Study Protocol

Official Title: Islet Transplantation in Type 1 Diabetic Patients Using the University of Illinois at Chicago (UIC) Protocol

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3 Introductory Statement

3.1 Basic Information

3.1.1 Name, Components, Pharmacological Class

Allogeneic human islets of Langerhans are a cellular therapy product with systemic effect derived from the pancreas of a cadaveric donor.

3.1.2 Formulation

The product will be formulated in serum-free transplant media (indicator-free CMRL 1066 with Hepes, without Bicarbonate and supplemented with Human Albumin).

3.1.3 Route of Administration

The islets of Langerhans mixture will be delivered slowly into the portal vein via a syringe attached to a catheter hub or using an infusion bag. Access to the portal vein is achieved by transvenous or percutaneous transhepatic access under fluoroscopic and ultrasound guidance. If a transvenous technique is used, access to the right jugular vein is obtained using a Microstik needle under ultrasound guidance. A guiding sheath is advanced through the right atrium and into the right hepatic vein. Position is confirmed with injection of contrast medium.

3.1.4 Objectives and Planned Duration of Proposed Clinical Investigation

The primary objective of the study is to demonstrate the safety and efficacy of allogeneic islet transplantation in Type 1 diabetic patients performed at the University of Illinois at Chicago (UIC).

The purpose is to demonstrate that islet transplantation achieves better glycemic control than state-of-the-art insulin treatment in the management of Type 1 diabetic patients with brittle control and a history of severe hypoglycemic episodes with hypoglycemia unawareness. The expected duration of the entire proposed clinical trial is five years.

3.2 Non-US Regulatory Actions

In Canada, islet transplantation is approved and funded clinical care through the Province Wide Services of the Government of Alberta, and is also funded in selected provinces. Research protocols outside of clinical care are regulated through Health Canada.

4 General Investigational Plan

4.1 Background and Previous Human Experience

4.1.1 Type 1 Diabetes

Type 1 diabetes results from the body's failure to produce insulin, the hormone that "unlocks" the cells of the body, allowing glucose to enter and fuel them. It is estimated that 1.4 Mio of Americans have Type 1 diabetes. Although life expectancy of patients with Type 1 diabetes has much improved since the introduction of insulin therapy , chronic complications, including blindness and renal failure, are hampering the quality of life and represent a multi-billion dollar annual burden on the health care system of industrialized countries (2). Keeping blood glucose levels under tight control represents the most effective way either to prevent the onset or to reduce the progression of the chronic complications of Type 1 diabetes (3, 4). At present, such a goal may be accomplished by treating patients with intensified therapy regimens consisting of multiple insulin injections, which involve accurate blood glucose monitoring. However, administration of subcutaneous insulin can never approximate pulsatile insulin secretory patterns of the normal ß-cell, and rarely attains normal blood glucose levels without the risk of major hypoglycemic episodes. In addition, intensive insulin therapy is only suitable for selected patients (5).

Pancreas transplantation is the only therapeutic modality which can stop the progression of diabetic complications (6) without increasing the incidence of hypoglycemic events (7). Unfortunately, this procedure, usually performed simultaneously with a kidney graft, has a high morbidity and a significant mortality rate (8). Pancreas transplantation, in spite of an important impact on the quality of life in successful cases (9), will be restricted to selected patients. In this context, islet transplantation appears to be the ideal solution, normalizing glucose metabolism without the risk of hypoglycemia (10) and avoiding the potentially life-threatening complications of whole pancreas grafts.

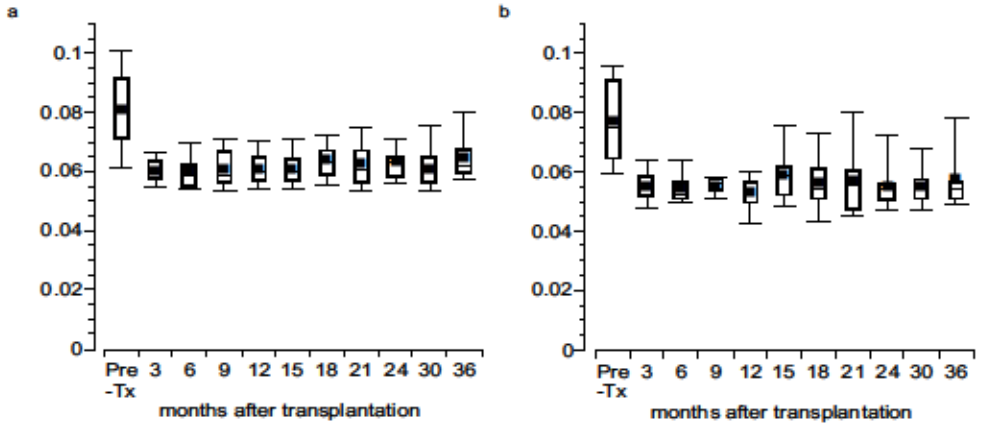
4.1.2 Allogeneic Islet Transplantation

A growing body of evidence over the past 15 years demonstrates that islet transplantation can restore insulin production and ameliorate glycemic instability in patients with Type 1 diabetes. From 1990 to 2001, more than 400 islet allotransplantations were reported to the International Islet Transplant Registry (ITR) (11). The last ITR analysis from 2001 reports sustained graft function (as defined by a basal C-peptide level of > 0.5 ng/mL or 0.17 nmol/L) at 1 month in 36% of patients who underwent transplantation from 1985 to 1989 and in 79% in transplant recipients from 1990-2000. Only 41% of the patients transplanted between 1990 and 2000 showed persistent graft function after one year. The rate of insulin independence remained very low with 20% of transplanted cases from 1990-2000 reaching insulin independence (11). At the time of writing, June 2009, the longest insulin-independence after allogeneic islet transplantation in a Type 1 diabetic patient reported to the registry exceeds 13 years (11, 12).

In North America from 1999-2005, thirty-one islet transplant programs conducted 592 allogeneic islet infusions (13). Increasing activity within islet transplant programs coincided with a breakthrough achieved by the Edmonton group that re-created enthusiasm for this approach among the medical and lay communities.

In the year 2000, the Edmonton group reported a series of seven consecutive patients reaching insulin-independence after transplantation of islets from multiple donors using a steroid-free, sirolimus-based immunosuppression (14). In contrast to previous series, in this report, non-uremic patients with brittle diabetes were chosen as recipients, and a minimum of 10,000 IEN/kg were infused per patient. By May 2003, 51 allogeneic islet transplantations had been performed at the University of Alberta in Edmonton. Overall patient survival was 100%. Insulin-free survival one and three years after islet transplantation was 79% and 50%, respectively, and 88% of patients presented with ongoing graft function at 3 years after transplantation. An intent to treat analysis comparing the outcome of islet and pancreas transplants (Figure 1) showed that levels of HbA1c remained within the normal range for the majority of patients during the 3-year follow-up period.

Figure 1 HbA1c before and after islet (a) and pancreas (b) transplantation. Presented as an intention to treat analysis with inclusion of patients with graft loss. The top, bottom, and line through the middle of the box correspond to the 75th percentile, 25th percentile, and 50th percentile respectively. The whiskers on the bottom extend from the 10th percentile and top 90th percentile. ■ represents the arithmetic mean. n represents the number of patients at the different follow-up time points



By November 2004, 65 patients had received islet transplantation through the Edmonton group. Forty-four patients completed the protocol to achieve insulin independence after 1-3 transplants. At 5-year follow-up, the majority (~ 80%) had C-peptide present, but only a minority (~ 10%) maintained insulin independence. The median duration of insulin independence was 15 months. HbA1c was well controlled in patients off insulin [6.4% (6.1-6.7)] and in those who resumed insulin and were C-peptide positive [6.7% (5.9-7.5)], and was higher in patients who lost all graft function [9% (6.7-9.3)]. Those who resumed insulin required half their pre-transplant daily dose. Post-transplant hypoglycemic scores improved significantly in both groups.

In 2005, the Minnesota group reported results of a 1-year prospective follow-up trial in which all eight subjects achieved insulin independence and freedom from hypoglycemia after single-donor, marginal-dose islet transplantation. Five recipients remained insulin-independent for longer than one year. Investigators related improved islet engraftment to peritransplant administration of the soluble tumor necrosis factor receptor etanercept. Tumor necrosis factor alpha is cytotoxic to human beta cells, and its selective inhibition in murine models has promoted reversal of diabetes after marginal-mass islet transplants (26).

In 2006, Shapiro et al. (15) reported results of a multinational prospective trial conducted at six centers in North America and three in Europe to explore the feasibility of reproducing the Edmonton protocol. The trial was a collaboration by the National Institutes of Health and the Immune Tolerance Network with a goal to establish centers of excellence to conduct future tolerance-based trials. The protocol standardized pancreas selection, islet processing, product-release criteria, recipient selection, and post-transplantation care. At the nine centers, 36 subjects received a total of 77 islet infusions. Forty-four percent reached the primary endpoint of insulin independence defined as HbA1c less than 6.5%, a glucose level after overnight fast not exceeding 140 mg/dL more than three times per week, and a 2-hour postprandial glucose level not exceeding 180 dL more than four times per week. By two years post transplant, 76% had become insulin dependent. C-peptide was detectable in 70% of subjects, and all subjects with residual islet function were protected against severe hypoglycemia and hyperglycemia. Investigators observed that previous experience at each site significantly affected achievement of the primary endpoint, and regionalization of islet processing centers could potentially reduce costs and improve outcomes. This trial demonstrated that islet transplantation performed by the Edmonton protocol can restore long-term endogenous insulin production and glycemic stability. Although insulin independence was not sustained, persistent islet function protected recipients from severe hypoglycemia and improved HbA1c levels.

4.1.3 Allogeneic Islet Transplantation at the University of Illinois (16)

During an initial Phase 1/2 study, we demonstrated safety and efficacy of islet transplantation at the University of Illinois and reported data to the Food and Drug Administration (FDA). The sample consisted of 10 adults with brittle Type 1 diabetes defined as metabolic lability despite optimal insulin management efforts. Subjects presented with hypoglycemic unawareness and normal kidney function, and met all enrollment criteria. In this section we summarize study results.

Subjects comprised two groups. Group 1 (n = 4) received the Edmonton protocol. Group 2 (n = 6) received the Edmonton protocol and additionally the soluble tumor necrosis factor receptor antagonist etanercept and the incretin analog exenatide. Subjects in Group 1 and 2 had similar age distribution (49.2 ± 11.3 and 44.8 ± 10.0 years, p = 0.5), diabetes duration (30.2 ± 9.2 and 27 ± 10.8 years, p = > 0.5), body weight (61.0 ± 4.3 and 63.8 ± 5.3kg, p = 0.5) and pre-transplant insulin requirements (39.3±2.9 and 32.1 ± 8.6 U/day, p= 0.5).

The 10 subjects received 18 islet transplants. Subjects in Group 1 received a mean total number of islets (EIN) of 1,460,080 ± 418,330 in 2 (n = 2) or 3 (n = 2) islet infusions. Subjects in Group 2 received a mean total EIN of 756,354 ±180,755 in 1 (n = 4) or 2 (n = 2) islet infusions.

All subjects had adverse events or experiences comparable to the types and rates reported by other centers. No patient died. No IND safety reports were required. No subjects experienced life-threatening adverse events during transplantation procedures. And no subject withdrew because of adverse experiences, dissatisfaction with the study, or for any other reason.

In Group 1, 2 subjects became insulin independent after two islet transplants, and 2 subjects became insulin independent after three transplants. All subjects in Group 1 completed 64 weeks of post-transplant evaluation and began a 5-year follow-up schedule.

In Group 2, exenatide facilitated insulin independence with fewer islets required initially, and all 6 subjects achieved insulin independence after one islet transplant. Three subjects completed 64 weeks of post-transplant follow-up. Two of the three remained insulin independent. The third subject resumed half the pre-transplant insulin dose at 19 weeks post-transplant following an illness and suboptimal immunosuppression, and awaited a second transplant. Three other patients in Group 2 were at various stages of follow-up. One subject was insulin independent at 40 weeks after first transplant. A second subject was insulin independent 40 weeks after second transplant. And the third subject was in the third week of follow-up after a second transplant and was weaning off insulin (at the time of writing 6 units/day). This subject was independent of insulin after first transplant, but severe gastroparesis forced her to discontinue exenatide after 10 weeks and to resume insulin at 17 weeks post transplant.

Among the 4 subjects who did not take exenatide (Group 1), the observed outcomes exceed the anticipated results and compare well with results published by the most experienced groups in the field. For example, in a recent report of results of the multi-center trial led by the Immune Tolerance Network (15) only one center out of nine achieved the primary endpoint of insulin-independence with appropriate glycemic control one year after the last transplant in 414 patients. In our study, to achieve insulin independence, subjects in Group 1 received either 2 (n = 2) or 3 (n = 3) sequential islet transplantations at least two weeks apart. By comparison, the 6 subjects who took exenatide (Group 2) achieved insulin independence with one islet preparation. Subjects in Group 2 temporarily experienced more nausea, vomiting, and weight loss than Group 1, and two subjects could not tolerate exenatide. The rest of the side effect profile was similar in both groups.

Our study indicated that islets prepared at UIC are safe and can consistently restore endogenous insulin secretion, which fulfilled the first purpose of our study. The results of the first four subjects show that UIC could reproduce the Edmonton protocol, which in the recent international trial could be reproduced by only one of nine groups. The completeness of data collection and reporting also fulfills the second purpose of the

study, namely to develop a data base for future trials investigating strategies to improve outcomes of allogeneic islet transplantation in diabetic patients. Such an improvement has already been achieved by adding exenatide and etanercept to the treatment of islet recipients in Group 2.

From our study we learned first, that the quality of the islet preparation is of utmost importance to achieve consistent graft function, and that UIC has the necessary know-how to produce such islet grafts. Secondly, we learned that islet graft function can be improved by pharmacological means, by achieving insulin independence with fewer islets when using a potent anti-inflammatory drug and an incretin analog. Therefore, we propose a Phase 3 study to demonstrate the efficacy of islet transplantation at University of Illinois at Chicago.

4.2 Indication to be Studied

Enrolling subjects must have Type 1 diabetes mellitus for more than 5 years, present with normal kidney function and have metabolic lability despite optimal intensive insulin management efforts. The enrollment inclusion and exclusion criteria for recipients and donors are discussed in more detail in Section 6.4 *Study Population*.

