® is known to cause fetal harm when administered to a pregnant woman. Subjects taking CellCept® must use two acceptable methods of contraception while taking CellCept®.

Cases of progressive multifocal leukoencephalopathy (PML), sometimes fatal, and pure red cell aplasia, have been reported in patients treated with CellCept®.

1.5.2.4 SIROLIMUS (RAPAMUNE®)

Sirolimus (Wyeth-Ayerst Laboratories) is associated with hypertension, increased creatinine, dizziness, increased cough, dyspnea, pharyngitis, rhinitis, abdominal pain, headaches, nausea, diarrhea, arthralgia, and hyperlipidemia. 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.

1.5.2.5 TACROLIMUS (PROGRAF®)

Tacrolimus (Astellas Pharm. Inc.) 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, neurological sequelae (including tremors, ataxia, and extremely rare central pontine myelinolysis), posterior reversible encephalopathy syndrome (PRES), progressive multifocal leukoencephalopathy (PML), interstitial lung disease, BK nephropathy, nausea, vomiting, and diarrhea. Please see product monograph for details. 1.5.2.6 CYCLOSPORINE (Sandimmune Neoral ®) Sandimmune® (Novartis) is associated with renal dysfunction, tremors, hirsutism, hypertension, and gum hyperplasia.

1.5.2.7 ETANERCEPT (Enbrel®)

Etanercept is a dimeric soluble form of the p75 TNFR that blocks TNF binding and reduces inflammation(Eason, Wee et al. 1995; Eason, Pascual et al. 1996; Wee, Pascual et al. 1997; Novak, Blosch et al. 1998; Chiang, Abhyankar et al. 2002). 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(Ormerod 2004; Ehlers 2005; Keane 2005), and based on post-marketing studies a warning has been issued about serious infections of sepsis, including fatalities, an increase risk of lymphoma and other malignancies in children and adolescents and leukemia, which have been reported with the use of Enbrel®. 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(Farney, Xenos et al. 1993). 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(Hering, Kandaswamy et al. 2005).

1.5.3 Transplant of Allogeneic Islets

Transplantation of islets is associated with the 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 have been discussed separately in the section entitled "Risks Associated with Study Procedures."

1.5.4 Study Procedures

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

Islets may be infused into the hepatic portal vein preferably by a percutaneous transhepatic approach or, if this is for some reason not desirable, by an open surgical approach.

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, or damage to gall bladder, or pleural effusion.

Open Surgical Approach
The risk with the open surgical approach is comparable to other minor intra abdominal surgery, including bleeding, infection, post operative ileus or thrombosis.

Hepatic Dysfunction and Steatosis
Transient abnormalities in liver enzyme tests have been observed immediately following intraportal islet transplantation(Wahoff, Papalois et al. 1995; Rafael, Ryan et al. 2003). Three of the 86 islet transplant recipients reported to CITR have experienced transient elevations of liver enzymes requiring prolongation of posttransplant hospitalization or admission(Close, Hering et al. 2004). 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 (Rafael, Ryan et al. 2003). No correlation between the increase in liver function tests and graft characteristics or graft function was found.

Periportal hepatic steatosis has been described following intraportal islet allotransplantation in 20% of the studied subjects (Markmann, Rosen et al. 2003; Bhargava, Senior et al. 2004)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 (Bhargava, Senior et al. 2004), 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
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 (Cameron, Mehigan et al. 1981). In all patients, the portal pressure had returned to normal and portal venograms were normal. Portal hypertension following intraportal infusion of unpurified allogeneic islet tissue resulted in a tear of the splenic capsule requiring splenectomy in one case (Hering and Ricordi 1999). Casey *et al* reported on changes in portal pressure following sequential islet infusions at the University of Alberta, and found that third islet infusions were associated with significantly greater

final portal pressures (18mmHg) than first or second infusions (12mmHg) (Casey, Lakey et al. 2002). The baseline pressures were normal in all cases, suggesting absence of chronic portal hypertension (Casey, Lakey et al. 2002).

Portal Vein Thrombosis

Transplanted islets release tissue factor and exhibit prothrombotic properties when infused to an intravascular site such as the portal vein (Moberg, Johansson et al. 2002). A partial portal vein thrombosis has been reported in one of six patients transplanted at the intramural NIH program (Hirshberg, Rother et al. 2003). In the Edmonton series, the risk of partial vein thrombosis was 3% in more than 100 intraportal islet infusions (Ryan, Paty et al. 2004). Early diagnosis and prompt management of branch vein portal occlusion with systemic heparinization may prevent clot propagation. Anticoagulation therapy may lead to intra-abdominal hemorrhage requiring transfusion and surgical intervention. (Rother and Harlan 2004)
Repeated intraportal islet infusions are generally contraindicated in patients that have experienced prior portal thrombus.

1.5.4.4 **GFR**

Risks associated with this 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: Iohexol has been widely used and has an excellent safety record. Very occasionally, allergic reactions to iohexol hay occur(Brown and O'Reilly 1991). Cr-EDTA: Whole-body radiation exposure will be less than 1% of the average annual exposure a person in the United States receives from natural background radiation.

1.5.5 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.

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. 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. If the CMV status of the donor and recipient is both negative, then Valganciclovir administration can be adjusted or eliminated..

1.5.6 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 rapid endotoxin assay is completed to confirm that the endotoxin content is less than 5EU/kg based on the recipient weight prior to proceeding with transplantation. For additional protection, broad-spectrum antibiotics are given prophylactically at transplant to further diminish the infectious risk. Cultures of the final islet preparation are sent in antibiotic-free media for microbial and fungal culture.

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

1.5.7 Sensitization of the Recipient to Donor Antigens

As with any allogeneic transplant, the islet transplant recipients may become sensitized to islet-donor histocompatability 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 (Alejandro, Angelico et al. 1997; Olack, Swanson et al. 1997; Roep, Stobbe et al. 1999). In the ITN-sponsored trial of islet transplantation using the Edmonton protocol of steroid-free immunosuppression, 5

of 36 subjects had evidence of elevated PRA post-transplant when measured by flow cytometry. Two of these 5 subjects experienced primary islet non-function. Moreover, data from 5 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 immunosuppression. 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.

1.5.8 Acceleration of Retinopathy with Acute Correction in Glycemic Control

In the DCCT study(DCCT Research Group 1991), about 10% of patients with preexisting 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 (7). Long-term follow-up of both groups suggested that successful pancreas transplantation may have a late beneficial effect that becomes evident only after 36 months.

1.5.9 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.

1.6 Known and Potential Benefits to Human Participants

1.6.1 Low Molecular Weight Sulfated Dextran

Optimal treatment of the islet recipient in the peri-transplantation period is difficult since the coagulation system needs to be prevented in order to protect the islets from IBMIR but at the same time the recipient needs to have an intact coagulation system in order to prevent bleeding from the puncturing of the liver during placement of the catheter in the portal vein. We believe the proposed approach including the use of LMW-DS in the transplantation medium and systemic treatment of the recipient with LMW-DS at a time after transplantation is clinically acceptable and at the same time holds promise in regard to controlling the IBMIR. Based on data from our *in vitro* and *in vivo* experimental studies, we anticipate that patients can be "cured" with significantly fewer islets and transplantations than have been needed in patients transplanted thus far.

1.6.2 Allogeneic Islet Transplantation

Successful islet transplantation alleviates T1D patients from life-threatening hypoglycemia and psychosocially debilitating glycemic lability(Ryan, Shandro et al. 2004). 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 (Ryan EA, Rachmiel Levine Diabetes and Obesity Symposium, October 6-9, 2004). Furthermore, as long as graft function is maintained, fear of hypoglycemia and anxiety are significantly lower after islet transplantation.(Johnson, Kotovych et al. 2004) Indeed, T1D participants in the DCCT who had persistent C-peptide production had a significantly reduced risk of severe hypoglycemia despite intensive insulin therapy.(Steffes, Sibley et al. 2003) Additionally, while most transplant recipients experience only a temporary reprieve from exogenous insulin therapy, a few have maintained insulin-independent graft function for more than 3 years. Novel strategies aimed at promoting the engraftment or survival of transplanted islets may lead to improved long-term graft function and further the duration of insulin-independence after transplantation, and hopefully lead to reductions in the secondary complications of T1D.

2. OBJECTIVES

2.1 Primary Objective

The primary objective is to evaluate the safety and efficacy of Low Molecular Weight Dextran Sulfate (LMW-DS) to enhance engraftment and prevent IBMIR in islet transplantation to Type 1 diabetic subjects.

2.2 Secondary Objective(s)

The secondary objective is to gather additional safety and efficacy information about the combination of Low Molecular Weight Sulfated Dextran with islet transplantation.

3. SELECTION OF PARTICIPANTS

3.1 Inclusion Criteria

Subjects must meet *all* of the following criteria to be considered eligible for participation in the study:

1. Patients between 18 to 65 years of age.
2. Subjects who are able to provide written informed consent and comply with the procedures of the study protocol.
3. Clinical history compatible with type 1 diabetes with onset of disease at < 40 years of age and insulin-dependence for ≥ 5 years at the time of enrollment, and a sum of patient age and insulin dependent diabetes duration of ≥28.
4. Absent stimulated c-peptide <0.3ng/ml [<0.099nmol/L] 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 90min after the start of consumption.
5. 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 enrollment.
6. At least one episode of severe hypoglycemia, defined as an event with one of the following symptoms: memory loss; confusion; uncontrollable behavior; irrational behavior; unusual difficulty in awakening; suspected 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, intravenous glucose, or glucagon administration, in the 12 months prior to study enrollment.
7. At least one of the following:
    a. 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 12 months prior to randomization;
    b. 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/hr ·wk^{-1}) during the screening period and within the last 6 months prior to randomization;
    c. A composite of a Clarke score of 3 or more or a HYPO score greater than or equal to the 75th percentile (423) in combination with a LI greater than or equal to the 75th percentile (329) during the screening period and within the last 12 months prior to randomization.

3.2 Exclusion Criteria

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

1. Known IgE mediated allergy to antibiotics and antifungal medications (ciprofloxacin, gentamycin, and amfotericin B) used in the culture medium.
2. Known hypersensitivity to dextran.
3. Body mass index (BMI) >30 kg/m^2
4. Insulin requirement of > 1.0 IU/kg/day
5. HbA1c >10%.
6. Untreated proliferative diabetic retinopathy.
7. Blood Pressure SBP >160mmHg or DBP > 100mmHg.

8. Measured glomerular filtration rate (GFR) using 51Cr-EDTA, 99technetium-DPTA, or iohexol <80 ml/min/1.73 m^2. The absolute (raw) GFR value will be used for subjects with body surface areas >1.73 m^2.
9. Presence or history of macroalbuminuria (>300mg/g creatinine).
10. Presence or history of panel-reactive anti-HLA antibodies >80% by flow cytometry. Subjects with panel reactive anti-HLA antibodies above background but ≤ 80%, can be included if the antigen specificity of the antibodies can be determined for future avoidance; however, if the antigen specificity of the antibodies cannot be determined they will be excluded
11. 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.
12. Active infection including hepatitis B, hepatitis C, or HIV.
13. Negative screen for Epstein - Barr Virus (EBV) by IgG determination.
14. Any history of malignancy except for completely resected squamous or basal cell carcinoma of the skin.
15. Known active alcohol or substance abuse.
16. Baseline Hgb below the lower limits of normal at the local laboratory; lymphopenia (<1,000/μL), neutropenia (<1,500/μL), or thrombocytopenia (platelets <100,000/μL).
17. Homocygotic Activated Protein C Resistance (APC-R).
18. History of hypercoagulability disorder or coagulopathy or international normalization ratio (INR) >1.5.
19. Known history of severe co-existing cardiac disease, characterized by ***any one*** of the following 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%.
20. Consistently abnormal liver function tests at the time of study entry. SGOT (AST), SGPT (ALT), Alk Phos or total bilirubin, with values >1.5 times normal upper limits on two consecutive measurements > 2 weeks apart.
21. Acute or chronic pancreatitis.
22. Patients with active peptic ulcer disease, symptomatic gallstones, or a history of portal hypertension.
23. Severe unremitting diarrhea, vomiting or other gastrointestinal disorders potentially interfering with the ability to absorb oral medications.
24. Receiving treatment for a medical condition requiring chronic use of systemic steroids, except for the use of ≤5mg prednisone daily, or an equivalent dose of hydrocortisone, only for physiological replacement.
25. Treatment with any-anti-diabetic medication, other than insulin, within 4 weeks of enrollment.
26. Use of any investigational agents within 4 weeks of enrollment.
27. Administration of live attenuated vaccine(s) within 2 months of enrollment.
28. Patients with any condition or any circumstance that in the opinion of the investigator would make it unsafe to undergo an islet transplant.
29. Treatment with any immunosuppressive regimen at the time of enrollment.
30. A previous islet transplant.
31. 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 an open label, stratified, randomized, multi- center study that will be conducted in 7 centers in Scandinavia (Norway, Sweden, Finland, and Denmark).

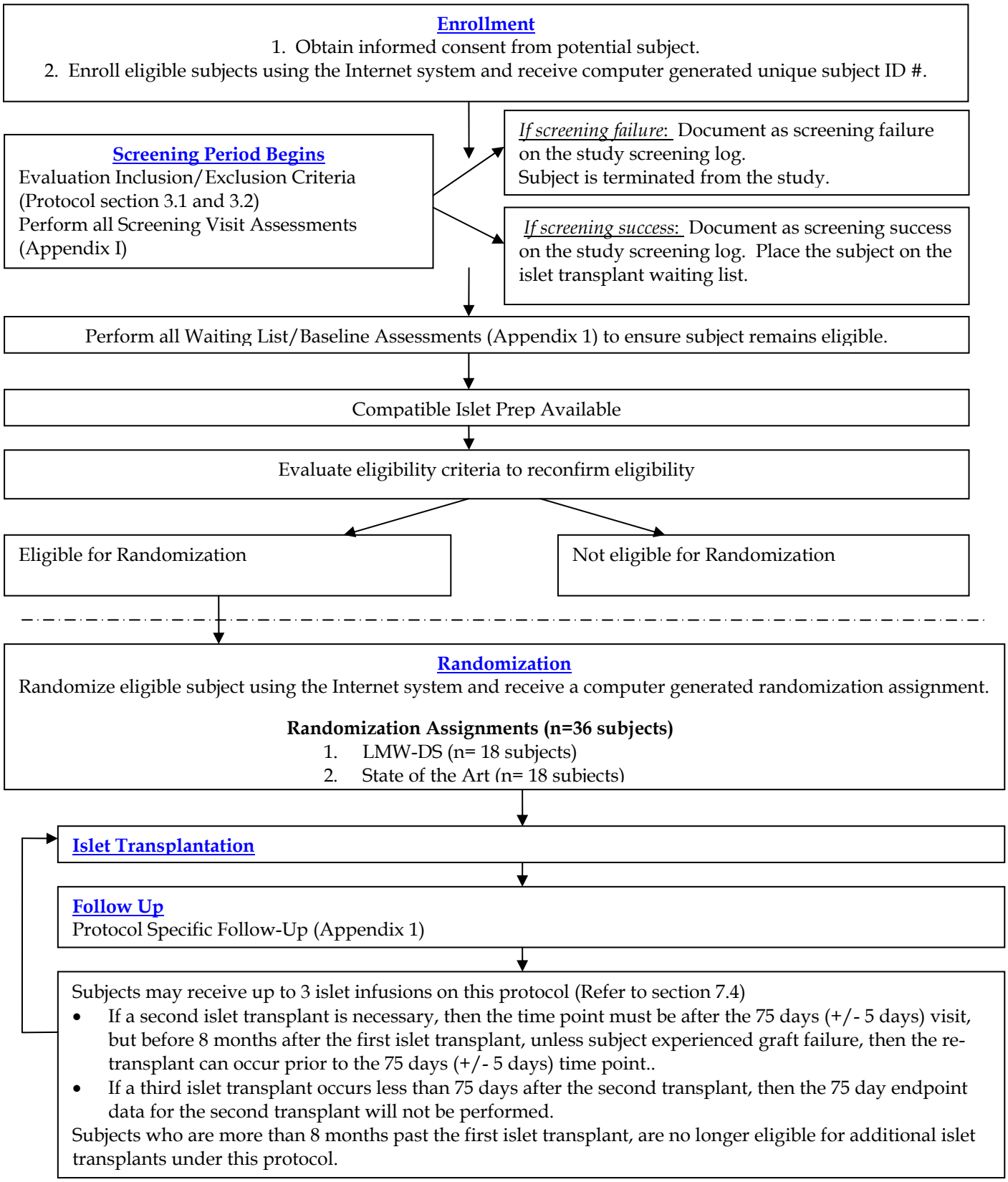


Figure 4: Study Design Schema: Enrollment, Screening, Randomization, and Transplantation

4.1 Study Endpoints

4.1.1 Primary Endpoint

The level of stimulated c-peptide at 90-minutes derived from the mixed-meal tolerance test (MMTT) at 75±5 days following the first islet infusion.

4.1.2 Secondary Endpoints

The target level for HbA1c chosen for this study is 7.0%. This value was chosen because it is the level recommended by the American Diabetes Association (ADA) and is considered to be the clinically relevant goal for subjects with T1D. A HbA1c level of 6.5% is the goal recommended by the American College of Endocrinology (ACE).

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

1. TAT complexes and C-peptide immediately prior to islet infusion, when 125 mL is left in the infusion bag (before rinsing) at 0, 15, 60, 180, 270 and 360 minutes after completion of the islet transplant, and 24 hours after completion of islet transplant;
2. Conduction Velocity and RR interval at screening, and month 12 after first and last islet transplant;
3. Portal pressure before and 15 minutes after completion of islet transplantation;
4. Liver enzymes (ALT, AST), one and seven days after all islet transplantation(s);
5. Quality of life (DTSQs, DTSQc, SF36 Questionnaires), 1 year after the first and final islet infusion, to be compared with the same test done as a part of the screening prior to being put on the waiting list (DTSQs);
6. Percentage of administered radioactivity found in the liver after the start of islet transplantation. Determined through the use of PET/CT (protocol section 9.1.1.11).

4.1.3 Efficacy Endpoints

At 75 ± 5 days following the first infusion:

- The percent reduction in insulin requirements
- HbA1c
- Mean amplitude of glycemic excursions (MAGE)
- Glycemic lability index (LI)
- Clarke hypoglycemia awareness score
- Ryan hypoglycemia severity (HYPO) score
- Basal (fasting) glucose and c-peptide and 90-min glucose derived from the mixed-meal tolerance test
- β-score
- C-peptide:glucose·creatinine ratio
- Acute insulin response to glucose (AIR_{glu}), insulin sensitivity, and disposition index derived from the insulin-modified frequently-sampled intravenous glucose tolerance (FSIGT) test
- Glucose variability and hypoglycemia duration derived from the continuous glucose monitoring system® (CGMS)
- The proportion of subjects with full islet graft function.

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

- The proportion of subjects with full islet graft function
- The proportion of subjects with an HbA1c <7.0% and free of severe hypoglycemic events from day 28 through day 365
- 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
- Acute insulin response to glucose (AIR_{glu}), insulin sensitivity, and disposition index derived from the insulin-modified frequently-sampled intravenous glucose tolerance (FSIGT) test
- Glucose variability and hypoglycemia duration derived from the continuous glucose monitoring system® (CGMS)
- The proportion of subjects receiving a second islet infusion
- The proportion of subjects receiving a third islet infusion

4.1.3.1 FULL ISLET GRAFT FUNCTION DEFINITION:

Islet transplant recipients are considered to have full islet graft function if all of the following criteria are met:

- Titrated off insulin therapy for at least 1 week (7 consecutive days) with the last day within the day 75 and day 365 windows;
- HbA1c <7.0% or a ≥2.5% decreased from baseline at the end of the 7 day period when titrated off insulin;
- Fasting capillary glucose level should not exceed 140 mg/dL (7.8 mmol/L) more than three times in the past week (based on measuring capillary glucose levels a minimum of 7 times in a seven day period);
- 2-hour post-prandial capillary glucose should not exceed 180 mg/dl (10.0 mmol/L) more than three times in the past week (based on measuring capillary glucose levels a minimum of 21 times in a seven day period);
- Fasting serum glucose level ≤ 126 mg/dL (≤7.0 mmol/L) from central lab results; 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;
- Evidence of endogenous insulin production defined as fasting or stimulated C-peptide levels ≥ 0.5 ng/mL (≥0.16 nmol/L) at the end of the 7 day period when titrated off insulin.

4.1.4 Safety Endpoints

At 75 ± 5 days and 365 ± 14 days following the first islet infusion:

- The incidence and severity of adverse events related to the islet infusion procedure including: bleeding (> 2 g/dl (20g/L) decrease in hemoglobin concentration); segmental portal vein thrombosis; biliary puncture; wound complication (infection or subsequent hernia); and increased transaminase levels (> 5 times ULN)
- The incidence and severity of adverse events 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 immune sensitization defined by detecting anti-HLA antibodies not present prior to transplantation
- The incidence of a change in the immunosuppression drug regimen

At 365 ± 14 days following the first islet infusion:

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

5. STUDY MEDICATIONS

5.1 Study Medication Regimen Design

STUDY MEDICATION REGIMEN	TREATMENT ARMS	SECTION (page)	TIME POINTS
Low Molecular Weight Sulfated Dextran (LMW-DS)	Arm 1: Experimental	5.2 (p37)	Administered as a bolus before the islet transplant infusion, with the islet transplant infusion, and as a continuous infusion (5 hrs) after the islet transplant.
Heparin	Arm 2: State of Art	5.3 (p39)	Administered with the islet transplant infusion and as a continuous infusion intraportally (5hrs) after the islet transplant infusion.
Anticoagulation Prophylaxis /Hematological Agents 1. Enoxaparinsodium (Klexzane®) 2. ASA (Trombyl® or Albyl-E®)	Arm 1: Experimental Arm 2: State of Art	5.4 (p39)	1. First dose administered from 2h after the Intraportal catheter is removed through Day 7 post-transplant. 2. Administered starting 24 hours post-transplant and continued as medically indicated.
Rabbit Anti-Thymocyte Globulin (ATG, Thymoglobulin®)	Arm 1: Experimental Arm 2: State of Art	5.5 (p39)	Administrated as an IV infusion on days –2, -1, 0, +1, and +2 at first transplantation.
Monoclonal Antibody IL-2 Receptor Blocker: *Basiliximab	Arm 1: Experimental Arm 2: State of Art	5.6 (p41)	Administered as an IV infusion on day of transplant and day 4, for the second and third transplant (if applicable) or may replace ATG at first transplantation if ATG has not been tolerated.
Cell Proliferation Inhibitor: *Mycophenolate Mofetil OR Sirolimus	Arm 1: Experimental Arm 2: State of Art	5.7(p40)	From day before first transplant, continued for the duration of the study.
Calcineurin Inhibitor: *Tacrolimus OR Cyclosporine	Arm 1: Experimental Arm 2: State of Art	5.8 (p40)	From day 1 after transplant, continued for the duration of the study.
Anti-Inflammatory Therapy: *Etanercept (Enbrel®)	Arm 1: Experimental Arm 2: State of Art	5.9 (p41)	Administered IV on day 0 and SC on days +3, +7, and +10 post-transplant.
Infection Prophylaxis: 1. Cefuroxim (Zinacef®) 2. Trimethoprim-sulfamethoxazole (BactrimTM/Septra®/Eusaprim®) **or** Pentamidine (Pentacarinat®) 3. Valganciclovir (Valcyte®) 4. Nystatin (Mycostatin®)	Arm 1: Experimental Arm 2: State of Art	5.10 (p41)	1. Once, immediately prior to transplant. 2. Administered once daily for a period of 6 months after transplant. **or** Nebulizer every 4 weeks for the first 6 months after transplant. 3. At time of discharge until 3 months after transplant. 4. PO 4 times daily from day -1 before transplant until 1 month after transplant.
Hypersensitivity Prophylaxis *Klemastin (Tavegyl®) or Dexchlorpheniramine	Arm 1: Experimental Arm 2: State of Art	5.10 (p42)	Once within 1 hour prior to transplant.