4.3 General Approach in Evaluation of the Product

4.3.1 Rational for Proposed Clinical Trial

Allogeneic islet transplantation is still considered an experimental procedure and can only be performed under an investigational plan reviewed by the FDA. The Edmonton protocol is currently the only protocol that has shown an acceptable safety and efficacy profile in a larger number of patients receiving allogeneic islet transplants (10, 17). The investigators have shown in a phase 1/2 trial at UIC the safety of the islet preparation, the procedure, and medical treatment. Therefore, the investigators propose this Phase 3 clinical trial protocol testing the safety and efficacy of allogeneic islet transplantation in Type 1 diabetic patients performed at the University of Illinois at Chicago.

4.3.2 General Approach for the Evaluation of the Study

The primary goal of this exploratory study is to demonstrate the safety and efficacy of allogeneic islet transplantation performed at the University of Illinois at Chicago. The study will collect safety and efficacy data from the islet isolation, the transplantation procedure, and the medical treatment during the one-year follow-up period after completion of the first and last islet transplant.

4.4 Trial Type and Number of Subjects to be Enrolled

The protocol presented herein is a Phase 3 clinical trial. It is a single center, open label, prospective trial in which 1-3 allogeneic pancreatic islet transplants per subject will be applied to a total of at least 50 study participants at the University of Illinois at Chicago. We plan to recruit 55 subjects and anticipate 10% attrition. A sample size of 50 subjects, will achieve sufficient power to conduct the proposed statistical analyses. For endpoint definitions and power analysis, please refer to Section 6.11 *Assessment of Endpoints*.

4.5 Potential Risks and Anticipated Adverse Reactions

Islet transplantation presents with procedure related risks and risk of medical complications consequent to immunosuppression. In a series of 54 islet transplants at the University of Alberta the following complications were reported (10):

- Procedure related:
  After 2 of the 54 procedures, partial thrombosis was detected in the portal vein circulation, which resolved after starting anticoagulation. Five subjects had bleeding related to the percutaneous portal vein access procedures: three required transfusion alone, and in one subject who had a partial thrombosis of the portal vein, an expanding intrahepatic and subscapular hemorrhage occurred while on anticoagulation, requiring transfusion and surgery. Elevated liver function test results were found in 46% of subjects but resolved in all.
- Medical treatment related:
  Complications related to the therapy have been hypercholesterolemia requiring statin therapy in 65%; a rise in creatinine in two patients, both of whom had preexisting renal disease; a rise in protein in four, all of whom had preexisting proteinuria; and antihypertensive therapy increased or started in 53%. Three of the 17 patients have required retinal laser photocoagulation.

There have been no cases of post-transplant lymphoproliferative disorder or cytomegalovirus infection, and no deaths. Current outcomes of islet transplantation in humans are discussed in more detail in Section 9 *Previous Human Experience with the Investigational Drugs.*

Adverse events observed during the Phase 1/2 trial at UIC are the following:

Serious Adverse Events

Date of Event	Description of Event	Related to Research	Date Reported to IRB-DMC	Outcome
1/05/06	DLP-010 underwent total abdominal hysterectomy for multiple ovarian cysts, abdominal pain, and heavy menstrual bleeding.	Possibly	1/19/06	Recovered
12/27/06	SRP-013 presented with acute renal failure. Researchers altered medication regimens until renal function returned to pretransplant status.	Possibly	1/19/06	Recovered
2/18/06	KAP-011 with myonecrosis of the right scalene was diagnosed and treated for staph aureus infection of the T-1 vertebral body, rib articulation, and soft tissue	Possibly	2/27/06	Recovered

Date of Event	Description of Event	Related to Research	Date Reported to IRB-DMC	Outcome
5/14/06	ATC-016 was diagnosed with intrahepatic hematoma one day posttransplant and remained hospitalized for observation.	Possibly	5/26/06	Recovered
7/5/06	ATC-016 with intrahepatic hematoma was admitted for evaluation after she tripped and fell onto her abdomen. The subject received 3 units of packed cells for low Hgb/Hct possibly related to rebleeding. Two days after discharge, she was readmitted to rule out infection. No infectious source was identified.	Possibly	8/3/06	Recovered
7/07/07	SRP-013 was admitted for pain management, treatment of oral herpes simplex virus, and recurrent dental abscesses.	Possibly	8/03/06	Recovered
7/21/06	SRP-013 was admitted for treatment of anemia. The subject received 2 units of packed cells and an intravenous iron infusion.	Possibly	8/03/06	Recovered

Adverse Events and Experiences of Post-transplant Subjects (N=10)

Category	Adverse Events / Experiences	Related to Research	Total Subjects (%)
Procedure related	Ascites	Possibly	1/10 (10)
	Bleed	Possibly	2/10 (20)
	Re-bleed after trauma	Possibly	1/10 (10)
Ears, Nose	Dry mouth	Possibly	5/10 (50)
	Nosebleeds	Possibly	2/10 (20)
Cardiovascular	Hypertension	Possibly	5/10 (50)
Gastrointestinal	Abdominal pain	Possibly	7/10 (70)
	Oral ulcerations	Possibly	8/10 (80)
	Diarrhea	Possibly	6/10 (60)
	Epigastric pain	Possibly	3/10 (30)
	Nausea with exenatide	Possibly	6/6 (100)
	Vomiting with exenatide	Possibly	6/6 (100)
Gynecologic	Dysfunctional uterine bleeding	Possibly	2/9 (22)
	Irregular menstruation	Possibly	1/9 (11)

Category	Adverse Events / Experiences	Related to Research	Total Subjects (%)
	Vaginal infection	Possibly	2/9 (22)
	Ovarian cyst	Possibly	1/9 (11)
Musculoskeletal	Arthralgia	Possibly	6/10 (60)
	Myalgia	Possibly	2/10 (20)
	Tendonitis	Possibly	2/10 (20)
	Myonecrosis	Possibly	1/10 (10)
Neurologic	Insomnia	Possibly	7/10 (70)
	Headache	Possibly	5/10 (50)
	Fatigue	Possibly	9/10 (90)
	Tremor	Possibly	3/10 (30)
	Depression	Possibly	3/10 (30)
	Anxiety	Possibly	1/10 (10)
	Concentration difficulty	Possibly	2/10 (20)
	Short term memory impairment	Possibly	1/10 (10)
	Lightheadedness/dizziness	Possibly	4/10 (40)
Dermatologic	Acneiform rash	Possibly	9/10 (90)
	Alopecia areata	Possibly	1/10 (10)
	Dermatitis	Possibly	4/10 (40)
	Cellulitis	Possibly	2/10 (20)
	Edema (pedal)	Possibly	4/10 (40)
	Venous stasis ulcers	Possibly	1/10 (10)
	Dry skin	Possibly	10/10 (100)
	Pruritus	Possibly	6/10 (60)
Endocrine/Metabolic	Hypoalbuminemia	Possibly	8/10 (80)
	ALT elevation	Possibly	9/10 (90)
	AST elevation	Possibly	10/10 100)
	ALP	Possibly	5/10 (50)
	Total bilirubin elevation	Possibly	3/10 (30)
	Total cholesterol elevation	Possibly	4/10 (40)
	LDL elevation	Possibly	2/10 (20)
	Triglycerides elevation	Possibly	2/10 (20)
	Hypophosphotemia	Possibly	4/10 (40)
	Hypomagnesemia	Possibly	6/10 (60)
	Hypoglycemia on exenatide	Possibly	3/10 (30)
	Weight loss	Possibly	10/10 (100)
Hematologic	Anemia	Possibly	10/10 (100)
	Leukopenia	Possibly	10/10 (100)
	Neutropenia	Possibly	7/10 (70)
	Platelet elevation	Possibly	5/10 (50)
	Thrombocytopenia	Possibly	4/10 (40)
Renal	Creatinine elevation	Possibly	3/10 (30)
	Renal failure, acute	Possibly	1/10 (10)
Infections	C-difficile	Possibly	1/10 (10)

Category	Adverse Events / Experiences	Related to Research	Total Subjects (%)
	CMV seroconversion	Possibly	2/10 (20)
	Osteomyelitis	Possibly	2/10 (20)
	Upper respiratory infection	Possibly	5/10 (50)
	Bronchitis	Possibly	2/10 (20)
	Pneumonia	Possibly	1/10 (10)
	Urinary tract infection	Possibly	5/10 (50)
	Viral stomatitis	Possibly	1/10 (10)
	Dental infection	Possibly	3/10 (30)
	Otitis media	Possibly	3/10 (30)
	Sinusitis	Possibly	2/10 (20)
	Influenza	Possibly	4/10 (40)
	Candida oral infection	Possibly	3/10 (30)
	Herpes zoster	Possibly	2/10 (20)

5 (Investigator brochure)

Not applicable

6 Protocol

6.1 Introduction

The replacement of insulin producing beta cells by islet transplantation has been shown to effectively control blood glucose levels in individuals with Type 1 diabetes. While the concept of islet transplantation is simple, its widespread clinical application has been hampered by a variety of technical and biological obstacles. Clinical trials from 1980 to 1998 in patients with juvenile diabetes had limited success, but showed that restoration of endogenous insulin secretion and, in some cases, even insulin independence was achievable through implantation of isolated islets (18-24). However, the variable and unpredictable outcome hindered the entry of islet transplantation to clinical reality. More recently, the successful clinical trials in islet transplantation in non-uremic Type 1 diabetic patients by the Edmonton group (14), focused much attention on the treatment of diabetes by islet transplantation. A more detailed review of previous and current experience with human islet transplantation is outlined in Section 9 *Previous Human Experience with the Investigational Drugs*

Before islet transplantation could become standard medical care, the results obtained by the Edmonton group had to be reproduced by other centers. Our own Phase 1/2 trial has reproduced those results and now requires a larger trial that, if confirming the results, may confer the possibility for a biological license application to make islet transplantation an accepted medical treatment to treat brittle Type 1 diabetic patients. In addition, this would facilitate longer and larger clinical trials to further improve the procedure.

6.2 Objective and Purpose of the Study

The primary objective of this study is to demonstrate the safety and efficacy of allogeneic islet transplantation in Type 1 diabetic patients performed at the University of Illinois at Chicago (UIC).

The purpose is to demonstrate that islet transplantation achieves better glycemic control than state-of-the-art insulin treatment in the management of Type 1 diabetic patients with brittle control and a history of severe hypoglycemic episodes with hypoglycemia unawareness. The series of transplants performed with the protocol described herein will provide the base for a biological license application in islet transplantation.

6.3 Study Design

The herein presented protocol is a Phase 3 study. It is a single center, open label trial in which 1-3 allogeneic pancreatic islet transplants per subject will be applied to a total of at least 50 study participants at the University of Illinois at Chicago

6.4 Study Population

This protocol is designed as a limited series of islet transplants for at least 50 Type 1 diabetic adult subjects considered to have brittle diabetes for which glycemic control using insulin is problematic if not impossible. The enrollment inclusion and exclusion criteria for recipients and donors are given below.

6.4.1 Recipient Inclusion Criteria

Enrolling subjects must have Type 1 diabetes mellitus for more than 5 years, complicated by the following situations that persist despite intensive insulin management efforts (Appendix *Pre-Screening*):

a. At least one episode of severe hypoglycemia in the past 3 years defined as an event with symptoms compatible with hypoglycemia in which the subject required the assistance of another person, and which was associated with either a blood glucose level < 50 mg/dL (2.8 mmol/L) or prompt recovery after oral carbohydrate, intravenous glucose, or glucagon administration
b. Reduced awareness of hypoglycemia, as defined by the absence of adequate autonomic symptoms at capillary glucose levels of < 54 mg/dL (3 mmol/L as reported by the subject

6.4.2 Recipient Exclusion Criteria

Subjects will be excluded from the study if one of the following conditions is present:

1. Diagnosis of co-existing cardiac disease, characterized by any one of these conditions:
    a. Recent myocardial infarction (within past six months), or
    b. Angiographic evidence of non-correctable coronary artery disease, or
    c. Evidence of ischemia on functional cardiac exam (with a stress echo test recommended for subjects with a history of ischemic disease).
    d. Heart failure > New York Heart Association II
2. Active alcohol or substance abuse includes cigarette smoking (must be abstinent for six months). Active alcohol abuse should be considered using the current National Institute on Alcohol Abuse and Alcoholism definitions.
3. Psychiatric disorder making the subject not a suitable candidate for transplantation, e.g., schizophrenia, bipolar disorder, or major depression that is unstable or uncontrolled on current medication. (A psychological or psychiatric consultation is required only if considered necessary by some current indication or history.)
4. History of non-adherence to prescribed regimens
5. Active infection including hepatitis C, hepatitis B, HIV
6. TB (by history or currently infected as evidenced by a positive QuantiFERON® - TB Gold test or under treatment for suspected TB)
7. Any history of malignancies except squamous or basal skin cancer. Any subject found to have squamous or basal cancer is required to have it removed prior to transplant.

8. Known family history of MEN2 or MCT
9. History of stroke within the past 6 months
10. BMI > 27 kg/m^2
11. C-peptide response to glucagon stimulation (1 mg iv) (any C-peptide ≥ 0.3 ng/mL)
12. Inability to provide informed consent
13. Age less than 18 or greater than 75 years
14. Creatinine clearance < 80 mL/min/1.73 m^2 by 24-hour urine collection. If corrected creatinine clearance is < 80 and serum creatinine is < 1.2 mg/dl, then a nuclear renal scan is required to determine glomerular filtration rate.
15. Serum creatinine consistently > 1.5 mg/dL
16. Macroalbuminuria (urinary albumin excretion rate > 300 mg/24h)
17. Baseline Hb < 12 gm/dL in women or < 13 gm/dL in men
18. Baseline liver function tests (LFT) outside of normal range (An initial LFT test panel with any values > 1.5 times normal upper limits will exclude a subject without a re-test. A re-test for any values between normal and 1.5 times normal should be made, and if the values remain elevated above normal limits, the subject will be excluded.)
19. Untreated proliferative retinopathy
20. Positive pregnancy test, intent for future pregnancy, or male subjects' intent to procreate, unwilling to follow effective contraceptive measures, or presently breast-feeding
21. Previous transplant (except islet transplant), or evidence of hyper sensitization on PRA (determined by demonstration of positive results for anti-HLA antibodies using solid phase immunoassay with soluble HLA Class I molecules as a target, or a general PRA panel with reactivity > 80%). All subjects require a negative crossmatch with the donor before transplant (UNOS requirement).
22. Insulin requirement > 0.7 IU/kg/day
23. HbA1c > 12%
24. Hyperlipidemia (fasting LDL cholesterol > 130 mg/dL, treated or untreated, and/or fasting triglycerides > 200 mg/dL)
25. Under treatment for a medical condition requiring chronic use of steroids
26. Use of Coumadin or other antiplatelet or anticoagulant therapy, or subject with PT-INR > 1.5. Low dose aspirin is allowed after transplantation.
27. History of Factor V deficiency
28. Currently smoking tobacco
29. Addison's disease
30. Symptomatic cholecystolithiasis
31. Acute or chronic pancreatitis
32. Symptomatic peptic ulcer disease
33. Severe unremitting diarrhea, vomiting, or other gastrointestinal disorders that could interfere with the ability to absorb oral medications
34. Treatment with antidiabetic medication other than insulin within 4 weeks of enrollment
35. Use of any study medication within 4 weeks of enrollment
36. Received live attenuated vaccine(s) within 2 months of enrollment

37. Any medical condition that, in the opinion of the investigator, might interfere with safe participation

6.4.3 Donor Organ Screening Criteria

All potential donors will be screened at the time an offer is made by an Organ Procurement Organization via telephone. Only the principal investigator (Dr. Oberholzer) or a trained, qualified designee will screen potential donors. The medical/social history form must be reviewed for risk of disease transmission. All donors will be screened for safety and for organ quality. All offers will be logged and tracked using a Donor Screening Form and logbook.