Table 1. Study Medication Regimen

5.2 Low Molecular Weight Sulfated Dextran (LMW-DS)

Please refer to applicable product labeling and Investigator Brochure for known and potential risks to human subjects associated with Low Molecular Weight Sulfated Dextran (LMW-DS).

5.2.1 Formulation, Packaging, and Labeling

Low Molecular Weight Sulfated Dextran (LMW-DS 20 mg/mL) is manufactured by Apoteket AB, Produktion & Laboratorier (Umeå, Sweden), with an average molecular mass 5000 Da. LMW-DS for the clinical trial will be produced in compliance with EU-GMP.

The LMW-DS (20mg/mL) will be provided in 50 mL glass vials with rubber stoppers. Each single-use vial contains 50 mL of LMW-DS in saline at a concentration of 20 g/L. Labeling will be in accordance with the local law and with trial requirements.

The trial product vials are to be stored at +2-8°C and is stable until the expiration date given on each vial. A temperature log must be kept and evaluated daily at the trial product storage facility. No other medication should be added to the solutions of LMW-DS. Partially used vials should not be reused.

5.2.2 Preparation, Administration, and Dosage

Inhibition of the IBMIR is probably most critical during the first 6 hours after infusion of the islets into the portal vein. If the IBMIR is allowed to proceed uncontrolled, the infused islets will to a large extent be entrapped in larger clots in the main branches of the portal vein. If so, the entrapped islets will fail to engraft and will instead be lost within the clots. On the other hand, if the islets can escape the IBMIR they will be entrapped in the smaller braches of the portal vein and in direct contact with the lining endothelial cells. This would markedly improve the engraftment process and increase the number of islets surviving the immediate post-transplantation period. Clinical grade LMW-DS approved for IV injections will be manufactured by the national pharmacy in Sweden. The stock 20g/L LMW-DS should be mixed with saline to obtain the decided concentration for infusion. The infusion will be prepared in the Radiology Units at each center. The study personnel will use the *Instruction for Administration of LMW-DS to Target an APTT of 150±10s worksheet* (this document is located in the study Manual of Procedures, as well as on the CIT Website www.isletstudy.org).

LMW-DS will be administered as a bolus of 4.5mg LMW-DS / kg to subjects randomized to protocol immunosuppression and LMW-DS.

1)One-third (1.5 mg/kg BW) administered intraportally immediately prior to islet transplantation.

2)Two-thirds (3.0 mg/kg BW) administered intraportally with the islet preparation.

There should be a time period of at least 15 minutes between placement of the portal catheter and the start of the bolus dose. The islets are given intraportally over a time period of about 32 minutes including rinsing of the islet bag with the washing buffer. The washing buffer will contain an amount of LMW-DS calculated from the subjects' body weight. It will be given at a fixed speed during the approximately last 12 of 32 minutes.

There will be a continuous infusion of LMW-DS targeting APTT of 150±10s directly after the islet infusion, and maintained for 5 hours. The infusion rate will be based on APTT immediately after the islet infusion. The infusion rate will be adjusted according to the *Instruction for Administration of LMW-DS to Target an APTT of 150±10s worksheet* (this document is located in the study Manual of Procedures, as well as on the CIT Website www.isletstudy.org).

This infusion should be given intraportally. If technical problems occur, the remaining dose can be given through a peripheral vein.

The APTT should be analyzed according to the *Instruction for Administration of LMW-DS to Target an APTT of 150±10s worksheet* (this document is located in the study Manual of Procedures, as well as on the CIT Website www.isletstudy.org), or more often if problems to adjust the infusions are encountered.

5.2.3 Warnings

Prolonged oral administration and infusion of LMW-DS (molecular mass 8k Da) have been associated with thrombocytopenia after 3-7 days. The thrombocytopenia led to bleeding complications (e.g. epitaxis) in some cases. In none of these studies, however, were serious bleeding events reported. Reversible alopecia was seen in 50% of the patients who received the LMW-DS for more than 8 days. In the present study, LMW-DS will not be used for more than 6 hours and side effects of the kind reported above are therefore not expected. Immobilized dextran sulfate has been used to treat patients with hyperlipidemia by plasmapheresis [21]. Anaphylactoid reactions with hypotension have been reported in patients treated with ACE-inhibitors due to activation of the contact system. Unlike high molecular weight dextran sulfate, however, soluble LMW-DS does not activate the contact system and is therefore not anticipated to produce this side effect. In a recent phase I study where LMW-DS was administered according to the proposed treatment protocol, the APTT was kept at 150±10s for 6 hours and no adverse events were reporting, including events related to bleeding.

5.2.4 Precautions

To avoid bleeding and to detect early signs of bleeding, the following measures should be taken:

1. The coagulation status, including APTT, PK, fibrinogen and platelet count, must be normal before the patient is randomized in the study.
2. Hemoglobin should be monitored before, immediately after, four hours after islet transplantation and also 2 hours after removal of the portal catheter.
3. The patient should have a central venous catheter.
4. CVP should be measured before and after placement of the portal catheter.
5. After transplantation, the patient will be kept in bed until 4 hours after the removal of the portal catheter. Blood pressure and pulse will be continuously monitored.
6. During treatment with LMW-DS or heparin, APTT will be monitored until APTT < 75 sec (LMW-DS), or < 50 sec (heparin). Not until then is the catheter removed.
7. First dose of Enoxaparinsodium will not be given if Hemoglobin is reduced with 10 or more percent between transplantation and two hours after removal of the portal catheter. If that is the case see below. If day +1 ultrasound shows no indication of bleeding and the patient is hemodynamically stable the first dose will be given then.

In the case of bleeding a step-wise plan to handle such an event is described below:

1. Stop administration of LMW-DS.
2. Buffer and/or plasma expander infusion.
3. Transfusion of erythrocytes, fresh frozen plasma and platelets.
4. Administration of Prothrombin complex or FVIIa (NovoSeven®).
5. Surgical intervention if severe bleeding cannot be stopped by steps 1-4.

The order of actions may be changed due to severity of bleeding.

5.2.5 Infusion Supervision

The infusion will be supervised by the clinical staff (clinical nurse, physician) at the participating institutions. A history of each infusion and any adverse side effects will be recorded and reported to the Data Coordinating Center using the appropriate case report forms.

Vital signs (blood pressure, pulse, and O_2 saturation (%) measured by a probe on the finger) will be monitored and results documented prior to the islet transplant infusion, in addition to 15, 30, 60, 120, 180, 240, and 300 minutes after initiation of the infusion. Additional vital signs may be obtained as clinically indicated.

5.2.6 Drug Accountability

The investigator is required to maintain adequate records of the disposition of the investigational agent, including the date and quantity of the product received, to whom the product was dispensed (subject-by-subject accounting), and a detailed accounting of any product accidentally or deliberately destroyed.

Records for receipt, storage, use, and disposition will be maintained by the study site. A dispensing log will be kept current for each subject. This log will contain the identification of each subject and the date and quantity of product dispensed. No trial products may be dispensed to any persons not enrolled in the trial. All records regarding the disposition of the investigational product will be available for inspection by the clinical trial monitor. All trial product vials should be retained for a period of 7 days after the administration to the participant, and then destroyed according to the institutions standard operating procedures.

All vials (used, partially used and unused) must be noted on the provided Drug Accountability Form. It is the investigator's responsibility to ensure completion this Drug Accountability Form. Used and unused trial drug vials must be stored separately.

5.3 Heparin

A dose of 70 U/kg body weight of recipient with the islet infusion, followed by a continuous intraportal infusion of heparin targeting an APTT of 50±10s for the next 5 hrs will be administered in patients who are randomized to receive State of the Art- Heparin therapy.

5.4 Anticoagulation Prophylaxis / Hematological Agent

5.4.1 Enoxaparinsodium (Klexane®)

Low Molecular Heparin will be administered at a dose of 30 mg SC BID through day 7 post-islet transplant, with the first dose given 2h after intraportal catheter is removed.

5.4.2 ASA (Trombyl® or Albyl-E®)

Acetylsalicylicacid will be administered at a dose of 75 mg PO qPM starting 24 hrs posttransplant and continued as medically indicated.

5.5 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 first dose will be administered over 6-12 hours and subsequent doses will be administered over 6 hours. If, for practical reasons, three doses can not be administered before transplantation the first and second will be administered on day -1 and 0 and the three remaining will be administered on day +1, +2 and +3. At least six hours between each infusion and also between infusion and transplantation is recommended.

Premedications will be used as follows:
#1: Paracetamol (i e Alvedon®) 1000 mg PO/PR ½ hr before and midway through the ATG infusion
#2: Klemastin (Tavegyl®) 1 mg PO ½ hr before and midway through the ATG infusion. Alternatively 5 mg Dexchlorpheniramine® is administered IV ½ hr before the 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)

Three pre-transplant infusions of ATG are suggested to be administered according to the following algorithm.

Time	Sample Day	Sample Time	Activity
0 hour	Day -2	1600	Viable islet prep into culture
0-12 hours	Day -2/Day-1	1600-0400	ATG #1 over 6-12 hours
12-18 hours	Day -1	0400-1000	Rest for 6 hours
18-24 hours	Day -1	1000-1600	ATG #2 over 6 hours
24-32 hours	Day -1	1600-2400	Rest for 8 hours
32-38 hours	Day 0	2400-0600	ATG #3 over 6 hours
38-44 hours	Day 0	0600-1200	Preparations for Transplant
44 hours	Day 0	1200	Islet Transplant

5.6 Monoclonal Antibody IL-2 Receptor Blocker

All subjects will receive *one monoclonal antibody IL-2 receptor blockers* (Basiliximab) for the second or third transplantation and if ATG is not tolerated at the first transplantation:

5.6.1 Simulect® (Basiliximab)

Intravenous Basiliximab, 20 mg, administered the day of transplantation and day 4 post transplant. Repeat for each subsequent transplantation.

Basiliximab dose may be adjusted for opportunistic infections or side effects listed in the SPC provided by EMEA product monographs (www.emea.eu.int).

5.7 Cell Proliferation Inhibitor

All subjects will receive *one of the following cell proliferation inhibitors* (MMF OR Sirolimus):

5.7.1 CellCept® (Mycophenolate Mofetil, MMF or equivalent)

MMF will be administered orally, beginning pre-transplant and continue throughout the study, at a dose of 500 –1500 mg BID. The starting dose is 1000 mg BID. The dose can be lowered or raised due to suspected side effects or signs of lack of efficacy. MPA-AUC measurements may be used to guide such decisions.

MMF dose may be adjusted for side effects listed in the SPC provided by EMEA product monographs (www.emea.eu.int); such as leukopenia, thrombocytopenia, anemia, GI toxicity and opportunistic infections.

5.7.2 Rapamune® (Sirolimus)

Sirolimus therapy will be administered orally, either as liquid or tablets., The sirolimus will be administered pre-transplant at a loading dose of 0.05-0.2 mg/kg BW, followed by the same dose days 1-2 and with 0.1 mg/kg once daily from day 2-3.

Doses are adjusted to achieve whole-blood trough levels:

Time Point	
Day 7-90	10 –15 ng/mL
> 3 months	7-10 ng/mL

Sirolimus dose may be adjusted for side effects listed in the SPC provided by EMEA product monographs (www.emea.eu.int); such leukopenia, thrombocytopenia, anemia, GI toxicity and opportunistic infections.

5.8 Calcineurin Inhibitor

All subjects will receive *one of the following calcineurin inhibitors* (Tacrolimus OR Cyclosporine):

5.8.1 Prograf® (Tacrolimus)

Tacrolimus is the preferred calcineurin inhibitor for this study. Tacrolimus will be administered orally; beginning the day after the first transplantation and continued throughout the study. Doses are adjusted to achieve whole-blood trough levels:

First, second and thirdTransplantation:

Time Point	
Day 1-90	10-12 ng/mL
Month 3-6	8-10 ng/ml
Month >6	6-8 ng/ml

If Sirolimus is used Tacrolimus levels may be reduced by 20%.