Safety screening will include criteria defined in the Suitability Determination for Donors of Human Cellular and Tissue-Based Products Final Rule, as well as current high-risk criteria for bloodborne pathogens defined by the Centers for Disease Control and Prevention. All donors with risk of disease transmission according to these guidelines will be deferred.

All screening criteria are outlined in SOP form. Safety screening exclusion criteria include the following:

- Men who have had sex with another man within the past five years
- Persons who have injected drugs for non-medical reasons within the past five years, including intravenous, intramuscular, or subcutaneous injections
- Persons with hemophilia or related clotting disorders who have received human-derived clotting factor concentrates
- Persons who have had sex for money or drugs within the past five years
- Persons who have had sex with any of the individuals described in the four items above within the past 12 months or with any individual suspected of having HIV or hepatitis B
- Persons who have been exposed within the past 12 months to known or suspected HIV, HBV and/or HCV infected blood through percutaneous inoculation (e.g., needlestick) or through contact with an open wound, non-intact skin, or mucous membrane
- Persons whose history, physical exam, or medical records reveal other evidence of high risk behavior and/or HIV, or hepatitis B or C infection such as: a diagnosis of AIDS and/or hepatitis B or C, unexplained weight loss, night sweats, blue or purple spots on the skin or mucous membranes typical of Kaposi's sarcoma, unexplained lymphadenopathy lasting > 1 month, unexplained > 38.6 degree Celsius temperature for > 10 days, unexplained persistent cough and shortness of breath, opportunistic infections, unexplained persistent diarrhea, male to male sexual contact, sexually

transmitted diseases, needle track marks or other signs of parenteral drug abuse, unexplained jaundice, or hepatomegaly

- Current inmates of correctional systems (including jails and prisons) and individuals who have been incarcerated for more than 72 consecutive hours during the previous 12 months
- Persons who have had close contact with another person having viral hepatitis within the past 12 months (contact which may allow exposure to blood/body fluids including sharing of kitchen/toilet facilities)
- Persons who within 12 months prior to donation have undergone tattooing, acupuncture, or body piercing in which shared instruments are known to have been used
- Persons with history/symptoms of spongiform encephalopathy (CJD) or known family history (blood relative) of a person with non-iatrogenic CJD
- Persons with dementia or degenerative neurologic disorders of viral or unknown etiology
- Persons who have received injections of human pituitary-derived growth hormone (pit-HGH)
- Persons known to have received transplants of human dura matter
- Persons who have had a xenotransplant of any type
- Septicemia

When the offer is made, blood type, consent, and serology results are verified and blood type is recorded. The informed consent for transplant/research is identified. Required serologies (HIV-1 and 2, hepatitis B surface antigen, HCV, HTLV I and II, and syphilis) are performed using FDA approved assays and must be negative/non-reactive (see Appendix *Letter of confirmation by collaborating organ procurement agencies*). A sample that tests positive for CMV may be accepted if the donor was asymptomatic prior to death.

In addition, the blood sample used for serological testing must not be plasma diluted. A sample will be considered plasma diluted if: a) the donor received more than 2000 cc of blood products/colloids during the 48 hours prior to the blood draw, or b) the donor received more than 2000 cc of crystalloids in the hour prior to the blood draw. In such cases, a Plasma Dilution Worksheet must be used to determine if the sample tested was hemodiluted. Only samples proven to be < 100% diluted will be acceptable.

Screening for organ quality will be done to help ensure an adequate amount of functional islets. Quality screening exclusion criteria include the following:

- Donor age < 15 or > 75 years
- Warm ischemia
- Cold ischemia > 12 hours
- Methanol toxicity
- History of diabetes
- BMI < 19

6.5 Test Material and Administration

6.5.1 Islet Dosing and Administration

A minimum amount of islets of about 10,000 IE/kg recipient body weight is required for engraftment and confirmed C-peptide expression based on the Edmonton series. This form of procedure may require multiple transplants in order to reach this desired minimum number of islets, as previous clinical studies were unable to reliably reach insulin independence with a single transplant.

Eligibility for second or third islet infusion

Subjects are eligible for further transplant if by the end of the fourth week after the first islet infusion or by the end of the fourth week after the second islet infusion they do not meet insulin-independence criteria. If a subject does not reach the definition of insulin independence by the end of the third month after the third transplant, the subject may be considered for an exceptional fourth islet infusion. However, the indication for an exceptional fourth transplant needs to be presented to the Data Monitoring Committee and approval obtained.

Eligibility for islet retransplantation and/or fourth islet transplant:

Subjects are eligible for subsequent islet infusions if after a period of insulin independence of at least 30 days, they present with declining islet function requiring reintroduction of exogenous insulin. Subjects may receive this additional islet infusion anytime during the first year after the last islet infusion, or anytime during the 5-year follow-up, as long as no exclusion criteria are present and avoiding any HLAs (human leukocyte antigens) against which the recipient may have developed donor specific antibodies.

No maximum number of delivered islets is specified, except that the number must exceed 10,000 IE/kg following 2 islet transplants, and packed cell volume may not exceed 10 mL per transplant.

Transplant Procedure

Islets will be isolated from human pancreases procured from deceased multi-organ donors as described in Section 7. The islet isolation procedure will be performed in the clean room facilities of the Cell Isolation Laboratory at the University of Illinois at Chicago Hospital (see Section 7.4 *University of Illinois at Chicago Islet Processing Facility*). Isolated human pancreatic islets will be stored for a maximum of 12 hours before transplantation (see Section 7.2 *Product Manufacturing*).

After the islet lot has been released by the PI, Dr. J. Oberholzer, or a co-investigator (see Section 7.6 *Product Testing*), and once the surgeon and subject are ready for infusion, the suspension is drawn directly into sterile 60 mL syringes. The 30 mL of transplant media containing the islets will be aspirated into the 60 mL syringe and the flask will be rinsed with 20 mL of transplant media. The 20 mL of transplant media will be aspirated in the 60 mL syringe. One 60 mL syringe will contain a maximum of 1 mL total packed islet cells. Alternatively to syringes, islets can be infused via a 600-mL iv bag-system.

The preparation will be labeled (see Section 7.3 *Product Label*), packed into sterile bags, and brought to the radiology suite via a hand carried insulating container at room temperature.

The radiology suite in which the procedure will be performed has access to emergency equipment, and the in-house anesthesiologist on call will be available to monitor the subject if necessary. Access to the surgical operation room is granted and a surgical team is available. The PI, Dr. J. Oberholzer, is a trained general and transplant surgeon with experience in the management of possible complications that could occur after islet transplantation such as bleeding from the puncture site or inability to access the portal vein by interventional radiology. In the absence of Dr. J. Oberholzer, a co-investigator will cover the service.

The subject will be admitted to the hospital as soon as an islet preparation is evaluated as potentially transplantable (sufficient islet mass and purity after purification). While release criteria of the islet cells are being evaluated, an eligible subject will be admitted and hospitalized in the Transplant Unit (7W UIC Hospital), and the immediate pre-transplant work-up will be performed as described in Section 6.6.4. The subject will remain in the Transplant Unit until the islet preparation is shown to fulfill the release criteria and is released for transplantation by the PI or a designee and the cross-match test is reported as negative. Then the subject will be brought to the radiology suite for transplantation. In the event that the islet preparation fails release criteria, the subject will be discharged from the hospital.

Access to the portal vein for islet transplantation is achieved by transvenous or percutaneous transhepatic access under fluoroscopic and ultrasound guidance by the Interventional radiologist co-investigator. If a transvenous technique is used, access to the right jugular vein is obtained using a 21 gauge micropuncture needle under ultrasound guidance. A guiding sheath is advanced across the right atrium and into the right hepatic vein under fluoroscopy. Position is confirmed with injection of contrast media (iodinated contrast or if allergic, CO2 or gadolinium). Close monitoring of the cardiac rhythm by continuous ECG and pulse oxymeter will be performed to allow rapid response to any cardiopulmonary events including cardiac dysrhythmias. Blood pressure and respiratory rate will be monitored intermittently (at least every 5 minutes. A sheath needle (Colapinto™, Cook, Bloomington, IN) is advanced anteromedially through the hepatic parenchyma under fluoroscopic guidance until access to a peripheral portal vein is obtained. The localization of portal vein puncture is confirmed similarly to the

percutaneous technique described below, and the sheath is advanced into the main portal vein.

For the percutaneous approach, a local anesthetic agent (lidocaine) is injected subcutaneously, and a 21 gauge needle is used to puncture a peripheral branch of the right portal vein. Tiny amounts of angiographic contrast media (iodinated contrast or if allergic, CO2 or gadolinium) are used to confirm satisfactory location of the puncture site in a peripheral portal vein. A thin, flexible guidewire is threaded into the main portal vein and the needle is exchanged for a 4/6 French Accustick™ catheter (Boston Scientific, Natick, MA). This catheter is threaded over the guidewire to position the tip in the main portal vein. Contrast portogram is obtained with minimal contrast. The catheter is then exchanged over a guidewire for a vascular introducer sheath positioning the sheath tip at the main portal vein to allow ease of infusion of islet cells. The portal pressure is monitored by attaching to an in-line pressure monitor via a 3-way stopcock after zeroing the monitor to room air pressure. Elevated absolute intraportal pressures (> 20 mmHg or > 27 cmH_2O) confirmed at the beginning of the procedure will be considered a contraindication for continuing with the transplant infusion.

If access to the portal vein cannot be gained by transvenous or transcutaneous approach, the subject will be brought to the operation theatre. A small laparotomy will be performed under local or general anesthesia, and portal access will be gained through cannulation of a mesenteric vein (24-26). The surgery will be performed by the PI, Dr. J. Oberholzer, or a co-investigator.

The islet preparation may consist of fractions of different purity with a total packed cell volume of less than 10 mL. If the total packed cell volume of the islet preparation is greater than 5 mL, the lot will be divided into two sub-lots during the purification process so that the higher purity fractions may be pooled separately from lower purity fractions and labeled accordingly. Each sub-lot will be divided into syringes (or bags) each containing a maximum of 1 mL of total packed islet cells (if syringes are used). If more than one sub-lot of the islet preparation is to be administered (i.e., where sub-lots are identified as having different islet purity), the lot with the higher purity will be administered first. Mixing sub-lots together for simultaneous administration is not permitted.

The islets will be injected slowly (each syringe over a period of at least 5 minutes) by Dr. J. Oberholzer or a co-investigator. During the injection, the syringe is turned constantly to avoid sedimentation of the islets and clumping. If the bag system is used, the islets are infused by gravity.

Portal pressure will be monitored before and after infusion of one syringe load (50 mL volume containing 1 mL of tissue). Any change in portal pressure will be documented, and if the intra-portal pressure rises above 22 mmHg, infusion of subsequent syringes must be held until the pressure falls below 18 mmHg. If the bag system is used, the portal pressure is taken intermittently, and if the intra-portal pressure rises above 22 mmHg, the infusion must be held until the pressure falls below 18 mmHg. The bag

system must be repetitively and gently shaken to keep the islet preparation in suspension and avoid clumping.

Following each infusion, if the portal pressure remains elevated above 22 mmHg for longer than 10 minutes, then no further infusion will be administered through the hepatic vein, and the procedure will be terminated.

The procedure, duration of injection, number of syringes injected and discarded, and portal pressures will be recorded in the case report form. Also, any adverse event occurring during the transplant process will be recorded in the case report form.

After successful completion of the islet infusion, the catheter and syringe or bag system will be rinsed with an additional 20 mL of transplant media, which is infused through the cannula over approximately 2 minutes, and a final portal pressure documented. Under ultrasound or fluoroscopic guidance with very minimal further contrast use, the sheath tip is withdrawn from the main portal vein into the liver parenchyma. Contrast media (iodinated contrast or if allergic, CO2 or gadolinium) is used to confirm extraportal position of the sheath. While the subject continues to be monitored, Avitene microfibrillar collagen hemostat (BARD, Davol Inc., Warwick, RI) paste mixed with diluted contrast is injected into the sheath to seal the liver parenchymal tract. Under fluoroscopic guidance the sheath is withdrawn while injecting the Avitene paste until the sheath is removed and Avitene paste seals the liver parenchymal tract to the peripheral portal vein. Alternatively, the catheter is withdrawn slowly over 30 minutes or more, allowing formation of an autologous blood clot. The catheter is then removed completely and the subject returns to the Transplant Unit with instructions to lie recumbent for 4 hours. Abdominal ultrasound and Doppler examination of the liver are performed the day after the procedure to exclude procedure related complications such as portal vein thrombosis or intra-abdominal bleeding (see Section 6.6.6 and Appendix *Post-Transplant Orders*).

6.5.2 Immunosuppression Dosing and Administration

Participants in the trial will receive immunosuppression using agents approved by the FDA for organ transplantation and evaluated for use in human islet transplantation under IND to prevent allotransplant rejection. Subjects will receive basiliximab, sirolimus, tacrolimus, and etanercept in the immunosuppressive regimens. Subjects who are sensitized to human leukocytes will receive thymoglobulin instead of basiliximab for the initial transplant. Sirolimus and tacrolimus will be given according to the Edmonton protocol as described by Shapiro et al. (14). In addition to the medications included in the Edmonton protocol, participants will receive etanercept as proposed by the University of Minneapolis group and based on experimental data indicating that TNF alpha receptor antagonist improves Islet graft function and engraftment (27). It should be noted that under this protocol, corticosteroids are omitted from the post-transplant immunosuppressive regimen. Mycophenolate mofetil may be used for subjects who do not tolerate the adverse effects of sirolimus or tacrolimus, Other immunosuppressant medications may be used for subjects who do not tolerate the adverse effects of sirolimus, tacrolimus. or mycophenolate mofetil.

Patients presenting with preformed antibodies against human leukocyte antigens will receive a more intense induction protocol with the addition of a polyclonal anti-T-cell antibody preparation (Thymoglobulin).

Beginning with the initial transplant, a steroid free immunosuppressive regimen will be administered to all subjects. Subjects will receive initial doses of basiliximab, sirolimus, low dose tacrolimus, and etanercept according to the following schedule (see Section 6.5.4).

Basiliximab (Simulect®) Regimen

Basiliximab will be administered at a dose of 20 mg iv given within two hours before transplant, and 20 mg iv at week 2 after transplant for a total of 2 doses. If a second or third transplant occurs and no basiliximab was given in the preceding seven days, then the dosing regimen will begin at the time of transplant. If basiliximab has been administered within the past seven days, then the dose at transplant is omitted, and one dose is given two weeks after the last dose of basiliximab. Basiliximab will not be administered for the initial transplant in patients who are sensitized to human leukocytes and receive thymoglobulin.

Rabbit anti-human thymocyte immunoglobulin (ATG) (Thymoglobulin®) Regimen

Thymoglobulin® will be administered to patients who are sensitized to human leukocyte antigens for the initial transplant only. The first dose will be 1 to 1.5 mg/kg IV given over 6 hours immediately pre-transplant. The second dose will be 1 to 1.5 mg/kg iv over 6 hours on day 1 after transplant. Three subsequent doses of 1 to 1.5 mg/kg iv over 6 hours will be given on days 2, 3, and 4 post-transplant.

Premedication will be as follows:

- Acetaminophen (Tylenol®) 650 mg po 1/2 hour before and midway through Thymoglobulin infusion
- Diphenhydramine (Benadryl®) 50 mg po1/2 hour before and midway through Thymoglobulin infusion
- Methylprednisolone (Solu-Medrol®) 1 mg/kg iv one hour before and midway through the first Thymoglobulin infusion

Sirolimus (Rapamune®) Regimen

Sirolimus will be administered at a loading dose of 0.2 mg/kg per day po immediately pre-transplant, and continued at a dose of 0.1 mg/kg/day each morning. Subsequently, trough serum blood levels of sirolimus will be analyzed by HPLC (two times per week initially, until trough levels are stable and within target range), and the dose will be adjusted to the target range of 12-15 ng/mL for the three months following the most recent islet infusion. When a subsequent transplant occurs, the loading dose is not used, and the subject continues on the current dosing regimen. After three months following last transplant, the target serum level will be lowered to 7-10 ng/mL.

Mycophenolate Mofetil (CellCept®) Regimen

Mycophenolate mofetil may be used for subjects who do not tolerate the adverse effects of sirolimus or tacrolimus, and will be administered at a dose of 500 mg to 1500 mg bid po for the duration of islet graft functioning.

Tacrolimus (Prograf®) Regimen

Tacrolimus will be administered at a dose of 1 mg po given immediately before transplantation, and will be continued at a dose of 1 mg po given bid. As soon as 12-hour trough tacrolimus levels (IMx assay, Abbott Laboratories) are available, the dose will be adjusted to maintain target trough levels of 3-6 ng/mL throughout the study. When a subsequent transplant occurs, the subject continues on the current dosing regimen (14).

Other Immunosuppressant Medications

Other immunosuppressant medications may be needed for subjects who do not tolerate sirolimus, tacrolimus, or mycophenolate mofetil, If azathioprine is planned, the subject may be tested for the presence of thiopurine methyltransferase (TPMT), the main enzyme responsible for inactivating toxic products of azathioprine metabolism. Test results may help to evaluate the risk for adverse effects of azathioprine. Patients with homozygous deficiency of TPMT have low enzyme activity are at increased risks for side effects such as panzytopenia (28, 29). Azathioprine will be administered at a dose of 100 to 150 mg daily. Patients with heterozygous deficiency have 50% of enzyme activity and have been shown to respond well and tolerate at reduced dose(30). Trough levels are not measured.

Etanercept (Enbrel®) Regimen

Etanercept will be administered at a dose of 50 mg iv before islet transplantation, and will be continued at a dose of 25 mg subcutaneously on the 3rd, 7th, and 10th post-transplantation days.

6.5.3 Other Medications Dosing and Administration

Exenatide (Byetta®) Regimen

Exenatide will be administered at a dose of 5 mcg subcutaneously given with I hour pre-transplant and post transplant bid for 1 week at any time within a 60 minute period before or after the morning and evening meals. After 1 week of therapy, if tolerated well, the dose will be increased to 10 mcg bid. Exenatide will be given for a total of 6 months after the last islet transplant and may be extended if necessary

In addition to prolonged immunosuppression, transplant subjects routinely need other medications (see Section 6.5.4). These include the use of prophylactic anti-infective drugs used in patients receiving prolonged immunosuppression. Anti-infective drugs used under this protocol include trimethoprim / sulfamethoxazole [Bactrim™ single

strength (80 mg TMP/400 mg SMX) 2 tablets po per week] for a minimum period of six months, and valganciclovir (Valcyte®) (450 mg po daily) for 3 months post-transplant. With the use of trimethoprim / sulfamethoxazole and valganciclovir, total WBC counts and kidney function will be monitored at routine visits as shown in Section 6.6.8 *Schedule of Evaluation*. If total WBC counts fall below 3,000, or if other conditions warrant a change in prophylaxis, sulfamethoxazole will be discontinued and substituted with pentamidine 300 mg by inhalation each month for a minimum of six months. Experienced personnel will administer pentamidine with caution, and necessary preparations will be taken to treat acute bronchospasm if it occurs.

During the transplant procedures, additional medications, local anesthetics, and contrast media are also used. Heparin is used during the transplant to reduce coagulation risks that may lead to liver thrombosis. And for 1 week following the transplant, subcutaneous longer acting, low molecular weight heparin (enoxaparin, Lovenox®) is used. Study subjects will be monitored for hemorrhage and bruising while receiving these anticoagulants. Subjects will be expected to self-administer sub-cutaneous doses of Lovenox® 30 mg bid for the week after each transplant or obtain the assistance of another person in doing so.

Other medications may be required according to medical conditions presenting during the study period, and will be given according to best medical practice.
For example, filgrastim (Neupogen®) 300 mg sc will be given to post transplant patients presenting with neutropenia secondary to sirolimus, valganciclovir, mycophenolate mofetil, and/or trimethoprim/sulfamethoxazole.

6.5.4 Schedule of Medication

Visit	TX	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Week	0	1	2	3	4	6	8	10	12	16	20	24	28	32	36	40	44	48	52
Medication																			
Basiliximab 20 mg iv 2 hours pre-tx and 2 weeks post-tx *	X		X																
Thymoglobulin 1 to 1.5 mg/kg iv for 5 doses * *	X																		
Tacrolimus 1 mg po bid. beginning day 1 post-tx	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Sirolimus 0.2 mg/kg loading dose 2 hours pre-tx , then 0.1 mg/kg po daily	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Etanercept 50 mg iv 1 hour pre-tx and 25 mg sc on post-tx days 3 7 and 10	X	X	X																
Trimethoprim-sulfamethoxazole 80/400 mg po twice/week	X	X	X	X	X	X	X	X	X	X	X	X							
Valganciclovir (Valcyte®) 450 mg po daily	X	X	X	X	X	X	X	X	X										
Enoxaparin 30 mg sc bid for 7 days	X	X																	
Cefazolin (Kefzol®) 1 gram iv 1 hr pre-tx and q6h for 24 hr after tx	X																		
Exenatide (Byetta®) 5 mcg sc bid for 1 week, then 10 mcg bid.	X	X	X	X	X	X	X	X	X	X	X	X							

Tacrolimus doses will be adapted to reach target trough levels of 3-6 ng/mL; Sirolimus doses will be adapted to reach target trough levels of 10-15 ng/mL during the first 3 months and 7-10 ng/mL thereafter.

**Thymoglobulin will be given only to sensitized patients, the first dose starting before transplantation, followed by 4 subsequent doses on post-transplant days 1, 2, 3, and 4. *Basiliximab will not be given at transplant or 2 weeks post transplant if Thymoglobulin is used.

6.6 Study Evaluations

All subjects will be evaluated and followed at the University of Illinois at Chicago Hospital, Medical Center (outpatient) and Clinical Research Center. Policies and procedures are in place for good clinical practice. Study evaluations and follow-up are detailed in Appendices *Pre-Screening*, *Detailed Visit Schedule, Transplant Order,* and *Post-Transplant Orders*, and summarized in Section *6.6.8 Schedule of Evaluation.*

6.6.1 Pre-Screening

Patients interested in participating in the study will provide Informed Consent to answer a questionnaire regarding their medical history as part of a pre-screening process to select potential candidates and exclude unsuitable subjects from further testing (see Appendix *Pre-Screening*).

6.6.2 Screening

Patients with diabetes who are potentially eligible based on questions asked during pre-screening and continue to express interest will continue with the screening process to determine eligibility. The screening will be organized in two phases (see Appendix *Detailed Visit Schedule*). During phase 1, subjects will provide written Informed Consent to participate in the clinical study and potentially receive investigational islet cell product. Subjects will undergo a detailed history and physical examination and have blood drawn to assess chemistry, hematology, and serologies. A 24-hour urine collection will be obtained, and subjects will have a glucagon stimulation test. Results of these tests will be reviewed, and subjects who remain eligible will undergo further evaluation in phase 2. The evaluations in this phase include additional blood work, cardiac testing, chest x-ray, abdominal ultrasound and Doppler, standard of care primary care screenings, current influenza and pneumococcal vaccines, and ultrasound of the thyroid.

Subjects meeting the inclusion criteria and not presenting with any exclusion criteria will be registered on the UNOS allogeneic pancreatic islet wait list.

The study procedures, risks and potential benefits will be discussed with potential subjects in lay language. The potential subject will have an opportunity to review the subject information and informed consent form and ask questions. Subjects will be assessed for standard islet transplantation at the local site prior to screening, and as a result, will have standard assessment testing per local standard of care, performed prior to signing consent documents. This must be clearly documented and verified within the subject's medical record.

6.6.3 Period on the Waiting List

During phase 3 of the screening period subjects are awaiting their first transplant and will have re-evaluations every 3 months (see Appendix *Detailed Visit Schedule*):

Tests scheduled every 3 months:

- HbA1C

- Comprehensive metabolic panel
- Microalbumin/creatinine ratio
- PRA

Tests scheduled if the subject has not been transplanted within 6 months:

- History and physical
- ECG
- Lab tests to be done in addition to the 3 months schedule
  - CBC
  - PT, PTT, INR
  - HIV
  - Hepatitis panel for hepatitis B and hepatitis C virus
  - VzVIgG if negative at screening phase 2
  - CMV if negative at screening phase 2
  - EBV if negative at screening phase 2
  - RPR
  - Lipid panel
  - Serum pregnancy test

The most recent values for these tests will be recorded in the pre-transplant records.

*6.6.4 Immediate Pre-Transplant Work-Up (***see Appendix *Detailed Visit Schedule and Transplant Order)***

Before the transplant the subject will be admitted to the Transplant Unit at the UIC Hospital and the following screening performed to ensure the subject is medically fit to undergo safely the transplant procedure:

a. History and physical exam

b. Laboratory studies - STAT

- Hematology
  - CBC and differential
  - PT-INR, PTT
  - ABO type and cross match
- Biochemistry
  - Total bilirubin, AST, alkaline phosphatase, albumin, total protein
  - Electrolytes, creatinine, glucose, BUN, magnesium, phosphorous
  - Urinalysis
- Microbiology
  - Serum CMV IgG & IgM, EBV IgG & IgM
  - C&S and fungi: sputum, urine and blood on subjects who were in hospital during the past week
- Serum pregnancy test

- Prospective donor-recipient cross match by flow and cytotoxicity sent from the Islet Processing Facility

6.6.5 Transplant Period

During the transplant procedure, the subject will be monitored by continuous ECG, and pulseoxymeter as well as clinically by the transplant surgeon. Capillary blood glucose will be measured every 30 minutes.

Although daily SMPG readings will not be required while in the hospital, the subject should be instructed to continue to complete the diary with all other relevant information (i.e. adverse events).

6.6.6 Early Post-Transplant Period (see Appendix *Post-Transplant Orders)*

After the islet transplant procedure, subjects will stay for a minimum of 24 hours in hospital with close monitoring in the UIC Hospital Transplant Unit (7W), offering intensive care unit equivalent conditions. Vital signs are monitored every 15 minutes for the first hour, then every 30 minutes for 4 hours, then every 4 hours thereafter until discharge. During the first 24 hours after islet transplantation, liver tests, amylase, lipase, and CBC will be monitored every 8 hours.

Starting at least 4 hours after the islet infusion, subjects will begin a series of treatments with low molecular weight heparin (enoxaparin sodium, Lovenox® 30 mg sc bid, which may be self-administered) for seven days. Hemoglobin will be monitored 3 times during the first week following the transplant procedure.

An ultrasound and Doppler evaluation of the portal system will be performed within 24 hours after each islet transplantation procedure to rule out the possibility of complication including portal thrombus or perihepatic hematoma. If portal vein thrombosis is encountered within a main right or left portal branch within the main portal vein (i.e., main portal vein, but not a segmental peripheral branch), this will be considered a serious adverse event (SAE), In case of portal thrombosis, the subject will receive therapeutic anticoagulation with heparin until reaching a therapeutic INR with oral anticoagulants for a period of 3 months. Ultrasound will be repeated until the portal vein thrombus is resolved.

Subjects will be discharged home 24 hours after transplant if afebrile, having stable hemoglobin, and provided post-transplant Doppler ultrasound is within normal limits. Subjects will be provided with contact information in the event of questions or adverse experiences following discharge.