Tacrolimus dose may be adjusted for side effects listed in the SPC provided by EMEA product monographs (www.emea.eu.int); such as nephrotoxicity, neurotoxicity, GI toxicity and opportunistic infections.

5.8.2 Sandimmune® or Neoral® (Cyclosporine)

Cyclosporine therapy will be initiated when Tacrolimus is not tolerable. Cyclosporine will be administered the day after the first transplantation and continue throughout the study. Doses adjusted to achieve whole-blood trough levels:

First, second and third Transplantation:

Time Point	
Day 1-90	200-250 ng/mL
Month 3-6	150-200 ng/ml
Month >6	100-150 ng/ml

Cyclosporine dose may be adjusted for side effects listed in the SPC provided by EMEA product monographs (www.emea.eu.int); such as nephrotoxicity, neurotoxicity, GI toxicity and opportunistic infections.

5.9 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.

5.10 Infection Prophylaxis

5.10.1 Infection Prophylaxis

All subjects will receive intravenous Cefuroxim (Zinacef®), 1.5g administered once immediately before each transplantation. Subjects with allergies to Cefuroxim will receive Klindamycin (Dalacin), 600mg intravenous or Ciprofloxacin 400mg intravenous immediately before each transplantation.

5.10.2 Pneumocystis jiroveci Prophylaxis

All subjects will receive prophylaxis for *Pneumocystis jiroveci* pneumonia will include one of the following:

1. Trimethoprim-sulfamethoxazole (Bactrim ™/Septra®/Eusaprim®) one tablet (Trimethoprin 80 mg/sulfamethoxazol 400mg) administered once daily (Dose adjusted according to renal function) for a period of 6 months after the transplantation.
2. Pentamidine inhalation treatment (Pentacarinat®), 300 mg via nebulizer every 4 weeks for the first 6 months post transplant.

5.10.3 CMV Prophylaxis

When tolerating PO or at discharge, subjects will receive Valganciclovir (Valcyte®) PO, 900 mg QD. Dose adjusted according to renal function, for 3 months post transplant. If the CMV status of the donor and recipient are both negative, then the valganciclovir administration can be adjusted or eliminated.

5.10.4 Fungal Prophylaxis

All subjects will receive Nystatin (Mycostatain®), 1 mL PO four times daily from day -1 before transplant until one month after transplant.

5.10.5 Hypersensitivity Prophylaxis

All subjects will receive 2mg IV of Klemastin (Tavegyl®) or 5mg IV of Dexchlorpheniramine within 1 hour prior to transplant.

5.11 Insulin Treatment

All subjects will receive IV insulin infusion during the first 2 days after islet transplantation in order to maintain plasma glucose levels between 4 and 8 mmol. All subjects will also receive SC insulin for an additional 4-8 weeks in order to achieve partial beta-cell rest and facilitate islet engraftment and aiming at the same glucose levels. When there is evidence of graft function and blood glucose is stable at the target level with HbA_{1C} approaching the normal range, exogenous insulin can be gradually reduced with no more than 20% of the pre-transplant daily insulin dose removed with 3 day intervals. If glycemic control deteriorates the dose shall be increased to the previous level.

5.12 Prohibited Medications

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

- steroid medication (save Solu-Medrol® with the first ATG dose, topicals and prednisone at a dose of $\leq$ 5mg daily, or an equivalent dose of hydrocortisone, for physiological replacement only)
- any medications in the macrolide antibiotic class
- other investigational products
- other immunosuppressive therapies
- immunomodulatory agents
- other anti-diabetic medications
- Any anti-coagulant apart from the ones defined in this protocol, for the first week after the islet transplant
- ACE inhibitors on the morning of the islet transplant.

5.13 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.

5.14 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, Adverse

Event 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 5.15 for a description of possible indications for premature discontinuation of study treatment.

5.15 Modifications or Discontinuation of Study Treatment

Should an islet product become unsuitable for transplantation or the scheduled islet transplant is cancelled subsequent to treatment with the first dose of ATG or Basiliximab, the subject will remain on the waiting list for the next available pancreas for islet transplantation. While on the waiting list and after the next available pancreas for islet transplant has been identified, the randomization assignment will remain the same.

When an organ becomes available, investigators should use clinical judgment and may refer to the CIT01 MOP to determine the amount and type of induction immunosuppression that should be administered at the time of the islet transplant.

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 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.

5.15.1 Modification of Study Treatment

At the discretion of the local investigator, immunosuppression protocols may be modified or discontinued if there are signs of reactions to any of the medications or if there is a serious infection.

5.15.2 Modification of Standard Immunosuppression

5.15.2.1 RABBIT ANTI-THYMOCYTE GLOBULIN-INDUCED ANAPHYLAXIS

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

5.15.2.2 RABBIT ANTI-THYMOCYTE GLOBULIN-INDUCED CYTOKINE RELEASE

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

5.15.2.3 NEUTROPENIA

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

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

- Reduce rabbit ATG by 50%.
- Reduce the prophylactic use of valganciclovir from 900 mg per day to 450 mg per day or hold valganciclovir.
- Reduce trimethoprim/sulfamethoxazole to 80/400 mg on Monday, Wednesday, and Friday or hold trimethoprim/sulfamethoxazole.
- Review and obtain current sirolimus trough levels and consider dosage adjustment if trough level are >12ng/mL.
- If subject is using mycophenolate mofetil or mycophenolate sodium in lieu of sirolimus, consider dose reduction.
- Consider administration of G-CSF.
- Monitor temperature BID.
- Follow up within 48-72 hours to obtain: repeat complete blood count (CBC) with differential, subject symptoms, and measured temperatures.

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

- Obtain Infectious Disease Consult.
- Hold rabbit ATG.
- Hold valganciclovir and trimethoprim/sulfamethoxazole.
- Review and obtain current sirolimus trough levels and consider dosage adjustment if trough level are >12ng/mL.
- If subject is using mycophenolate mofetil or mycophenolate sodium in lieu of sirolimus, consider dose reduction.
- Administer G-CSF.
- Monitor temperature BID.
- Follow up within 48-72 hours to obtain: repeat CBC with differential, subject symptoms, and measured temperatures.

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

- Hold rabbit ATG.
- Hold administration of trimethoprim/sulfamethoxazole and/or valganciclovir.
- Review and obtain current sirolimus trough levels and hold dose if trough level are >12ng/mL.
- If subject is using mycophenolate mofetil or mycophenolate sodium in lieu of sirolimus, consider holding dose.
- Obtain CMV antigenemia or PCR for CMV.
- Consider fluoroquinolones in afebrile subjects.
- Consider clotrimazole.
- Administer G-CSF.
- Monitor temperature BID.

- Follow up within 24 hours to obtain repeat CBC, subject symptoms, and measured temperatures.

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

- The subject will be hospitalized under neutropenic precautions and Infectious Disease/Hematology consult will be obtained.
- Hold rabbit ATG.
- Hold administration of trimethoprim/sulfamethoxazole and/or valganciclovir.
- Review and obtain current sirolimus trough levels and hold dose if trough level are >12ng/mL.
- If subject is using mycophenolate mofetil or mycophenolate sodium in lieu of sirolimus, consider holding dose.
- Obtain CMV antigenemia or PCR for CMV.
- Administer G-CSF.

5.15.2.2 THROMBOCYTOPENIA

If the subject is found to have a platelet count (PLT) of <50 x 10^9/L, ATG will be withheld until PLT >50 x 10^9/L, then resume at a 50% reduced dose. *If the PLT is between 50 and 75 x 10^9/L, reduce anti-thymoglobulin dose by 50% until PLT is >75 x 10^9/L.*

If the PLT is <50 x 10^9/L, sirolimus will be withheld for 24 hours, then resumed at a 50% reduced dose. If PLT fails to return to >50 x 10^9/L within one week, sirolimus is to be withheld until PLT >50 x 10^9/L, after which sirolimus is resumed at 50% of the dose that preceded the drop in PLT to <50 x 10^9/L.

5.15.2.3NEPHROTOXICITY

An increase in serum creatinine (sustained 33%), warrants an evaluation with the institutions nephrologist. Evaluation should at least include GFR, urinary culture, tests for albuminuria, red/white blood cells in urine and an ultrasound. Possible harmful effects of medication should also be evaluated.If the lowering of function is thought to be attributable to the treatment with calcineurin inhibitor the trough level of this compound should be lowered to the lower limit of the span given in section 5.7.If this is not enough and other causes are ruled out the patient can be switched from Prograf/Sandimmune to Sirolimus. Renal function should be reevaluated within 3 months.

5.15.3 Premature Discontinuation of Study 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: Islet allograft failure will be defined as absence of insulin production by transplanted islets, as evidenced by absence of c-peptide <0.1nmol/L (<0.3 ng/mL). This will be determined by (1) c-peptide <0.1nmol/L (<0.3 ng/mL) on random testing, followed by (2) c-peptide<0.1nmol/L (<0.3 ng/mL) at baseline, and at 60 and 90 minutes after MMTT. C-peptide levels obtained in the course of the MMTT levels will be run at the core laboratory in Seattle, WA. Subjects with graft failure do not need to complete the day 75 metabolic assessments.

4. A unexpected related serious adverse event. 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

schedule outlined in Appendix 2. Data from these subjects will be used in the intent-to-treat analysis.

6. Criteria for Premature Termination of the Study

6.1 Participant Withdrawal Criteria

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 her/his islet graft and becoming sensitized if s/he chooses to discontinue immunosuppressive therapy and return to her/his original method of insulin management.

6.1.1 Premature Termination from the Study

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

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

6.2 Study Stopping Rules

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

1. The Medical Monitor finds any unexpected fatal or life-threatening adverse event possibly related to the use of the test therapy;
2. Primary non-function (PNF) occurs in 3 or more consecutive subjects at 2 or more participating clinical sites. PNF is defined as graft failure that occurs between 3-7 days post-transplant. Participants with graft failure do not need to complete the day 75 metabolic assessments.
3. There are 6 consecutive study subjects with a c-peptide less than 0.3 ng/mL (0.099nmol/L) (on random testing, at baseline and 1-3 hours post-MMTT) at 75 days post-transplant.
4. Any event(s) which in the opinion of the Medical Monitor or Protocol Chair indicates the need for DSMB review; or
5. The DSMB recommends termination of protocol enrollment and further transplants on a study-wide basis based on a review of the data and finding evidence that such action is necessary. Statistical guidelines for terminating the study based on monitoring guidelines are provided in section 10.

After the protocol is placed on hold, no additional transplants within the trial will be performed at any participating clinical site until the CIT Steering Committee and DSMB meet either in person or by conference call to review in depth the results and circumstances surrounding the islet functional failure or SAE to determine whether the trial enrollment of new subjects and conduct of additional transplants could be safely resumed.

6.3 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 institutional review board (IRB), the NIAID, the NIDDK, and the NIDDK DSMB, if any one of the following occurs:

1. Any possibly study-related grade 5 adverse event; or
2. Two serious adverse events related to the islet infusion procedure (e.g. bleeding, thrombosis, gall bladder injury); or
3. Two consecutive primary non-functioning transplants defined as graft failure that occurs between 3-7 days post-transplant.

After any site is placed on hold, no additional transplants will be performed under this protocol 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 protocol also at other affiliated sites.

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

The site investigator or designated study personnel will explain the research study in lay terms and discuss available modes of treatment individually with every potential study patient referred to the Nordic Network for Clinical Islet Transplantation. If the patient is willing to participate and once the written consent is obtained, the subject is enrolled and the screening period begins. Patients who have been previously assessed for standard islet and/or pancreas transplantation at the participating sites may ultimately be enrolled in this trial and, as a result, may have some testing done prior to signing the consent document.

Enrollment into the study is done using an electronic internet entry system that can be accessed using any computer connected to the Internet. Authorized personnel will be required to log into the system using their assigned user identification and password. Allocation to a unique subject identification number is computer generated and assigned at the time of enrollment.

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. Results from assessments completed prior to signing informed consent, must be current within the windows stated in the table below.

Screening Assessments	*Allowable timeframe prior to the date of consent*
EBV IgG	*No limit. Positive test result required for eligibility.*
Retinopathy evaluation; Physical exam; Chest x-ray; Abdominal Ultrasound; ECG; Myocardial Scintigram; Conduction Velocity and RR intervals; Serology (HIV, Hepatitis B, Hepatitis C); CMV IgG and IgM; Coagulation Status (APTT, PK, fibrinogen, platelets)	*Within one year*
CBC w/ differential; Serum Chemistry Panel and CRP; Urine Albumin; Fasting Lipid Profile (Total, LDL, HDL, Triglycerides); GFR	*Within 6 months*
Quality of Life Questionnaires; Medial and Diabetes History Assessment; Panel Reactive Antibody (Alloantibodies); Record recipient HLA and Blood Type; Pregnancy Test; HBA1c; MMTT; FSIGT; LI; HYPO; Clarke Score; c-peptide to Glucose Creatinine ratio; Serum to Archive; PBMC and Plasma to Archive; RNA to Archive.	*After informed consent has been obtained*

Table 2. Screening Assessments Windows

The screening period ends when all information/results needed for evaluation of inclusion and exclusion criteria are available. If the screening is a success the subject should be placed on the waiting list for transplantation. A general waiting list for all subjects entering the study will be kept at the Rudbeck Laboratory in Uppsala, Sweden.

If there is a screening failure the subject should be documented as screening failure on the study screening log, and the subject is terminated from the study.

7.2 Waiting List / Baseline Visit

During the period when the subject is awaiting for their first transplant, they will return to the study site for clinic visits every 6 months. Waitlist assessments will be repeated at pre-defined intervals as detailed below. Results from assessments done closest to the date of randomization will be used as the subject's baseline values.

Waitlist Assessments	*Timetable for repeat testing while on the waiting list*
Retinopathy Evaluation; Physical Exam; Chest x-ray; ECG; Coagulation Status (APTT, PK, fibrinogen, platelets); CGMS; Serology (HIV, Hepatitis B, Hepatitis C	*Once a Year*
CBC with differential; Serum Chemistry Panel; Glycemic Lability- LI (repeat only if used as inclusion criteria); Glycemic Lability (MAGE); Alloantibodies; Quality of Life Questionnaires (SF36, DTSQ); HbA1C; Blood Sugar Records and Hypo sheets; Full Hypo Score; Clarke Score.	*Once every 6 months*
Baseline Assessments	
Re-evaluation of the eligibility criteria; Record concomitant medications; CMV and EBV by PCR; Autoantibodies; RNA and Plasma to Archive; Alloantibodies; Physical Exam; CBC with differential; Serum Chemistry Panel; Coagulation Status (APTT, PK, fibrinogen, platelets); Blood Sugar Records and/or Hypo sheets; Serology (HIV, Hepatitis B, Hepatitis C); Chest x-ray; ECG; Cross Match; Serum Pregnancy Test; HbA1C	*At the time the pancreas becomes available, prior to transplant*

Table 3. Baseline/Waiting List Assessment Windows

7.3 Randomization and Study Treatment Schedule

Once a compatible islet prep becomes available, then the site personnel will re-confirm the eligibility criteria. Eligible subjects will be randomized (1:1), to the experimental arm or control arm. A total of 36 subjects will be randomized; 18 subjects to an experimental arm and 18 subjects to a control arm.

7.3.1 Experimental Arm ("LMW-DS" Study Arm)

Eligible type I diabetic (T1D) subjects randomized to the "LMW-DS" arm will receive islets fulfilling release criteria from the islet isolation lab. Randomized subjects will receive protocol immunosuppression and LMW-DS, excluding heparin treatment, and in addition receive 1) and 2) below:

1. A bolus dose of 1.5 mg LMW-DS/kg BW given intravenous, another 3.0 mg LMW-DS/kg BW distributed in the islet bags given intraportally, and
2. Continuous intravenous infusion of LMW-DS started immediately after islet infusion and maintained for 5 hours, carefully adjusted to target an APTT of 150±10s according to Instructions for Administration of LMW-DS to Target APTT of 150+/- 10s Worksheet (available in the Manual of Procedures, as well as on the CIT Website).

7.3.2 Control Arm ("State of the Art")

Eligible Type I diabetic subjects randomized to the "State of the Art" arm will receive islets fulfilling release criteria from the islet isolation lab. In addition, randomized subjects will receive protocol immunosuppression without LMW-DS, including anticoagulative treatment with heparin. A dose of 70 U/kg body weight of recipient with the islet infusion, followed by a continuous intraportal infusion of heparin targeting an APTT of 50±10s for the next 5 hours will be administered.

7.4 Transplantation

At least 5000 IEQ/kg shall be given at the first transplantation, and 4000 IEQ/kg for the second or third transplant.

7.4.1 First Islet transplantation (1)

The primary endpoint is determined based on the first islet transplantation. If necessary, a second islet transplant may occur at least 75 days after the first transplantation, and a third islet transplant may occur at least 28 days after the second islet transplant. All subsequent transplants must take place prior to 8 months after the first islet transplant.

Islets are suspended in medium and injected through a catheter placed in the portal vein, either through transhepatic cannulation or through a minimal surgical incision.

7.4.2 Criteria and Timing for Subsequent Islet Transplant

If repeated transplantation (s) are needed the subject will follow the same protocol and will remain in the randomized study group previously assigned. Subjects who do not meet criteria for a subsequent transplant will enter the reduced follow-up schedule (Appendix 2).

Partial Graft Function: Islet transplant recipients who do not meet criteria for full islet graft function, but have either a basal or stimulated c-peptide level ≥0.1 nmol/L (≥0.3ng.mL), will be considered partial islet graft function.

Graft Failure

Islet transplant recipients who have graft failure (absence of insulin production, with (c-peptide <0.1 nmol/L (<0.3 ng/mL) are eligible to undergo a second islet transplant if they meet the eligibility criteria described below:

- Prior to day 75, repeat transplantation within the CIT protocol must be approved by the CIT Steering Committee, based upon review of clinical information provided by the transplanting site to the DCC.
- After day 75, repeat transplantation must be approved by the Nordic Network Steering Committee, based on clinical information provided by the site. The decision of the Nordic Network Steering Committee will be provided to the sponsor.

Clinical information provided must include:

1) Results of graft failure assessments
    a) random c-peptide <0.1 nmol/L (<0.3 ng/mL)
    b) c-peptide <0.1 nmol/L (<0.3ng/mL) at baseline, and at 60 and 90 minutes after MMTT.
2) Post transplant clinical data
3) Potency testing from the first transplant product
4) Additional assessments as requested from the CIT Steering Committee.

Eligibility criteria include:

1. Subject has been compliant with study monitoring and prescribed immunosuppressive therapy;
2. No evidence of a serious and life-threatening infection, adverse event, or other condition that precludes attempting an intraportal injection or continuation of the post-transplant treatment regimen;
3. No evidence of post-transplant lymphoproliferative disorder (PTLD);
4. No evidence of progressive renal dysfunction, defined as creatinine rising above 2.0 mg/dL (177 µmol/L) with calcineurin inhibitor trough levels within maintenance levels;
5. No evidence of hypersensitization, allergic responses, or other potentially serious drug reactions to medications required by the protocol.
6. Less than 8 months has passed after the first transplantation.
7. Absence of any medical condition that, in the opinion of the investigator, will interfere with a safe and successful second islet transplant.

If after the second islet infusion both basal and stimulated C-peptide levels remain <0.1nmol/L (<0.3 ng/mL), these recipients will be considered treatment failures with no islet graft function, and immunosuppression will be withdrawn.

Islet transplant recipients who do not meet criteria for full islet graft function **after the second islet infusion**, but before 8 months from the first infusion will be considered for a **third islet infusion**.

The option of a **third islet infusion** under this protocol will be considered if the subject is ≥28 days (+/- 3 days) following the second islet infusion, and if all of the following conditions are met:

1. The subject remains without full islet graft function;
2. There is evidence of partial graft function (C-peptide > 0.1nmol/L (>0.3ng/mL);
3. No evidence of post-transplant lymphoproliferative disorder (PTLD);
4. The CIT Principal Investigator and Site Principal Investigators have determined that there were no relevant protocol deviations at the site;
5. The subject has been compliant with study monitoring and prescribed immunosuppressive therapy;
6. No evidence of a serious and life-threatening infection, adverse event, or other condition that precludes attempting an intraportal injection or continuation of the post-transplant treatment regimen;
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 upper limit of the normal range prior to the third transplant.
10. Less than 8 months has passed after the first islet transplantation.

Participants who have completed 8 months follow up post first infusion will no longer be eligible for additional islet infusions funded under this protocol.

7.5 Follow Up Schedule and Procedures

Subjects will undergo clinical evaluation by the investigator and/or their designee at screening, baseline, transplantation, days 1, 3, 7, 14, 21, 28, 75, and months 6 and 12 (Appendix 1). Clinical safety will be monitored through routine physical examinations and appropriate laboratory assessments. During the study, subjects will have repeated clinical and laboratory evaluations, as specified in the Schedule of Events. The timing of all follow-up assessments will reset at the time of subsequent transplants (i.e., the day of the second transplant becomes day 0, and all assessments are conducted in relation to this day).

Evaluations should be made for sirolimus, cyclosporine and tacrolimus levels, as applicable. Immunosuppression levels will be monitored from time of transplantation until the end of the study.

7.6 Subject Self Monitoring Plasma Glucose

Investigators should instruct subjects to measure and keep notes of pre and postprandial plasma glucose and requirement of insulin, from the time of enrollment until completion of the study. Data should be recorded before breakfast, at lunch and dinner, and then two hours after each of these meals, and once in the evening. If the subject becomes free of insulin it is sufficient to record the data of blood/plasma glucose two days per week up until 12 months post transplant. All subjects will use the Ultra One Touch® glucose monitoring units, provided by the study center. Subject diaries should be collected as specified in the Schedule of Events, and kept as source documentation.

7.7 Visit Windows

VISIT NO.	VISIT	VISIT WINDOW
01	Screening/Enrollment	Date written informed consent is obtained until all information / results for evaluation of inclusion/exclusion criteria are complete.
02	Baseline Visit / Waiting List	According to the pre-defined schedule detailed in the Schedule of Events (Appendix 1)
03	Transplant	Date of the islet transplantation
04-05	Day 1, Day 3	± 0 days
06-09	Days 7, 14, 21, and 28	± 3 days
10	Day 75	± 5 days
11-12	Month 6, Month 12	± 14 days

Table 4. Visit Windows

If the patient requires additional islet transplantation(s), the previous follow-up period stops and a new visit period starts, beginning with the Baseline Visit.

7.8 Study Treatment Assignment Procedures

7.8.1 Blinding and Randomization

This is an open-label study; therefore, no treatment codes are required for the assignment of study drug. Randomized treatment assignments will be developed by CIT-DCC statisticians and stored on the CIT-DCC server. The CIT-DCC will maintain a central web-based randomization system for the study. When a subject has been determined to be eligible, the site personnel will access the appropriate web page and complete the randomization procedures. At that time the system will verify that the patient is indeed eligible. If the patient is eligible, the site personnel and the islet laboratory (Rudbeck Laboratory, Uppsala, Sweden) will receive an email notification that the subject was successfully randomized, and will be provided the randomization assignment. The date and time of the randomization will be recorded in the database. Once a treatment assignment has been provided, the patient becomes a part of the intention-to-treat population. All randomized subjects will be included in the primary analysis for the study and will be assigned to the treatment to which they were randomized. A parallel and equivalent electronic telephone based system will provide back-up in the unlikely event that the web-based system is not available.

8. SAFETY MONITORING

Adverse events (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, principal investigators, and the ethics committee (EC). 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 current version of the Terminology Criteria for Adverse Events in Trials of Adult Pancreatic Islet Transplantation CIT-TCAE. This document, created by the Clinical Islet Transplantation (CIT) Consortium, modifies the National Cancer Institute (NCI), *Common Terminology Criteria for Adverse Events (CTCAE) version 3.0* (June 10, 2003), to ensure applicability in the setting of Islet Transplantation.