6.6.7 Post-Transplant Period (see Appendix *Detailed Visit Schedule)*

Following transplant of islet cells, subjects will monitor their own glucose levels at home. Subjects will be asked to record results in a log for review at the next study visit. Blood samples for analysis (see Appendix *Detailed Visit Schedule*) are drawn three times per

week for the first two weeks and twice a week until the end of the 10th week (see Section 6..6.8 *Schedule of Evaluation*).

Subjects will come to the Clinical Research Center for visits and tests 3 times a week for the first two weeks, then twice per week for another 2 weeks, then weekly until week 12 after transplantation, then gradually tapered to every month during the first year. Additional visits may be necessary. The last study visit will be 52 weeks after transplantation. Additional evaluations may be required for medical conditions presenting during the study period and according to best medical practice.

When a second or third transplant is completed, the follow-up visit schedule is re-started to permit careful follow-up during the immediate post-operative period. In the event a second transplant is not needed due to successful engraftment, insulin independence, and stable blood glucose levels, the follow-up schedule will be maintained at monthly intervals following week 12 through the first year study period according to the S*chedule of Evaluation* in Section 6.6.8.

As in any other transplant situation, medical conditions that arise (e.g., new serious infection, malignancy, compliance issues, etc.) will automatically trigger a re-evaluation to determine if the subject remains qualified for continuous immunosuppression and subsequent transplants, if applicable.

The following laboratories will perform analyses of blood and urine samples:

CMV antibody panel, EBV antibody panel, C-peptide, insulin, anti-islet cell antibodies, anti-IA2, anti-GAD65, and anti-insulin antibodies will be analyzed at:
ARUP Laboratories
500 Chipeta Way
Salt Lake City, Utah 84108

CMVqPCR and EBVqPCR will be analyzed at:
ViraCor
1210 NE Windsor Drive
Lee's Summit, MO 64086

Inflammatory and detailed lipid panels will be analyzed at:
CRL Medinet, Inc.
8433 Quivira Road
Lenexa, KS 66215

Autologous crossmatch and donor crossmatch will be analyzed at:
Gift of Hope Organ & Tissue Donor Network Histocompatibility Laboratory
600 N. Industrial Drive
Elmhurst, IL 60126-1520

PRA and HLA will be analyzed at:
University of Illinois at Chicago Tissue Typing Laboratory

808 S. Wood Street
Chicago, IL 60612

All the other analyses will be performed at:
UIC Pathology Laboratory
1819 W. Polk Street (MC 847)
Chicago, IL 60612.

The following exams and blood test will be performed according to the *Schedule of Evaluation* (see Section 6.6.8 and Appendix *Detailed Visit Schedule*):

- History, and adverse event and toxicity assessments (reviewed at each visit and recorded at any time necessary)
- Physical exam including vital signs (blood pressure, heart rate, respiratory rate, temperature
- Body mass index (BMI: body weight in kilograms over square of height in centimeters)
- Electrocardiogram (ECG)
- Comprehensive metabolic profile (CMP):
  - Na^+, K^+, Cl^-, Ca^{++}, CO2, creatinine, BUN, glucose, total protein, albumin, total bilirubin, direct bilirubin, ALP, AST, ALT, alkaline phosphatase
- Complete blood count (CBC):
  - Hemoglobin, hematocrit, red blood cells, white blood cells, differential,, platelets
- Serum pregnancy test
- Urine analyses
  - Urinalysis
  - Creatinine, microalbumin, total protein
  - M/c ratio (microalbumin-creatinine ratio) 24-hour specimen at phase 1 screening, and random specimens thereafter unless a 24-hour specimen is medically indicated by elevated results from random sampling
- Drug levels
  - Tacrolimus and sirolimus trough levels to reach target levels defined in Section 6.5.2
- Cytomegalovirus (CMV) viral load by real time PCR
- Epstein-Barr virus (EBV) viral load by real time PCR
- HbA1c, basal C-peptide, fructosamine
- Blood sugar self monitoring (BS-SM)
- Metabolic tests to evaluate insulin-secretory capacity of the islet graft:
  - The following metabolic tests will be done on separate days. The tests are performed after an overnight fast greater than or equal to 10 hours; subjects may drink water. For subjects still on insulin injections, long-acting insulin may be taken no less than 8 hours prior to the test; no food or short-acting insulin may be taken within 5 hours of the test. Humalog should not be taken within 4 hours of the test. If a subject's blood glucose reading is 15 mM or greater (> 270 mg/dL) on the morning of the test, the test will be

rescheduled rather than to perform a procedure which will greatly increase the glucose levels. Additionally, subjects must have consumed at least 150 gm carbohydrate in their diet for the 3 days leading up to the test.

IVGGT, intravenous glucose tolerance test

- An intravenous line is inserted on each arm, and 300 mg/kg of glucose (using a 50% glucose solution) is injected as rapidly as the subject tolerates and within less than 3 minutes. Blood samples for glucose, C-peptide, and insulin measurement are drawn at time -10, -1 (before injection), 2, 3, 4, 5, 6, 8, 10, 15, 20, 30, 40 minutes after glucose injection.
- The acute insulin response will be defined as the mean of the 3, 4 and 5 minutes insulin values with the mean of the basal values (–10 and 0 minute) subtracted.

- GST, glucagon stimulation test
  - Glucose, C-peptide, and insulin levels are measured before and six minutes after injecting a bolus of 1 mg of glucagon (Glucagen®, Novo Nordisk Pharma SA) or the equivalent intravenously.

- OGGT, oral glucose tolerance test
  - Starting at time 0 the subject consumes a drink containing 75 gm of glucose (in 300 mL solution) over a 5-minute period. Blood samples for glucose, C-peptide, and insulin are drawn before and at 30, 60, 90 and 120 minutes after the subject begins the drink.

- MMTT, mixed meal tolerance test
  - A fasting blood sample for serum glucose, insulin, and C-peptide is drawn just before the test. At time 0, the subject is given Ensure High Protein® or Boost® to drink in the amount of 6 mL per kg body weight to a maximum of 360 mL, which is to be consumed within 5 minutes. Ninety minutes from time 0, another blood sample is drawn for serum glucose, insulin, and C-peptide.

- Detailed lipid panel
  - Total cholesterol, direct LDL cholesterol, HDL cholesterol, triglycerides, apolipoprotein B, apolipoprotein A1, free fatty acids, and lipid fraction ratio
- Lipid panel
  - Cholesterol, HDL, triglycerides, LDL cholesterol
- Inflammatory panel
  - CRP, MCP, MMP9, CAM, VCAM, fibrinogen, PAI-1 (antigen), TPA
- B-type natriuretic protein (BNP)
- ABO/RH (UNOS requires 2 independent blood type tests)
- Panel reactive antibodies (PRA)
- Antibodies: anti-GAD 65, anti-IA2, anti-islet cell, and anti-insulin
- IL2 receptor saturation
- Archive serum

- Chest x-ray
- Abdominal ultrasound and Doppler
- Thyroid ultrasound (at screening and 52 weeks after final transplant)
- Nuclear renal scan if 24-hour urine corrected creatinine clearance is < 80 mL/min/1.73m^2 and/or serum creatinine is < 1.2 mg/dL on repeated measurement
- Primary care screening referrals:
    - Women:
        - Mammography annually from age 40
        - Pap test according to current American Cancer Association guidelines
    - Men:
        - Prostate specific antigen (PSA) annually beginning at age 50
    - All subjects
        - Fecal occult blood testing (FOBT) annually
        - Dual x-ray absorptiometry scans (DXA) every 2 years
        - Skin cancer screening annually
        - Dental examination yearly
        - Vision and retina examinations annually
        - Flu vaccine annually
        - Pneumovax according to current CDC guidelines for patients with diabetes or immunocompromising conditions: At least one vaccine and a one-time revaccination if the vaccine was administered > 5 years ago

6.6.8 Schedule of Evaluation for UIC Islet Transplant

Visit	A[1]	A[2]	A[3A]	A[3B]	Tx	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Week post transplant	0					1	2	3	4-5	6-7	8-9	10-11	12	16	20	24	28	32	36	40	44	48	52
History, physical exam	X			X	X	3	3	2	2	2	2	2	X	X	X	X	X	X	X	X	X	X	X
Vital signs, weight	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Body mass index	X				X				X				X				X						X
BS-SM 7/day	X	X	X	X	X	X	X																
BS-SM 4/day								X	X	X	X	X	X										
BS-SM 2/day														X	X	X	X	X	X	X	X	X	X
CBC	X			X	X	3	3	2	2	2	2	2	X	X	X	X	X	X	X	X	X	X	X
CMP, direct bilirubin	X		X	X	X	3	3	2	2	2	2	2	X	X	X	X	X	X	X	X	X	X	X
Magnesium					X																		
Phosphorous					X																		
Amylase					X																		
Sirolimus trough level					X	3	3	2	2	2	2	2	X	X	X	X	X	X	X	X	X	X	X
Tacrolimus (FK506) trough level					X	3	3	2	2	2	2	2	X	X	X	X	X	X	X	X	X	X	X
Alternative immunosuppressant**					X	3	3	2	2	2	2	2	X	X	X	X	X	X	X	X	X	X	X
CMV q PCR							X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
EBV q PCR									X		X		X		X		X		X		X		X
Lipid panel	X			X					X		X		X					X				X	
Basal C-peptide	X					3	3	2	2	2	2	2	X	X	X	X	X	X	X	X	X	X	X
HbA1c	X		X	X		X				X			X			X			X			X	X
Fructosamine		X				X				X			X			X			X			X	X
Detailed lipid panel		X																					X
Inflammatory panel		X																					X
Antibodies	X												X			X						X	
IL2 receptor saturation									X		X		X	X	X	X	X	X	X	X	X	X	X
BNP		X				X									X								X
HCG pregnancy (serum)	X			X	X										X								X
Archive research serum		X				X									X								X
Blood type (ABO) (2)	X	X																					
Type, crossmatch					X*																		
PT, PTT, INR	X			X	X																		
QuantiFERON® TB Gold test	X																						
HIV antibody	X			X																			
RPR	X			X																			
Hepatitis panel	X			X																			
EBV panel		X		X*	X*																		
CMV IgG, IgM		X		X*	X*																		
VZVIgG		X		X*																			
PRA	X		X	X			X						X			X			X			X	
Autologous cross match	X																						

Visit	A[1]	A[2]	A[3A]	A[3B]	Tx	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Week post transplant	0					1	2	3	4-5	6-7	8-9	10-11	12	16	20	24	28	32	36	40	44	48	52
HLA		X																					
Donor crossmatch					X																		
Urinalysis		X	X		X								X			X							X
24-hour urine creatinine clearance	X																						
Urine culture, sensitivity					#																		
M/C ratio (random)		X	X	X							X				X					X			X
Sputum culture, sensitivity					#																		
Blood culture, sensitivity					#																		
Glucagon stimulation test	X									X						X							X
Mixed meal test		X														X							X
OGTT										X						X							X
IVGTT										X						X							X
ECG	X			X											X								X
Chest x-ray		X																					X
Abdominal ultrasound, Doppler		X				X																	
Cardiology Consult		X																					
CGMS		X																					X
Primary care	X																						X
Thyroid Ultrasound		X																					X

Visit A: screenings for inclusion
Visits 1 and 2 include 3 evaluations per week; Visit 3 includes 2 evaluations per week; Visits 4 to 7 include 2 evaluations per 2 week period (1 visit per week)
Vital signs: blood pressure, heart rate, respirations, temperature
CMP: comprehensive metabolic profile
CBC: complete blood count
BNP: B-type natriuretic peptide
Antibodies: anti-GAD 65. anti-IA2, anti-islet cell, anti-insulin
IL2 receptor saturation at investigator's discretion
PRA: panel reactive antibodies
Only if hospitalized within the past week
* Only if negative at Screening II
CGMS: continuous glucose monitoring system at Screening A[2], 1 year after first transplant, 1 year after final transplant

**** Alternative immunosuppressant trough level only if applicable**

5-Year Follow-Up

Subjects who complete the original 52-week post-transplant evaluation period will be asked to continue follow-up evaluations every three months for five years for safety and efficacy monitoring. All subjects can voluntarily withdraw from follow-up at any time.

The following examinations and blood tests will be performed according to the *Schedule of 5-Year Follow-Up* (Section 6.7.1). Additional evaluations may be required for medical conditions presenting during the study period and according to best medical practice. Should the function of the islet graft decline over time and the subject return to insulin therapy, repeat transplant may be considered.