8.1 Definitions

8.1.1 Adverse Event (AE)

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 (SAE)

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 a protocol-mandated procedure do not need to be reported as an SAE, unless the hospitalization is prolonged due to complications.
4. Persistent or significant disability.
5. 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.
6. Congenital anomaly or birth defect.
7. Other conditions specified in the protocol.

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.

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

8.1.3 Unexpected Adverse Event

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

(SPC), for an authorized medicinal product in the European Community, which is being used according to the terms and conditions of the marketing authorization.

8.2 Adverse Events

8.2.1 Collecting Procedure

AEs that are associated with a protocol-mandated procedure, that is not part of the normal standard of care, and hypoglycemic events will be collected beginning immediately after the consent document has been obtained. All other AEs will be collected 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. AEs will be followed until the time the event is resolved, stabilized, or the participant completes or withdraws from the study, whichever occurs first.

AEs may be discovered through any of these methods:

- Observing the subject.
- Questioning the subject, this 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.

8.3 Recording Procedure

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

8.3.1 Grading and Attribution

GRADING CRITERIA

The study site will grade the severity of adverse events experienced by CIT study subjects according to the criteria set forth in the current version of the *Terminology Criteria for Adverse Events in Trials of Adult Pancreatic Islet Transplantation.* This document (referred to herein as the *CIT-TCAE* manual) provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all adverse events.

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 adverse event
Grade 2 = moderate adverse event
Grade 3 = severe and undesirable adverse event
Grade 4= life-threatening or disabling adverse event
Grade 5 = death

Grade 2 and higher adverse events will be reported. Grade 1 events do not require reporting.

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

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.

Table 5. General Grade Definitions

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

DEFINITION OF ATTRIBUTION

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

The relatedness, or attribution, of an adverse event to an investigational product will be determined by the site investigator. The site investigator will also record the determination of attribution on the appropriate CRF and/or SAE report form. The relationship of an AE to the study treatment will be defined by using the descriptors provided below.

Code	Descriptor	Definition
UNRELATED CATEGORY		
1	Unrelated	The adverse event is clearly not related to the investigational agent(s).
RELATED CATEGORIES		
2	Unlikely	The adverse event is doubtfully related to the investigational agent(s).
3	Possible	The adverse event may be related to the investigational agent(s).
4	Probable	The adverse event is likely related to the investigational agent(s).
5	Definite	The adverse event is clearly related to the investigational agent(s).

Table 6. CIT-TCAE attribution of adverse events

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

8.3.2 Outcome Categories and Definitions:

All AEs classified as serious or severe or possibly/probably related to the trial product must be followed until the patient has recovered, stabilized, recovered with sequelae or died, and until all queries related to these adverse events have been resolved.

All other AEs must be followed until the patient has recovered or stabilized or until 30 days after the end of the trial, and until all adverse event related queries for the patient have been resolved.

Furthermore, the sponsor will at examination of the SAE reports evaluate which reports that should be reported to the concerned competent authorities and Ethic Committees according to the criteria's outlined below.

- **Recovered**= Fully recovered or by medical or surgical treatment the condition has returned to the level observed from the first trial related activity after the patient signed the informed consent.
- **Stabilized**= This outcome should only be used for cancer events and chronic conditions that cannot be normalized by medical or surgical treatment. This term should only be used when the patient has completed the protocol.
- **Recovered with Sequelae**= As a result of the AE, the patient suffered persistent and significant disability/incapacity (e.g. became blind, deaf, paralyzed). Any AE recovered with sequelae should be rated as an SAE.
- **Not yet recovered**
- **Fatal**
- **Unknown**

8.4 Serious Adverse Events

8.4.1 Collecting Procedure

Serious adverse events (SAEs) will be collected following the subject's written consent to participate in the study until 30 days after the participant completes or withdraws from the study. SAEs will be followed until the time the event is resolved, stabilized, or until 30 days after the subject completes or withdraws from the study, whichever comes first.

8.4.2 Recording Procedure

SAEs will be recorded on the AE CRF and on the SAE form. The DCC will complete the CIOMS form for submission to the European Health Authorities.

8.4.3 Reporting Procedure

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

8.4.3.1 REPORTING CRITERIA FROM SPONSOR TO HEALTH AUTHORITY

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

- **No reporting.** This requirement applies if the AE is deemed not serious by the DCC medical reviewer and the NIAID / NIDDK medical monitor.
- **Standard reporting** (i.e., will be included in the annual report to the health authorities). This requirement applies if the AE is classified as any of the following:
    1. Serious, expected, and drug related.
    2. Serious, expected, and *not* drug related.
    3. Serious, *unexpected,* and not drug related.

- **Expedited reporting.** This requirement applies if the AE is considered serious, unexpected, and drug related. 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.

8.4.3.2 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. The investigator must ensure that these events are entered on the 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.

8.4.3.3 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 from the clinical site. The sponsor is responsible for disseminating appropriate reports to the health authorities, and all investigators in the study. SAEs per 21 CFR 312.32 definitions, except elective hospitalizations, will be reported to the Health Authority by the study sponsor (NIAID) in accordance with applicable regulations.

8.4.3.4 NOTIFYING THE DATA AND SAFETY MONITORING BOARD

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

8.4.3.5 NOTIFYING ETHICS COMMITTEE

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

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

8.4.4 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.

8.5 Suspected Unexpected Serious Adverse Reactions (SUSARs)

All suspected adverse reactions related to the investigational medicinal product (IMP, i.e. LMW-DS) that are both unexpected and serious are subject to expedited reporting.

The sponsor shall report all the relevant safety information regarding fatal or life-threatening SUSARs to the concerned competent authorities and Ethic Committees as soon as possible but not later than 7 calendar days after the sponsor has first knowledge of the minimum criteria for expedited reporting. In

each case relevant follow-up information should be sought and a report completed and communicated to the competent authorities and Ethic Committees within an additional eight calendar days.

The sponsor shall inform all investigators concerned of relevant information about SUSARs that could adversely affect the safety of subjects.

8.6 Other safety issues requiring expedited reporting

All non fatal and non life-threatening SUSARs must be reported to the competent authority and the Ethics committee in the concerned Member States as soon as possible but no later than 15 calendar days after the sponsor has first knowledge of the minimum criteria for expedited reporting. Further relevant follow-up information should be given as soon as possible. The same time frame shall also be used for expedited reporting of safety issues that might alter the current benefit-risk assessment of the IMP or that would be sufficient for considering changes in the IMP administration or in the overall conduct of the trial, for instance;

- Single case reports of an expected serious adverse reaction with an unexpected outcome (e.g.: a fatal outcome)
- An increase in the rate of occurrence of an expected serious adverse reaction, which is judged to be clinically important
- Post-study SUSARs that occur after the patient has completed a clinical trial and are reported by the investigator to the sponsor

9. MECHANISTIC ASSAYS

9.1 Metabolic Testing

All subjects will use a study provided One Touch Ultra glucometer or an approved glucometer and/or CGMS unit identified in the MOP for measuring capillary glucose levels. Investigators should instruct subjects to measure and keep notes of pre and postprandial plasma glucose and requirement of insulin. Data should be recorded before breakfast, at lunch and dinner, and then two hours after each of these meals, and once in the evening. If the subject becomes free of insulin it is sufficient to record the data of blood/plasma glucose two days per week up until 12 months post transplant. Subject diaries should be collected as specified in Appendix I, Schedule of Events, and kept as source documentation. Glucometers will be collected and measurements downloaded as specified in the Schedule of Events.

9.1.1 Study Endpoints

Designated primary and secondary endpoints will be employed to compare each treatment group ("LMW-DS" and "State of the Art"). Because the assessment of islet graft function is dependent on complex physiologic relationships between the graft and its recipient, a number of different tests will be performed. The stimulated c-peptide for the Mixed Meal Tolerance Test (MMTT) will be used as the primary endpoint, and additional stimulatory tests of islet graft function utilizing 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 quality of life (QOL) will be assessed as additional secondary endpoints. (See section 4.1 for endpoint description).

9.1.1.1 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 5-9 mmol/L and avoiding hypoglycemia.

9.1.1.2 GLYCEMIC CONTROL

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

9.1.1.3 GLYCEMIC LABILITY

Glycemic lability will be assessed by both the mean amplitude of glycemic excursions (MAGE) and the lability index (LI).

The MAGE requires 14 – 16 capillary blood glucose (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 ($mmol/L^2/hr \cdot wk^{-1}$). A LI greater than or equal to the 90th percentile (433 $mmol/L^2/hr \cdot wk^{-1}$) of values derived from an unselected group of type 1 diabetes (T1D) patients is evidence for severe glycemic lability.

9.1.1.4 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 seizures, 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.

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

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.

9.1.1.5 MIXED-MEAL TOLERANCE TEST (MMTT)

Basal (fasting) and stimulated glucose and c-peptide levels will be determined using the MMTT. Subjects will be instructed not to eat or inject short-acting (or bolus) insulin after 8 PM the night before the test. Evening or bedtime administration of long-acting insulin will be permitted, as will consumption of water. Subjects receiving CSII (insulin "pump" therapy) may remain on the basal rate of insulin. Subjects will arrive fasting to the transplant or diabetes clinic where the capillary BG will be checked. If the BG is < 70 mg/dL (3.9 mmol/L) or > 136 mg/dL (8.0 mmol/L), the test will be rescheduled for the next possible day. If the BG is 70 – 136 mg/dl (3.9-8.0 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 Resource Protein Drink (or a nutritionally equivalent substitute) to consume in 5 minutes starting at time = 0. Then, at time =15, 30, 60, 90 and 120 minutes, stimulated glucose and c-peptide levels will again be drawn.

Each blood sample for c-peptide and glucose determination will be drawn according to University of Washington (Seattle, WA) standard protocol. Each sample will be shipped frozen to the University of Washington for measurement by the core laboratory.

9.1.1.6 β-Score: a composite index of post-transplant graft function

The β-score will be determined from the HbA1c, insulin requirements, fasting (basal) glucose, and stimulated c-peptide as developed by Ryan et al. 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.

9.1.1.7 THE C-PEPTIDE TO GLUCOSE, CREATININE RATIO

The C-peptide to glucose, 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. 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 with the β-score.

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

The acute insulin response to glucose (AIR_{glu}), insulin sensitivity (S_I), and disposition index (DI) will be determined using the FSIGT test. This assessment provides a composite measure of β-cell function, the disposition index (DI), which relates the effect of insulin sensitivity (S_I) 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 (Rickels MR et al., unpublished data). These results require confirmation by longitudinal analysis.

The test will start between 8 and 10am. The subjects must have fasted overnight, and have abstained from alcohol and followed a proper diet maintained for 3 days prior to the test. If the subject is on insulin, an ordinary NPH dose may be given at 21:00 the day prior to the test, or alternatively 50% of their regular night dose of glargine or determir. If needed, they are kept euglycemic overnight with an IV insulin infusion according to local algorithm.

Before the test is started, plasma glucose must be between 3.9-7.8 mmol/L in a pre-transplant subject, and between 3.9-6.4 mmol/L in the post-transplant subject.

The insulin-modified FSIGT test involves blood sampling at baseline (t = -10, -5, and -1 min) and at t = 1, 2, 3, 4, 5, 7, 10, 12, 14, 16, 18, 20, 22, 25, 30, 40, 50, 70, 100, 140, & 180 minutes post-injection of glucose at t = -30 seconds with an injection of insulin (0.03 U/kg over 30 seconds) 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 basline (t=-10, -5, and -1 min) and the 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 the University of Washington (Seattle, WA) standard operating procedures and shipped frozen for measurement in the core laboratory. The acute insulin response to glucose (AIR_{glu}) is calculated as the incremental area-under-the-curve for insulin between 0 and 10 minutes post-injection (the same calculation can be performed for C-peptide). Glucose effectiveness (S_G), a measure of insulin-independent glucose disposal, and insulin sensitivity (S_I), a measure of insulin-dependent glucose disposal, are derived from Bergman's minimal model using MinMod Millenium® software, and further allow for determination of the disposition index ($DI = AIR_{glu} \cdot S_I$).

9.1.1.9 CONTINUOUS GLUCOSE MONITORING SYSTEM® (CGMS)

Glucose variability and hypoglycemia duration will be determined using CGMS (Medtronic Minimed, Northridge, CA). CGMS involves the subcutaneous (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 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.

9.1.1.10 QUALITY OF LIFE (QOL)

Quality of life (QOL) will be assessed using the DTSQs and DTSQc, and the SF36 patient questionnaires.

9.1.1.11 POSITRON EMISSIONS TOMOGRAPHY (PET)

In a subset of subjects (n=8, 4 LMW-DS, 4 Control) at selected sites, a minority (100 000 IEQ) of islets will be labeled with FDG and a PET/CT scan will be performed at the first transplantation. Procedures will follow established SOPs from the Nordic Network. Subjects will be informed about this sub-study after randomization and a separate informed consent will be obtained.

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.

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 Local Center Laboratory

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 the individual centers. HLA typing should not be repeated if previous test results are readily available. A negative crossmatch is required in order for transplantation to occur.

9.2.2 Core Laboratories

9.2.2.1 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. The University of Pennsylvania and The Rudbeck Laboratory will provide core laboratory services for alloantibody assessments.

9.2.2.2 AUTOANTIBODY

The role of autoantibody in graft loss remains unclear. The Barbara Davis laboratory in Denver, CO will provide core lab service for autoantibody assessments.

9.2.2.3 ARCHIVED SERUM, CELLS, RNA, AND PLASMA

Specimens that are collected for the purpose of archiving will be registered in the biobank, re-labeled and shipped from Uppsala, Sweden to the NIDDK repository. The study Manual of Procedures details the procedures for de-identifying and batch shipping specimens to the United States.

In order to ensure that we will ultimately gain as much information as possible from these trials, and due to the ongoing development of, e.g., T cell assays, serum, cells and RNA will be archived for future analyses.

Serum (NIDDK Repository)

- Blood will be collected to obtain serum. Specimens will be collected, processed, and shipped frozen according to procedures outlined in the CIT-01 Laboratory Manual.

Peripheral Blood Mononuclear Cells (PBMC) and Plasma (NIDDK Repository)

- Blood will be collected to obtain recipient PBMC and Plasma. The specimen will be collected, processed and shipment frozen according to procedures outlined in the CIT-01 Laboratory Manual.

RNA (NIDDK Repository)

- Blood will be collected to obtain RNA. Specimens will be collected, processed, and shipped frozen according to procedures outlined in the CIT-01 Laboratory Manual.

RNA and Plasma (The Rudbeck Laboratory)

- Blood will be collected to obtain RNA. The specimen will be collected, processed, and shipped frozen to The Rudbeck Laboratory, according to procedures outlined in the CIT-01 Laboratory Manual.

9.2.2.4 THROMBIN-ANTITHROMBIN COMPLEXES, C3A AND C-PEPTIDE

IBMIR is triggered by tissue factor expressed by the islets of Langerhans. After infusion of the islet into the portal vein the IBMIR is triggered by tissue factor (TF). IBMIR is reflected in the generation of the coagulation and complement markers, thrombin-antithrombin (TAT) and C3a, and release of C-peptide from the islet indicates islet damage. C-peptide, TAT and C3a are assayed by ELISA. There are no international calibrators for TAT or C3a which necessitates that these samples are measured at the same site if inter-laboratory variations are to be avoided.

Thrombin-antithrombin (TAT) complexes have been instrumental in demonstrating IBMIR in subjects. Initial high levels have also been shown to correlate with low levels of fasting C-peptide 7-14 days after transplantation indicating poor function. Complement activation and generation of C3a is associated with a strong IBMIR. Release of C-peptide directly after the islet infusion reflects damage to the islets.

The Rudbeck Laboratory in Uppsala, Sweden will provide core lab service for TAT, C3a, and C-peptide assessments. In order to correlate expression of proinflammatory or procoagulant markers on islets with recipient response in the early post-transplant period, EDTA anti-coagulated blood (3 mL at each time point) will be collected immediately prior to islet infusion, when 125 mL is left in the infusion bag (before rinsing), and at 0, 15, 60, 180, 270, 360 minutes after the completion of the islet infusion, and 24 hours post-infusion for assessment of TAT, C3a and c-peptide levels. The EDTA-blood should be centrifuged and the plasma stored at -70°C. The samples shall be shipped (without previous thawing) on dry ice to The Rudbeck Laboratory in Uppsala, Sweden.

9.2.2.5 ADDITIONAL ANALYSES

Additional analysis will also be performed to further characterize the IBMIR, growth factors and additional inflammatory parameters. These analyses, the time points and the amount of blood required are summarized in Appendix I- Schedule of Events.

10. STATISTICAL CONSIDERATIONS AND ANALYTICAL PLAN

10.1 Analysis Samples

The primary analysis will use the intent-to-treat principle and all subjects will be analyzed in the group to which they were randomized. Safety analyses will be based on treatment received. A secondary per protocol analysis will include data for those subjects who provided data at 75 days and who had no major protocol deviations.

10.2 Study Endpoint Assessment

The details of the analyses will be provided in the statistical analysis plan (SAP). The following provides an overview of the planned analyses.

10.2.1 PRIMARY ENDPOINT

The primary endpoint is the level of stimulated c-peptide at 90-minutes derived from the mixed-meal tolerance test (MMTT) at 75±5 days following the first islet infusion. The difference in the means between the two treatment groups will be used as the measure of efficacy. Because the sample size is small, it is not practical to adjust for treatment center. The primary analysis will be based on an independent samples two-sided t-test. A difference will be declared statistically significant if $p \leq 0.05$. The effect size will be estimated by the observed difference in the means for the two treatments. The estimated effect size and a 95% confidence interval for the effect size will be reported. If there is compelling evidence that the normal distribution does not hold then a logarithmic or square root transformation will be done before using the t-test. Appropriate adjustments will be made to compute the estimated effect size and its confidence interval.

The primary endpoint should be available in all randomized subjects. However, should a value not be available for a subject then a value will be imputed in one of two ways. If a value is observed for a subject after the 75 day period but before any subsequent islet infusion then that value will be used in the analysis. If no later value is available (e.g. the subject dies or withdraws from the study) then the lowest value observed for all subjects will be imputed for that subject. All imputations will be reported with the primary analysis. A secondary sensitivity analysis using multiple imputation methods will be performed to examine the effect of the choice of this imputation strategy.

10.2.2 SECONDARY ENDPOINTS

The secondary endpoints are defined in section 3.1.2.

HbA1c levels will be categorized for example, as either normal (<= 7.0 %), or non-normal (>7.0%). Dichotomous variables such as HbA1c (normal/non-normal) and rate of severe hypoglycemic events will be analyzed using logistic regression analysis with a term for treatment. Should a dichotomous endpoint not be evaluated for a particular individual then a failure will be imputed unless an evaluation is done at a time longer than 75 days after transplant and before an additional islet cell infusion, in which case that later value will be imputed.

Continuous variables such as QOL scales will be analyzed by a t-test. If there is compelling evidence that the normal distribution does not hold then a logarithmic or square root transformation will be done before using the t-test. If lack of normality persists, non-parametric tests will be used.

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

Regression models for longitudinal data (mixed models) will be used to examine the differences between groups in each of the response variables (HbA1c level and C-peptide levels) where measurements are repeated over time. Mixed models for dichotomous variables will be used to model full islet graft function over time. Survival analysis models will be used to compare time to becoming insulin dependent and to identify risk factors through Kaplan-Meier estimates and Cox regression models.

10.3 Patient and Demographic Data

10.3.1 Baseline Characteristics and Demographics

Summary descriptive statistics for baseline and demographic characteristics will be provided for all enrolled subjects, according to the NIH guidelines.

Statistical presentation for baseline and demographic characteristics may be further summarized and will be further defined in the statistical analysis plan (SAP).

10.3.2 Medical History

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

10.3.3 Use of Medications

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

10.3.4 Study Completion

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

10.4 Sample Size and Power Calculations

The selected sample size is 18 subjects in each of the 2 arms. Historical data on 10 subjects from The University of Alberta suggest a standard deviation in the control group of approximately 0.9 nmol/L. Power calculations assumed that a two-sided 5% level t-test with equal variances would be used for the analysis. Based on a standard deviation of 0.9 nmol/L, this study will have 90% power to detect a difference of 1.0 nmol/L between the mean for the LMW-DS treatment group and the mean for the control group. The study will be able to detect a difference of 0.9 nmol/L with 80% power.

10.5 Interim Analyses to Ensure Patient Safety

The NIDDK DSMB will be convened to review safety data periodically. When an official interim analysis is requested the DCC will prepare interim analyses that will include distributions of primary and secondary endpoints, biomarkers and adverse events by treatment group. Safety reports will

summarize all reported serious adverse events and adverse events. Adverse events will be summarized for each body system and for all body systems combined. Additional analyses may be requested by the DSMB.

10.6 Statistical Guidelines for Terminating the Study

We have prepared a monitoring plan based on using the Lan and Demets alpha spending method with an O'Brien-Fleming spending function [43]. This strategy accounts for the multiple analyses associated with the interim monitoring and allows the DSMB to request analyses at times that are not fixed at the beginning of the study. The method is very conservative early and therefore has very little effect on the overall power of the study. The details of the monitoring plan are included in the SAP.

Table 6 displays an example using this strategy with planned interim analyses when 12 subjects (6 in each group) have finished the 75 day follow-up period, a second interim analysis when 24 subjects (12 in each group) have finished, and a final analysis when all 36 subjects have completed the 75 day follow-up. These calculations were based on a 5% two-sided t-test to compare two independent means and no stopping for futility. The program PASS [44] was used to calculate the values in the table.

This strategy recommends stopping after 12 subjects have completed 75 days if the standard z-test for a difference in the two means exceeds 3.71 in absolute value. This is equivalent to requiring that the p-value for the test is less than or equal to 0.0002. The plan would recommend stopping after 24 subjects, if the absolute value of the test statistic exceeds 2.51. This requires an observed p-value less than or equal to 0.0120. If the plan recommends stopping after either interim analyses and the difference is in favor of the LMWDS group then one would conclude that LMWDS is superior to the control. If the difference is in favor of the comparison group then one would conclude that LMWDS is inferior to the control. The stopping boundaries for this example are displayed in Figure 5 [45].

At the final analysis one would conclude that LMWDS is superior to the control if the difference is in favor of LMWDS and the p-value is less than or equal to 0.0463. If the difference is in favor of the comparison group and the p-value is less than or equal to 0.0463 then one would conclude that the LWMDS is inferior to control. If the p-value is greater than 0.0463 then one would conclude that there is not enough evidence to conclude that the two interventions are different.

Interim Analysis	Number of subjects completing 75 day follow-up	Boundary Values For Z Test		Associated p-value
		Lower Boundary	Upper Boundary	
1	12	-3.71	3.71	0.0002
2	24	-2.51	2.51	0.0120
3	36	-1.99	1.99	0.0463

Table 7. Stopping Boundary Values

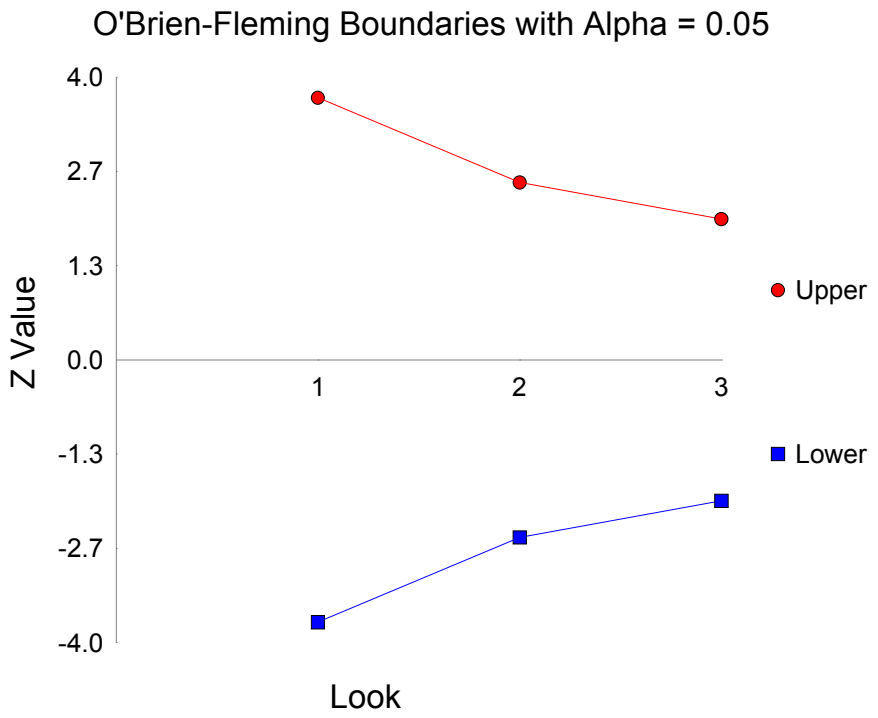


Figure 5. Stopping Boundary

Should the effect be found to cross the monitoring boundary before the end of the study, the DSMB will advise NIH on whether enrollment should be terminated early. Subjects already enrolled in the study will continue with their assigned treatment unless the DSMB recommends otherwise.