- History and adverse event and toxicity assessments (reviewed at each visit and recorded at any time necessary)
- Physical exam including vital signs (blood pressure, heart rate, respiratory rate, temperature)
- Body mass index (BMI, body weight in kilograms over square of height in centimeters
- Electrocardiogram (ECG)
- Comprehensive metabolic profile (CMP):
  - Na^+, K^+, Cl^-, Ca^{++}, CO2, creatinine, BUN, glucose, total protein, albumin, total bilirubin, ALP, AST, ALT, amylase
- Complete blood count (CBC):
  - Hemoglobin, hematocrit, red blood cells, white blood cells, differential
- Urine analyses
  - Urinalysis
  - Creatinine, osmolality, microalbumin, total protein
  - Microalbumin-creatinine ratio)
- Drug levels
  - Tacrolimus and sirolimus, and alternative trough levels if applicable to reach target levels as defined in Section 6.5.2
- Cytomegalovirus (CMV) viral load by real time PCR
- Epstein-Barr Virus (EBV) viral load by real time PCR
- HbA1c, basal C-peptide, fructosamine
- Blood sugar self-monitoring (BS-SM)
- Metabolic tests to evaluate insulin-secretory capacity of the islet graft:
  - IVGTT – intravenous glucose tolerance test
  - GST - glucagon stimulation test
  - OGTT – oral glucose tolerance test
  - MMTT - mixed meal tolerance test
- Detailed lipid panel:
  - Total cholesterol, direct LDL cholesterol, HDL cholesterol, triglycerides, apolipoprotein B, apolipoprotein A1, free fatty acids, and lipid fraction ratio
- Lipid panel:
  - Cholesterol, HDL, triglycerides, LDL cholesterol
- Inflammatory panel:

    - CRP, MCP, MMP9, CAM, VCAM, fibrinogen, PAI-1 (antigen), TPA
- B-type natriuretic protein (BNP)
- Panel reactive antibodies (PRA)
- Antibodies: anti-GAD 65, anti-IA2, anti-islet cell, and anti-insulin
- IL2 receptor saturation
- Archive serum
- Thyroid ultrasound (annually)
- Abdominal ultrasound and Doppler
- Primary care screening referrals:
    - Women:
        - Mammography annually from age 40
        - Pap test according to current American Cancer Association guidelines
    - Men:
        - Prostate specific antigen (PSA) annually beginning at age 50
    - All subjects
        - Fecal occult blood testing (FOBT) annually
        - Dual x-ray absorptiometry scans (DXA) every 2 years
        - Skin cancer screening annually
        - Dental examination yearly
        - Vision and retina examinations annually
        - Flu vaccine annually
        - Pneumovax according to current CDC guidelines for patients with diabetes or immunocompromising conditions: At least one vaccine and a one-time revaccination if the vaccine was administered > 5 years ago

6.6.9 Schedule of 5-Year Follow-Up

Visit	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Months	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45	48	51	54	57	60
History, physical exam, vitals	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BMI	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ECG				X				X				X				X				X
CMP, direct bilirubin	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CBC	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urinalysis		X		X		X		X		X		X		X		X		X		X
CMV q PCR	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
EBV q PCR		X		X		X		X		X		X		X		X		X		X
M/C ratio		X		X		X		X		X		X		X		X		X		X
IVGTT				X				X				X				X				X
Glucagon stimulation test				X				X				X				X				X
OGTT				X				X				X				X				X
Mixed meal test				X				X				X				X				X
HbA1c and fructosamine	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Basal C-peptide	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BS-SM twice weekly fasting	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Detailed lipid panel				X				X				X				X				X
Inflammatory panel				X				X				X				X				X
Lipid panel		X				X				X				X				X		
BNP		X				X				X				X				X		
Archive serum		X		X		X		X		X		X		X		X		X		X
Abdominal ultrasound Doppler				X				X				X				X				X
Tacrolimus, Sirolimus	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
PRA				X				X				X				X				X
Antibodies				X				X				X				X				X
IL2 receptor saturation	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Primary care screenings, vaccines				X				X				X				X				X
Thyroid Ultrasound				X				X				X				X				X

BMI: body mass index; Vitals: blood pressure, heart rate, temperature, weight; ECG: electrocardiogram; CMP: comprehensive metabolic profile; CBC: complete blood count; M/C ratio: microalbumin to urine creatinine ratio (random); IVGTT: intravenous glucose tolerance test; OGTT: oral glucose tolerance test; BNP: B-type natriuretic peptide; PRA: panel reactive antibodies; Antibodies: anti GAD65, anti IA2, anti islet cell, anti insulin. IL2 receptor saturation at investigator's discretion.

6.7 10-Year Follow-Up

Subjects who complete the 5-year post-transplant evaluation period will be asked to continue follow-up evaluations every three months for an additional five years for safety and efficacy monitoring. All subjects can voluntarily withdraw from follow-up at any time.

The following examinations and blood tests will be performed according to the *Schedule of 10-Year Follow-Up* (Section 6.8.1). Additional evaluations may be required for medical conditions presenting during the study period and according to best medical practice. Should the function of the islet graft decline over time and the subject return to insulin therapy, repeat transplant may be considered.

- History and adverse event and toxicity assessments (reviewed at each visit and recorded at any time necessary)
- Physical exam including vital signs (blood pressure, heart rate, respiratory rate, temperature)
- Body mass index (BMI, body weight in kilograms over square of height in centimeters
- Electrocardiogram (ECG)
- Comprehensive metabolic profile (CMP):
  - Na^+, K^+, Cl^-, Ca^{++}, CO2, creatinine, BUN, glucose, total protein, albumin, total bilirubin, ALP, AST, ALT, amylase
- Complete blood count (CBC):
  - Hemoglobin, hematocrit, red blood cells, white blood cells, differential
- Urine analyses
  - Urinalysis
  - Creatinine, osmolality, microalbumin, total protein
  - Microalbumin-creatinine ratio)
- Drug levels
  - Tacrolimus and sirolimus trough levels to reach target levels as defined in Section 6.5.2
- Cytomegalovirus (CMV) viral load by real time PCR
- Epstein-Barr Virus (EBV) viral load by real time PCR
- HbA1c, basal C-peptide, fructosamine
- Blood sugar self-monitoring (BS-SM)
- Metabolic tests to evaluate insulin-secretory capacity of the islet graft:
  - IVGTT – intravenous glucose tolerance test
  - GST - glucagon stimulation test
  - OGTT – oral glucose tolerance test
  - MMTT - mixed meal tolerance test
- Lipid panel:
  - Cholesterol, HDL, triglycerides, LDL cholesterol
- B-type natriuretic protein (BNP)
- Panel reactive antibodies (PRA)
- Antibodies: anti-GAD 65, anti-IA2, anti-islet cell, and anti-insulin
- IL2 receptor saturation

- Archive serum
- Thyroid ultrasound (annually)
- Abdominal ultrasound and Doppler
- Primary care screening referrals:
  - Women:
    - Mammography annually from age 40
    - Pap test according to current American Cancer Association guidelines
  - Men:
    - Prostate specific antigen (PSA) annually beginning at age 50
  - All subjects
    - Fecal occult blood testing (FOBT) annually
    - Dual x-ray absorptiometry scans (DXA) every 2 years
    - Skin cancer screening annually
    - Dental examination yearly
    - Vision and retina examinations annually
    - Flu vaccine annually
    - Pneumovax according to current CDC guidelines for patients with diabetes or immunocompromising conditions: At least one vaccine and a one-time revaccination if the vaccine was administered > 5 years ago

6.7.1 Schedule of 10-Year Follow-up

Visit	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Months	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45	48	51	54	57	60
History, physical exam, vitals	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BMI	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ECG				X				X				X				X				X
CMP, direct bilirubin	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CBC	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urinalysis		X		X		X		X		X		X		X		X		X		X
CMV q PCR	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
EBV q PCR		X		X		X		X		X		X		X		X		X		X
M/C ratio		X		X		X		X		X		X		X		X		X		X
IVGTT				X				X				X				X				X
Glucagon stimulation test				X				X				X				X				X
OGTT				X				X				X				X				X
Mixed meal test				X				X				X				X				X
HbA1c and fructosamine	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Basal C-peptide	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BS-SM twice weekly fasting	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Lipid panel		X		X		X		X		X		X		X		X		X		X
BNP		X				X				X				X				X		
Archive serum		X		X		X		X		X		X		X		X		X		X
Abdominal ultrasound Doppler				X				X				X				X				X
Tacrolimus, Sirolimus	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
PRA				X				X				X				X				X
Antibodies				X				X				X				X				X
IL2 receptor saturation	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Primary care screenings, vaccines				X				X				X				X				X
Thyroid Ultrasound				X				X				X				X				X

BMI: body mass index; Vitals: blood pressure, heart rate, temperature, weight; ECG: electrocardiogram; CMP: comprehensive metabolic profile; CBC: complete blood count; M/C ratio: microalbumin to urine creatinine ratio (random); IVGTT: intravenous glucose tolerance test; OGTT: oral glucose tolerance test; BNP: B-type natriuretic peptide; PRA: panel reactive antibodies; Antibodies: anti GAD65, anti IA2, anti islet cell, anti insulin. IL2 receptor saturation at investigator's discretion.

6.8 Withdrawal or Discontinuation

All subjects can voluntarily withdraw from the study at any time. In addition, the Investigator may discontinue subjects from the study under the conditions noted below. Individuals who prematurely leave the study may elect to continue immunosuppressive therapy under the care of their own physicians, and at their own expense. If a subject with functioning transplanted islets chooses not to continue immunosuppressive therapy, he/she must be informed of the risk for losing the islet graft and returning to his/her original method of insulin management.

6.8.1 When and How to Stop Sequential Transplants

Subjects will be not receive any subsequent transplants if either no suitable donor pancreata were made available during that period or the subject has become otherwise ineligible for a second transplant. These subjects will be encouraged to remain in the study for safety and efficacy monitoring.

Subjects will be immediately withdrawn from subsequent transplants for serious and unexpected adverse experiences that appear to be related to the treatment regimen. Subjects will also be withdrawn if they are no longer willing or able to comply with the protocol.

Subjects who become pregnant during the study period will be recommended to stop immunosuppression. If a subject is unwilling to stop immunosuppression, appropriate information must be given in regard to the risk of fetal malformations with the use of immunosuppression. In any case, sirolimus needs to be stopped and replaced by mycophenolate mofetil because of lack of documented experience with the use of sirolimus during pregnancy.

Subjects who withdraw or are discontinued from subsequent transplants will not be replaced, i.e., the total number of subjects transplanted will not be more than 50

6.8.2 Type and Timing of Data Collected from Withdrawn Subjects

Subjects who withdraw from the study for any reason will be asked to continue follow-up evaluations on their original protocol schedule, except for unnecessary tests not related to their clinical care (e.g., monitoring of blood levels for medications no longer being taken). At a minimum, the subject's health status information will be collected so that some safety and efficacy information may be obtained through the original follow-up period.

If a subject intends to withdraw and forego further follow-up examinations, he/she will be asked to complete at least a single comprehensive examination for study exit equivalent to the final one-year evaluation, or as much of that exam as he/she is willing to complete. Documentation of a subject's decision and reason to not continue or complete requested testing will be recorded in the case report form.

6.8.3 Follow-Up for Subjects Withdrawn from Treatment

Subjects will be followed on an intention-to-treat basis; data from all enrolled subjects who are assigned a participant identifier and who undergo islet transplantation will be collected, irrespective of outcome, for the scheduled minimum follow-up period of one year.

6.9 Adverse Events Definitions and Monitoring

6.9.1 Adverse Event (AE)

An adverse event is any unfavorable and unintended diagnosis, symptom, sign (including an abnormal laboratory finding), syndrome, or disease which either occurs during the study, having been absent at baseline, or if present at baseline, appears to worsen.

The length of time between screening and first transplantation may be 6 months or more. Therefore, non-serious adverse events will be recorded on the case report forms (CRF) for the period starting with hospitalization for the first transplantation and during the subsequent study period because these events are relevant to the islet cell transplantation. All serious adverse events (see below) regardless of time relative to transplantation will be recorded on the case report form

6.9.2 Serious Adverse Event (SAE)

A serious adverse event (experience) is defined (21 FCFR 312.32) as any adverse experience that suggests a significant hazard, contraindication, side effect, or untoward medical occurrence that:

1. Results in death
2. Is life-threatening
3. Requires or prolongs hospitalization, including emergency room care
4. Causes persistent or significant disability or incapacity
5. Results in congenital anomaly
6. Requires intervention to prevent permanent impairment or damage or in the judgment of the investigator represents significant hazard to the study participant.

6.9.3 Assessment of Causality

The relationship or attribution of a medical event or toxicity, or a serious adverse event (being possibly related or not) to an investigational product or therapy represents the best estimate of the principal investigator or sub-investigator at the time of reporting the causal relationship.

The investigator's determination of treatment-relatedness (attribution) for each medical event or toxicity should be recorded in the source documentation and on the case report form. For this protocol, the investigator must place the attribution in either the "possibly related" or "not possibly related" categories. (This collapses the five suggested NCI-CTC categories—definite, probable, possible, unlikely, and unrelated—into a binary choice of possibly related or not.). For each event, the attending physician or clinician in

conjunction with the research nurse who examined and evaluated the subject should assign the attribution. Data managers who are removed from the clinical assessment of the subject must not perform this important task.

6.9.4 Severity of Adverse Event

Severity grades are assigned by the investigator to indicate the severity of medical events and toxicities. This protocol will use the Collaborative Islet Transplant Consortium *Terminology Criteria for Adverse Events (TCAE) In Trials of Adult Pancreatic Islet Transplantation, Version5.0 (03 August 2011)* for reporting and grading medical events. The purpose of using the TCAE system is to provide a standard language to describe toxicities and medical events in islet transplantation, to facilitate tabulation and analysis of the data, and to facilitate the assessment of the clinical significance of treatment-related toxicities and medical events.

- The TCAE provides a detailed list of terms and a grade that closely describe the medical event or toxicity. While over 300 terms are described, each with a graded severity scale, the investigator may need to insert new terms when an applicable term cannot be found in the list.

Adverse events *not* included in the TCAE listing should be recorded and graded 1 to 5 according to the *General Grade Definitions* provided below (see Table 1):

Table 1. General Grade Definitions for Items Not in TCAE Listing

Grade	Descriptor	Definition
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)
2	Moderate	Mild to moderate limitation in activity; some assistance may be needed; no or minimal intervention/therapy required, hospitalization possible
3	Severe	Marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalization possible
4	Life-Threatening or Disabling	Extreme limitation in activity, significant assistance required; significant medical/therapy intervention required, hospitalization or hospice care probable
5	Death	Death

6.9.5 Monitoring and Reporting Adverse Events

The investigator will monitor participants for the occurrence of adverse events during the course of the study. In addition, a Data Monitoring Committee (DMC) will assist in the

monitoring of patient safety (see Section 6.12.2). All non-serious adverse events reported from the time of hospitalization for the initial islet cell transplantation and subsequent study period will be recorded on the case report form. All serious adverse events regardless of time relative to transplantation will be recorded on the case report form. The investigator will instruct subjects prior to transplantation to report any physical changes or new symptoms that they notice during the course of the study.

Physicians and health care personnel involved in the subject's medical care will be instructed to report all serious adverse events (defined above) as soon as possible to:

José Oberholzer, MD
Associate Professor of Surgery
University of Illinois
840 South Wood Street, Suite 402 (MC 958)
Chicago, IL 60612
Tel: 312 996 6771
Email: jober@uic.edu

When a serious adverse event is considered reportable to regulatory agencies, Dr. Oberholzer will prepare the initial report and forward copies to the FDA, the Institutional Review Board (IRB), and the Data Monitoring Committee. Under FDA regulations (21 CFR 312.32) the sponsor of an IND application, in this case the investigator, is required to report in writing within 15 days to the FDA any serious and unexpected adverse experiences, and by telephone and facsimile within 7 days any unexpected fatal or life-threatening event. For reporting events to the FDA, an FDA3500-Medwatch form will be filled out and submitted to the appropriate authorities with copies to the UIC IRB.

According to monitoring procedures in place at University of Illinois at Chicago, the investigator must report all deaths, clinically significant infections, and hospitalizations, regardless of potential association with the investigational therapy to the IRB. Other serious adverse events and toxicities are also submitted to the IRB.

Serious events will be followed until resolved or considered stable. The following attributes must be assigned: description, date of onset and resolution (if known when reported), severity, assessment of relatedness to test therapy, and action taken.

According to institutional procedures at the study site, outcomes of serious adverse events are also reported to the IRB throughout the study period. Regular updates at scheduled visits include completion of the toxicity grading, which should reflect the status (improvement, stability, or worsening) of each adverse event reported. When requested, the investigator must summarize in writing the details regarding remarkable adverse events, their treatment and resolution for review by the IRB and relevant regulatory authorities.

All transplant procedure related complications are reviewed on a weekly morbidity and mortality meeting within the Department of Surgery at the University of Illinois at Chicago and supervised by the Chief of General Surgery at UIC.

The principal investigator and co-investigators will apply their clinical judgment whether or not an adverse event is of sufficient severity to require that the subject should immediately be removed from treatment related to the transplantation (e.g., subsequent islet transplantation, immunosuppressive therapies, etc.) (see Section 6.9 S*ubject Withdrawal or Discontinuation*). If necessary, the investigator must suspend any trial treatments and institute any necessary medical therapy to protect a subject from any immediate dangers. Review of safety events by ethics review committee or IRB, or the FDA or relevant local regulatory authorities may also suspend further trial treatments.

A subject may also voluntarily withdraw from treatment due to what he/she perceives as an intolerable adverse event or for any other reason. If voluntary withdrawal is requested, the subject should be asked to continue (at least limited) scheduled evaluations, complete an end-of-study evaluation, and be given appropriate care under medical supervision until the symptoms of any adverse event resolve or his/her condition becomes stable.

Reporting of Pregnancy

Subjects of childbearing potential, both male and female, must be either surgically sterile or use adequate contraception and express an intent not to become pregnant while participating in this study. For participants who are not surgically sterile, investigators will recommend contraception methods using either a) hormonal and barrier method combination, or b) abstinence (for both male and female subjects).

Documentation that counseling was provided regarding the importance of contraception during participation in the study will be recorded in the subject's medical chart.

Pregnancy occurring while the subject is participating in this study will be reported to the IRB and measures taken as described in Section 6.9 *Withdrawal or Discontinuation*.

6.9.6 Enrollment and Transplantation Stopping Rules

The investigator will stop study enrollment and islet transplantation in the event that the following events occur:

- 2 subjects experience hepatic vein thrombosis following transplantation, or
- 3 final islet preparations show positive culture results, or
- 1 subject experiences death related to the procedure

In addition, the DMC will review eligibility and safety data through 2 weeks after transplantation and agree that islet transplantation can continue with the next subject (see Section 6.12.2).

The study investigator will notify the FDA and IRB that enrollment and transplantation have been stopped. Transplanted subjects will continue to be monitored for safety and efficacy and continue to receive immunosuppressive therapy to maintain their transplants. The investigators and DMC will review all safety data on all subjects enrolled as well as safety data on subjects experiencing these events. These data and assessments of the investigator and DMC will be submitted to the IND for FDA review

and discussion. Enrollment will resume and the 2nd or 3rd islet cell transplantation be conducted only when the FDA has reviewed the clinical safety data and notified the investigator that the procedure can continue.

6.10 Assessment of Endpoints

The primary objective of the study is to demonstrate the safety and efficacy of allogeneic islet transplantation in improving glycemic control in Type 1 diabetic patients performed at the University of Illinois at Chicago.

6.10.1 Safety

Safety of islet cell transplantation in Type 1 diabetes depends primarily on determining the incidence of serious and unexpected complications or adverse events. Additionally, the ability of the cell isolation laboratory to produce uncontaminated islet cell preparations with minimal endotoxin content will be considered as a safety variable. The surgical implantation procedure has associated risk and complications of the islet cell infusion will be followed closely. The post-transplant immunosuppression regimen also has expected toxicities, both acute and chronic. Laboratory measures and signs and symptoms will be followed at specified intervals (see Section 6.6.8 *Schedule of Evaluation* and Appendix *Detailed Visit Schedule*). Some of these measures are often more subjective and variable in nature, but should provide a clear indication of relative safety for subjects enrolled in this study.

All study participants who receive an islet cell transplant will be followed for safety for 1 year following the last transplantation. The safety of the islet transplantation and associated immunosuppressive therapies will be evaluated by analysis of adverse experiences, clinical laboratory tests, and physical examination. Safety events will be analyzed by their incidence, severity, and relationship to islet cell transplantation. In particular, the following parameters are of primary interest in assessing the clinical safety of islet cell transplantation:

Specification of Safety Parameters
One year after first and the last islet transplants we will evaluate:

a. Procedure Related Events
The incidence and severity of adverse events related to the islet infusion procedure including:

- Bleeding (> 2 g/dl decrease in hemoglobin concentration)
- Portal vein thrombosis, branch or main
- Biliary puncture
- Wound complication (infection or subsequent hernia)
- Increased transaminase levels (> 5 times upper level of normal)

b. Immunosuppression Related Events

The incidence and severity of adverse events related to immunosuppression including allergy:

- Reduction in GFR (> 25% reduction in estimated GFR from baseline by Cockroft and Gault formula)
- Increase in urinary albumin excretion
- New onset microalbuminuria (albumin > 30 mg/day confirmed by 24 hour urine collection) in patients who were previously within normal limits
- For those patients with baseline microalbuminuria (30-300 mg/day), new onset overt albuminuria (greater than 300 mg/day confirmed by 24 hour urine collection)
- Addition or intensification of antihypertensive therapy
- Oral ulcers
- Lower extremity edema
- Gastrointestinal toxicity (diarrhea)
- Neutropenia (neutrophils < 1.3 thous/µL)
- Anemia (hemoglobin men < 12.1, women < 11.7 g/dL)
- Thrombocytopenia (platelets < 150 thous/µL)
- Infections (viral, bacterial, or fungal)
- Neoplasms, benign or malignant

Severity will be graded according to Collaborative Islet Transplant Consortium *Terminology Criteria for Adverse Events (TCAE) In Trials of Adult Pancreatic Islet Transplantation, Version5.0 (03 August 2011)*

c. Islet Preparations for Transplant
The following parameters will be assessed to determine the safety of each islet preparation for transplant:

- Microbial contamination (preliminary Gram stain and subsequent culture results)
- Endotoxin content
- Final packed cell volume (mL)

A copy of the islet release sheet containing all the relevant information on the islet graft characteristics, unique batch identification number, and compliance with the release criteria will be included in the case report form.

6.10.2 Efficacy

Specification of Efficacy Parameters

a. Primary Efficacy Endpoint:

The proportion of subjects with an HbA1c ≤ 6.5% and free of severe hypoglycemic events at 1 year after the first islet cell infusion

Standard insulin treatment can rarely improve glycemic control in brittle Type 1 diabetic patients who are already on state-of-the-art insulin therapy. In general these patients require insulin reduction to reduce hypoglycemic episodes, which would likely lead to higher HbA1c. In contrast, islet transplantation can reduce hypoglycemic episodes without increasing HbA1c.

Definition of severe hypoglycemic event: An event with symptoms compatible with hypoglycemia in which the subject required the assistance of another person and which was associated with either a blood glucose level < 50 mg/dl (2.8 mmol/L) or prompt recovery after oral carbohydrate, intravenous glucose, or glucagon administration.

For the primary endpoint (composite HbA1c ≤ 6.5% and absence of severe hypoglycemic events), we expect 80% of the transplant population to achieve this endpoint. Therefore, assuming a sample size of 50, a two-sided 95.0% confidence interval for a single proportion will extend ± 0.111 from the observed proportion. When the sample size is 40, a two-sided 95.0% confidence interval for a single proportion will extend ± 0.124 from the observed proportion. Proportion and surrounding confidence interval will be reported.

Figure 2. Sample Size Curve for 95% Confidence Interval for Proportion

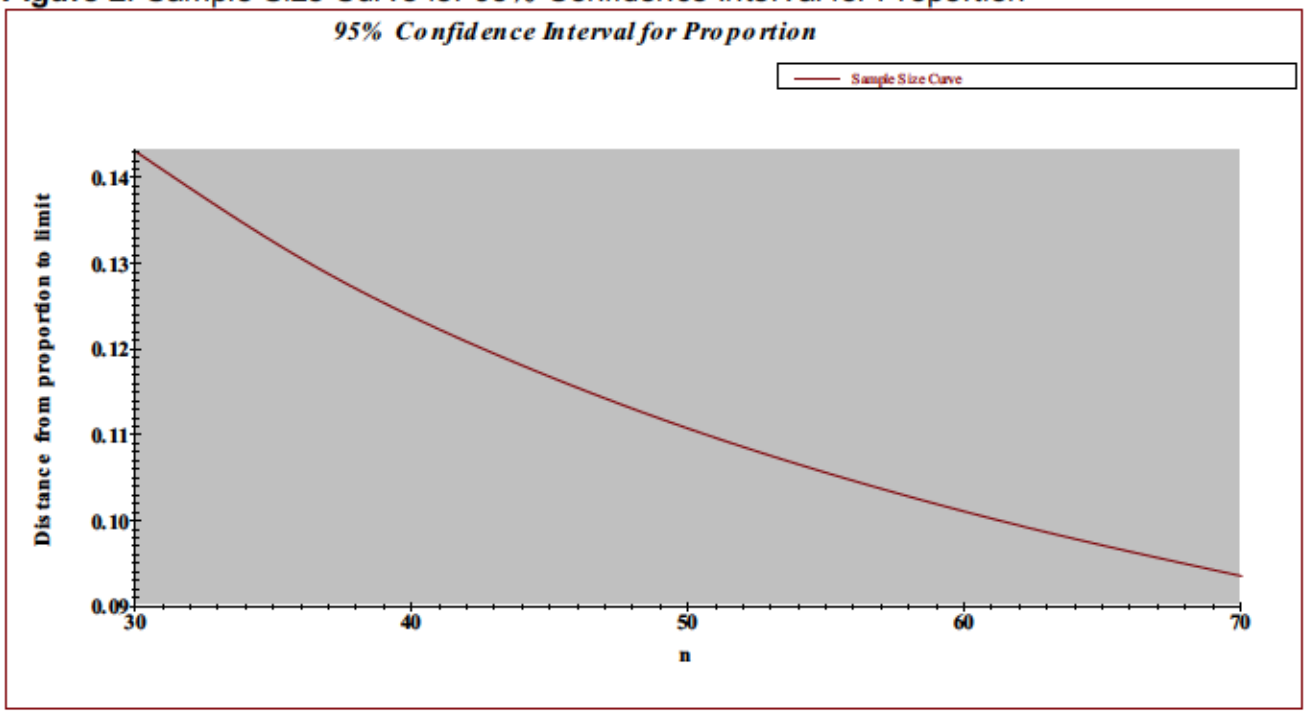
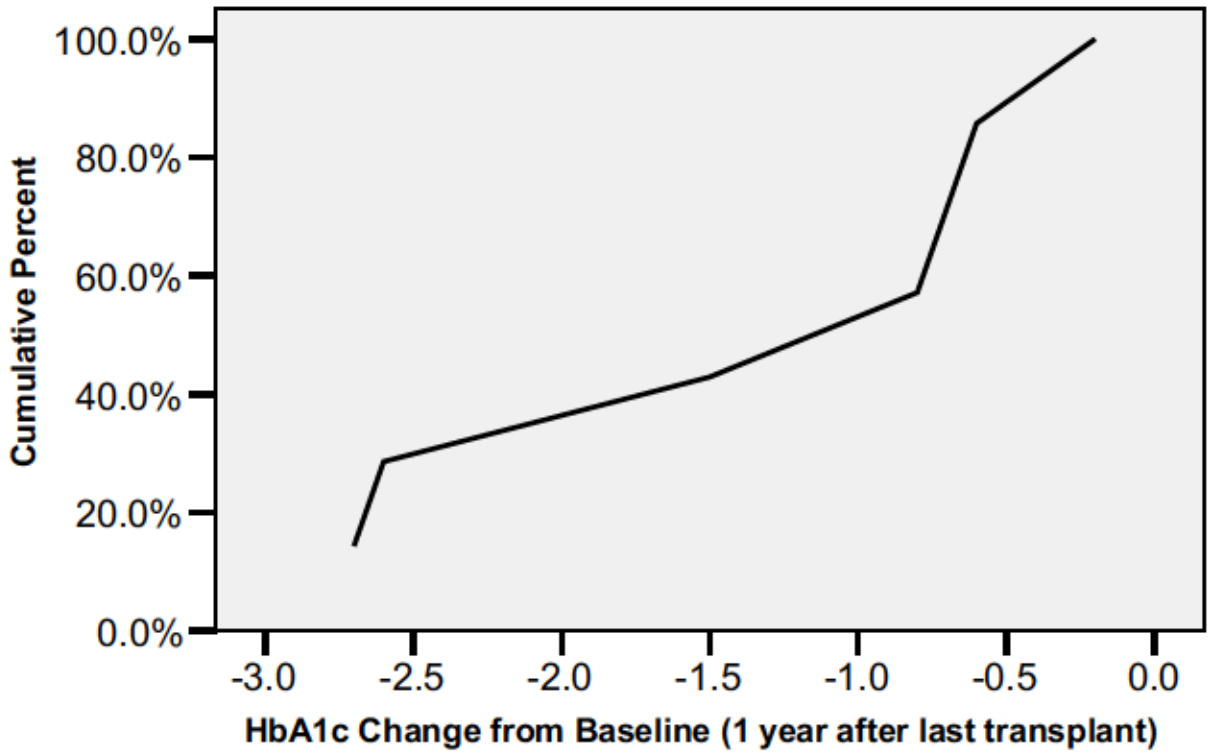


Figure 3 illustrates the cumulative percent of patients achieving HbA1c reduction from baseline in Phase1/2. As shown, all patients experienced a reduction in HbA1c (decrease of 0.6 to 2.7%). Furthermore, all patients presented with a HbA1c ≤ 6.5 one year after final transplant.

Figure 3. Cumulative Percent of Patients Attaining HbA1c Reduction from Baseline at 1 Year after Last Transplant (N=7).



b. Secondary Efficacy Endpoints:
The secondary efficacy endpoints will focus on more detailed analysis of glycemic control, and will include the following:

1. **Insulin independence**: We will evaluate the proportion of subjects presenting with insulin independence while fulfilling the primary endpoint.