10.7 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. 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 identify 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 Principal Investigator 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.

During the course of the study, all data will be entered, stored, and managed in a relational database supported by database servers at the CIT-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 Case Report Forms (CRF).

Once the analysis phase is complete, all clinical and mechanistic data will be archived for a minimum of 10 years. After 10 years, the data will be de-identified and archived for a minimum of 5 years. The data will be archived in the ImmPort System managed by the Northrop Grumman Information Technology Health Solutions team http://www.immport.org/immportWeb/home/home.do). The ImmPort System is a long-term, sustainable archive of data generated by investigators funded through the National Institutes of Allergy and Infectious Diseases (NIAID).

In order to maintain security, all data will be encrypted using the Secure Sockets Layer (SSL) protocol. This protocol allows an encrypted link to be established between the CIT-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 CIT-DCC personnel and certified clinical center coordinators to make changes. Changes can be initiated by CIT-DCC monitors, CIT-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 CIT-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 current good clinical practices (cGCP), as delineated in *Guidance for Industry: E6 Good Clinical Practice Consolidated Guidance*[46], 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 the NIAID/NIDDK. Any amendments to the protocol or to the consent materials must also be approved by the IRB/IEC 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 documents not in the primary language of the subject must be translated into the subject's 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 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 Declaration of Helsinki

This study will be conducted in accordance with the Declaration of Helsinki.

13.4 Permission to Review Source Records

In accordance with the European Guidelines on Good Clinical Research Practice, it is desirable to check case report forms against original patient records. Therefore, the investigator agrees that the sponsor, the sponsor's representatives, the CRO, its employees or agents will have the right to audit and review pertinent medical records relating to this clinical trial.

13.5 Unanticipated Events

Any changes in the study or unanticipated events involving risks to the subjects must be reported promptly to the Ethics Committees.

13.6 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.

APPENDIX 1: SCHEDULE OF EVENTS

				DAY								MONTH		1 Yr post 1st Tx [2]
Time points	**Screen**	**WL**	**BL**	**Transplant**	**1**	**3**	**7**	**14**	**21**	**28**	**75 [1]**	**6**	**12**	
Visit Number	**01**	**02**		**03**	**04**	**05**	**06**	**07**	**08**	**09**	**10**	**11**	**12**	**Y1**
Visit Windows (Days)	**N/A**			**±0**	**±0**	**±0**	**±3**	**±3**	**±3**	**±3**	**±5**	**±14**	**±14**	**±14**
GENERAL ASSESSMENTS														
Informed consent	X													
Evaluation of Eligibility Criteria	X		X											
Quality of Life (SF36, DTSQ) [24]	X	X									X [1]		X	X
Medical and Diabetes History	X													
Retinopathy Evaluation	X	X												X
Physical Exam and Vital Signs (Weight, Height [3], BMI [3], Temperature, Pulse, Blood Pressure)	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Chest X-Ray	X	X	X											
Abdominal Ultrasound	X			X	X									
ECG, Myocardial Scintigram	X	X[4]	X[4]											
Conduction Velocity and RR intervals	X												X	X
Immunosuppression Medications				→	→	→	→	→	→	→	→	→	→	→
Concomitant Medications			→	→	→	→	→	→	→	→	→	→	→	→
Assessment of AE, SAE, Infections, Hospitalizations, Rejections	→	→	→	→	→	→	→	→	→	→	→	→	→	→
Blood Sugar Record eCRF	X[5]	X[5]	X[5]								X[5]	X[5]	X[5]	X[5]
Glucometer Download	X[5]	X[5]	X[5]	X[5]			X	X	X	X	X[5]	X[5]	X[5]	X[5]
Insulin Requirement (Total U/24 h) [15]	X[5]	X[5]	X[5]	X[5]			X	X	X	X	X[5]	X[5]	X[5]	X[5]
Portal Pressure				X [6]										
LOCAL LABORATORY ASSESSMENTS														
HLA, Blood Type	X													
Cross Match			X											
CBC with differential [7]	X	X	X	X [7]	X [7]	X [7]	X	X [7]	X [7]	X	X	X	X	X
Serum chemistry panel [8] and CRP	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Coagulation Status (APTT, PK, fibrinogen, platelets)	X	X[9]	X	X[9]	X	X	X	X	X	X	X		X	X
Urine Albumin	X										X		X	X
Fasting Lipid Profile (Total, LDL, HDL, Triglycerides)	X			X						X	X	X	X	X
Glomerular Filtration Rate (GFR)	X										X [10]		X	X
Serum Pregnancy Test	X		X[11]											
Fast. & Postprandial Plasma Glucose						X	X	X	X	X[12]				
Fasting & post-prandial C peptide						X	X	X	X	X[12]				
HIV, Hepatitis B, Hepatitis C [13]	X	X[13]	X[13]										X	
CMV IgG, IgM	X												X	
EBV IgG	X													
CMV by PCR			X								X	X		
EBV by PCR			X											
IMMUNOSUPPRESSION TROUGH LEVELS														
Tacrolimus Or Cyclosporine Levels [14]				X	X	X	X	X	X	X	X	X	X	X
Sirolimus Levels [14]				X	X	X	X	X	X	X	X	X	X	X
STUDY TREATMENT														
Islet Transplant Waiting List		X												
Identify Compatible Donor		X												
Randomization				X										

Refer to section 7.2 Wait List/ Baseline Visit Assessments for frequency of testing during the WL period.
Assessments marked in BL column must be completed prior to transplant.

	Pre-Transplant			DAY								MONTH		1 Yr post 1st Tx [2]
Time points	**Screen**	**WL**	**BL**	**Transplant [1]**	**1**	**3**	**7**	**14**	**21**	**28**	**75 [1]**	**6**	**12**	
Visit Number	**01**	**02**		**03**	**04**	**05**	**06**	**07**	**08**	**09**	**10**	**11**	**12**	**Y1**
Visit Windows (Days)	**N/A**			**±0**	**±0**	**±0**	**±3**	**±3**	**±3**	**±3**	**±5**	**±14**	**±14**	**±14**
CENTRAL METABOLIC ASSESSMENTS														
HbA1C (%) [16]	X	X	X							X	X	X	X	X
Mixed-Meal Tolerance Test [16]	X										X	X	X	X
Insulin Modified FSIGT test [16]	X										X [1]		X	X
CALCULATED METABOLIC ASSESSMENTS														
Glycemic Lability (MAGE)		X [5]									X [1]		X	X
Glycemic Lability (LI)	X	X[25]									X [1]		X	X
Full HYPO Score	X	X[25]									X [1]		X	X
Clarke Score	X	X									X [1]		X	X
β-Score [17]											X	X	X	X
C-Peptide to Glucose, Creatinine Ratio [18]	X										X	X	X	X
LOCAL METABLOIC ASSESSMENT														
Positron Emissions Tomography (PET) [19]				X										
Continuous Glucose Monitoring System ®	X	X									X [1]		X	X
MECHANISTIC ASSAYS														
Alloantibodies [20, 22]	X	X	X								X[20]	X[20]	X[20]	X[20]
Autoantibodies (GAD, IA2, IAA) [21]			X								X	X	X	X
TAT Complex, C-peptide, and C3a [22]				X	X									
ARCHIVED SPECIMENS														
Serum [23]	X										X	X	X	
PBMC and Plasma [23]	X										X	X	X	
RNA [23]	X										X	X	X	
RNA and Plasma [22]			X			X		X			X			X
Refer to section 7.2 Wait List/ Baseline Visit Assessments for frequency of testing during the WL period.														

1 **DAY 75 (Primary Endpoint):** If needed, repeated transplant(s) will be performed more than 75 days after the first transplantation. The previous follow-up period stops and a new visit period starts, beginning with the baseline visit. After a second or third transplant the 75 day follow up will NOT include QoL Surveys, MAGE, LI, CLARKE, HYPO, FSIGT, or CGMS.

2 **YEAR 1 POST INITIAL TRANSPLANT:** If 1-year post initial transplant (Visit Y1) falls within a visit window of a scheduled re-transplant study visit, complete only the eCRFs for the 1-year post initial transplant visit. If Y1 visit does not fall within a re-transplant visit, complete the Y1 visit in addition to the scheduled re-transplant visit.

3 Height and BMI assessment completed at screen, baseline and 12 months only.

4 Myocardial Scintigram not required at visit 02.

5 Diary Log eCRF completion is completed using blood sugar record, hypo sheets, glucometer download, and insulin requirements, this is required at each visit.

6 Portal pressure: Before islet infusion, and 15 minutes after completion of the islet transplant.

7 The CBC with differential should be completed within 2-hours prior to planned transplant. Differential not required at the following visits: 04, 05, 07, and 08

8 Sodium, Potassium, Creatinine, Glucose, Albumin, Alk Phos, ALT, AST, LDH, T. Bilirubin

9 Coagulation status must be normal prior to transplant. Hemoglobin should be monitored before, immediately after, four hours after islet transplant, and 2 hours after removal of the portal catheter. CVP should be measured before and after placement of the portal catheter. Blood pressure and pulse should be monitored continuously until 4 hours post removal of portal catheter. During treatment with LMW-DS or heparin, APTT will be monitored.

10 GFR completed at screening and at 75 days (+/- 5 days) after the FIRST islet transplant, and 365 days (+/- 14 days) after first and final islet transplant.

11 Pregnancy test within 3 days of randomization

12 Before and after breakfast. No insulin before the second measurement.

13 Serology panel include s HBcAb, HBsAg, HBsAb, HCV Ab, HIV. Do not repeat Hepatitis B tests if HBsAb was previously positive.

14 Applicable immunosuppression levels.

15 Total daily dose (IU), day before visit (24 h)

16 Central laboratory assessment: University of Washington (Seattle, WA)

17 Results based on the HbA1c, Insulin requirement, and fasting (basal) serum glucose and stimulated C-peptide from the MMTT (Ryan, et al)

18 Results based on the fasting (basal) serum glucose and c-peptide from the MMTT and the simultaneous serum creatinine.

19 This is a substudy to be completed in a subset of subjects (n=8, 4 LMW-DS, 4 Control) at selected sites.

20 Central Laboratory assessment: U. Pennsylvania (Philadelphia, PA)

21 Central laboratory assessment: The Barbara Davis Center Laboratory (Denver, CO)

22 Central laboratory assessment: The Rudbeck laboratory (Uppsala, Sweden)

23 Central Laboratory: The NIDDK Repository

24 Only SF36 completed during the baseline / wait list visits. DTSQs completed during the screening visit and DTSQc completed at the 12 month post first and final islet transplant.

25. Repeat the LI or HYPO during the Baseline/Waitlist period only if the inclusion criteria for study enrollment (inclusion criteria #7b or #7c) were used for inclusion in the study.

APPENDIX 2. REDUCED FOLLOW-UP SCHEDULE

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

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 one year 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-year (+/- 14 days) post **initial** transplant:

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

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

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

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