Definition of insulin-independence:

- Absence of exogenous insulin injection
- HbA1c ≤ 6.5%
- 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.
- Fasting plasma glucose level ≤ 126 mg/dL (7.0 mmol/L); if the fasting plasma glucose level is > 126 mg/dL (7.0 mmol/L), it must be confirmed in an additional one out of two measurements.
- 2-hour postprandial 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.
- Evidence of endogenous insulin production defined as fasting or stimulated C-peptide levels 0.5 ng/mL (0.16 nmol/L)

2. **Hypoglycemic episodes** will be measured by the number and severity of hypoglycemic episodes quantified by the Ryan HYPO Score (31) (see Appendix *HYPO Score sheets*), and the percent reduction will be reported.

3. **Glucose variability and hypoglycemia duration will be measured by continuous glucose monitoring system (CGMS)** for a 3-day period at three different time points: 1) at screening, 2) one year after first islet transplant, and 3) one year after last islet transplant. The following measurements and analysis will be reported:
    1. Mean glucose concentration
    2. Percent of time in the following ranges:
        < 60 mg/dl
        60-140 mg/dl
        141-200 mg/dl
        > 200 mg/dl

6.10.3 Analysis of Endpoints

The schedule of assessments for efficacy parameters is described in Section 6.6.8 *Schedule of Evaluation*. All data will be entered in the case report form and will also be entered into a secure electronic subject chart used at the University of Illinois at Chicago (PowerChart by Cerner, Missouri, USA). All correspondences, blood work and examinations are automatically recorded in PowerChart.

All subjects who receive an islet transplant will be analyzed on an intent to treat basis. Subjects not tolerating exenatide or any other study medication and completing the study will be included for endpoint analysis. Descriptive information on subjects who withdraw from any study medication will be provided; it is unlikely that the study would have enough statistical power to allow for a subset analysis.

We plan to recruit and transplant all subjects within the first three years of this study and to complete all sequential transplants and follow-up by the end five years. All subjects will have a minimum follow-up of one year after the completion of islet transplantations. All subjects will be offered a 5-year follow-up.

6.11 Monitoring of Patient Safety

6.11.1 Informed Consent

The investigator is responsible for obtaining informed consent from all subjects in accordance with U.S. federal regulations (21 CFR 50 and 21 CFR 312.60) (see

Appendix *Letter of consent*). An individual meeting with each subject will give detailed information about the purpose, risks, and comforts involved and potential benefits of the study. The subject will be involved an explanation and discussion of the contents of each page of the consent form to ensure understanding of the consent form.

The subject will sign and date a written Informed Consent. The original signed and dated copy of the Informed Consent will be maintained at the study site. The names of the subjects enrolled during this study will be considered confidential.

6.11.2 Patient Eligibility and Safety

Data Monitoring Committee

In order to assist in the monitoring of the safety of subjects participating in this study, the investigator will establish a Data Monitoring Committee (DMC). The DMC will consist of 3 members who have training in medicine and/or organ transplantation. Some members may provide temporary coverage for the investigator or co-investigator while they are off-site (e.g., vacation) but otherwise will not be involved in the medical care of subjects. In the event that a DMC member is covering an investigator, that member will recluse him/herself from voting on DMC decisions. At all times, only one member may cover an investigator at a time so that 2 members are available for review of safety data and to make any decisions.

For each subject, the DMC will review the eligibility and safety data approximately 2 weeks following each islet cell transplantation. In the event that a second transplantation will be done in the same subject during the first 2 weeks following the first transplantation, the DMC will review the eligibility and available safety data for this subject prior to his/her second transplantation. The DMC must review the safety data through week 2 following transplantation on the preceding subject and agree that islet cell transplantation can continue in the subsequent subject. In this way, an interval of 2 or more weeks will exist between subjects undergoing islet transplantation to allow the review of safety events. This applies to the first 2 subjects in this cohort. If no serious events were observed, subsequent transplants can be performed in another patient during this 2-week review phase following a transplant.

The DMC will meet every 3 months to review the collected clinical data in order to assess safety. The DMC may also meet in an emergency situation when requested by the investigators or should the study meet a stopping rule (See Section 6.10.6).

It should be noted that in addition to the formal monitoring of safety by the DMC, the IRB will be notified of each serious adverse event. As described above, the IRB has the right to suspend the clinical study at any time for safety reasons.

Subject Eligibility

Only subjects fulfilling the inclusion criteria and not presenting any of the exclusion criteria will be entered into the study. The inclusion and exclusion criteria will be evaluated by the principal investigator, Dr. Oberholzer. Fulfillment of eligibility criteria will be reviewed by the DMC (see above) and the study monitor (see Section 6.13.2).

6.12 Regulatory Standards

6.12.1 Case Report Form

A case report form (CRF) will be completed for every subject who signed a written Informed Consent form and undergoes an islet cell transplantation..

The principal investigator must sign and date a case report certification statement upon completion of the study by the subject. This signature will indicate that thorough inspection of the data has been made and will certify the contents of the case report forms.

The investigator or institution will retain all original source documentation (e.g., laboratory results, treatment records, audit queries, etc.) unless specified otherwise by the protocol. All data will be entered in the study chart and will also be entered into a secure electronic patient chart used at the University of Illinois at Chicago (PowerChart by Cerner, Missouri, USA). All correspondences, blood work and examinations are automatically recorded in PowerChart, which is protected by HIPAA. The results as they become available will be entered on the appropriate case report forms.

Case report forms will be reviewed at the study site by a clinical monitor who will make a decision as to their acceptability in regard to completeness and accuracy of data recording. Audit queries will be generated for omissions, corrections, and clarifications.

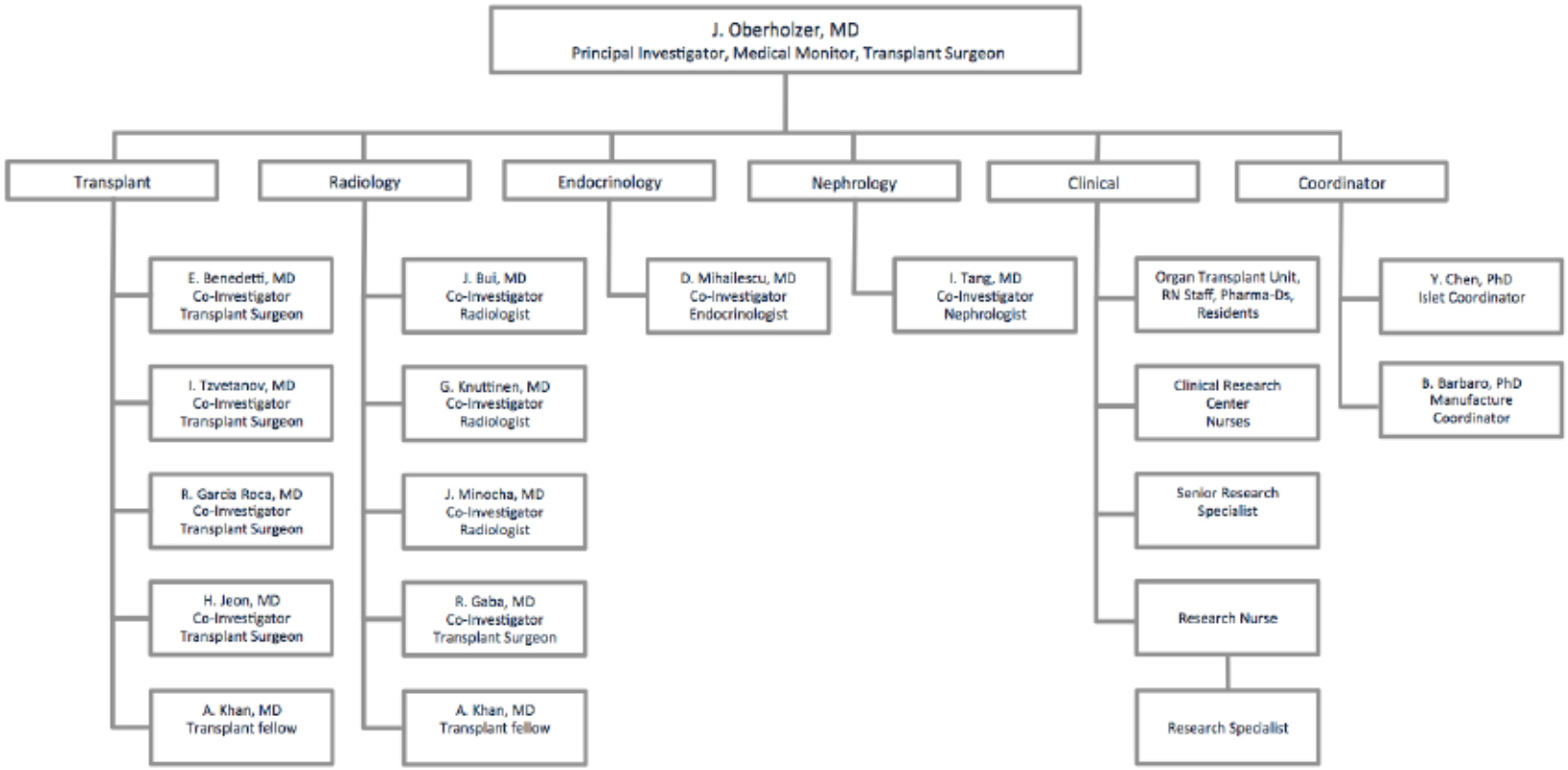
A copy of the Transplant Release Form containing all the relevant information on the islet graft characteristics, unique batch identification number and compliance with the release criteria will be included in the case report form. There is traceability in all steps of the transplant procedure and adverse event reporting, which will respect the mutual anonymity of the donor and the recipient. The traceability system with inclusion of the UNOS donor identification number enables the path taken by each donation to be traced from the donor to the recipient or to disposal and vice versa.

6.12.2 Clinical Monitoring of the Study

The study will be monitored in compliance with the relevant parts of 21 CFR and according to ICH GCP Guidelines.

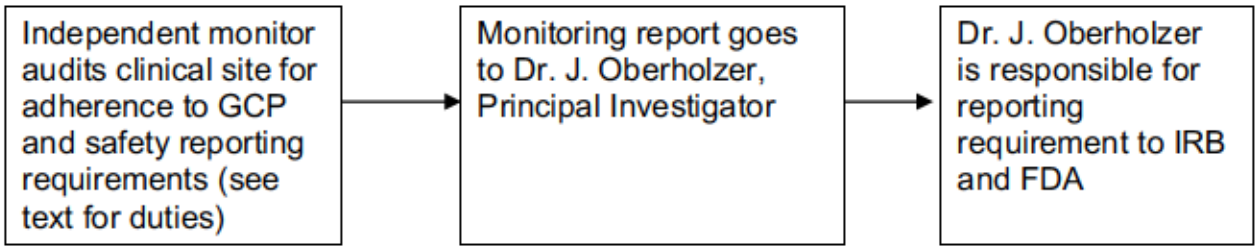
The following organizational chart depicts the principal investigator's oversight of the clinical study:

Organizational Chart



Study Conduct (Site)

An independent monitor knowledgeable in Good Clinical Practice (GCP) guidelines and regulations will monitor the clinical study and report to Dr. Oberholzer.



The monitor will visit the study site prior to study initiation and periodically thereafter to monitor the acceptability of the facilities, the agreement between CRF entries and original source documentation, adherence to the clinical protocol including documentation of study procedures and adherence to the treatment plan, adherence to GCP and to applicable FDA regulations, and the maintenance of adequate clinical records. The monitor will also verify documentation of informed consent and study eligibility for all subjects enrolled. The monitor will review all CRFs to verify that data are recorded appropriately including any changes and corrections to the CRF. The monitor will have access to participant records, medication sheets, laboratory data, and other source documentation.

In addition to the initiation visit, the clinical site will be monitored yearly during the study and at study completion or termination. Monitoring visits during the study conduct may occur after the 2nd and 5th subject have received their first islet cell transplantation. The investigator may increase the number or change the timing of these visits depending upon the occurrence of safety events. At each visit, the investigator will cooperate with the monitor in their review and verification of all CRFs. The monitor will ensure that all required safety reports are submitted to FDA and IRB within the required timeframes. The monitor will also ensure that any study protocol modifications/amendments are submitted to FDA and IRB as required.

All information contained in a subject's CRF must have corresponding source documentation. This source documentation includes but is not limited to notes taken at study visits recording the date of the visit, vital signs, physical findings, adverse events, and concomitant medications; laboratory reports; hospital records; and clinic records. Any correction of errors in the CRF will be first reviewed with the investigator prior to correction. The reason for any correction to the CRF will be noted along with the date and initials of the person making the correction.

The records of the subject may be audited by the FDA. The investigator agrees to allow access to the required subject records in the event of such an audit.

Within a reasonable time following completion of the study, a final study report will be written and submitted to FDA.

6.12.3 Study Subject Confidentiality

The medical and research record will be confidential to the extent permitted by law. Some of the research information will be part of the subject's permanent medical records. Study subjects will be identified by a code for the research records, and personal information from research records will not be released without written permission by the subject except:

- If necessary to protect the subject's rights and welfare (for example, if the subject is injured and needs emergency care or when the UIC Institutional Review Board monitors the research or consent process)
- If required by law

In compliance with regulatory guidelines regarding the monitoring of clinical studies, it is required that data generated as a result of the study be available for inspection on request by the study monitor and regulatory agencies. These shall include all study-relevant documentation, including medical histories to verify eligibility, laboratory test results to verify transcription accuracy, treatment and diagnostic reports, admission/discharge summaries for hospital admissions occurring while the subject is on-study, and autopsy reports (if available) for deaths occurring during or in temporal proximity to the study.
The subject will not be identified in any publication about this study. However, the FDA, IRB, and the investigators of the study at UIC may review the records, and as a result the subject's name will be seen. However, the persons reviewing the record will be bound to the rules of confidentiality and will not reveal the identity to others.

As part of the required content of the informed consent, all subjects will be asked for consent for access to the subject's medical record by the study monitor, FDA, and IRB.

6.12.4 Records

The FDA requires that an investigator retain records for a period of 2 years following the date a New Drug/Biologic Application or Product License Application is approved for the drug for the indication for which it is being investigated; or, if no application or license is to be filed, or if the application or license is not approved for such indication, until 2 years after the investigation is discontinued (21 CFR 312.62).

The investigator will ensure that the following records are maintained: subject files containing copies of completed case reports and supporting documentation and a copy of the signed, Informed Consent form.

Investigator files will include copies of the executed form FDA 1572, Curricula Vitae for the principal investigator and co-investigators, copy of the IRB approval of the protocol ***and Informed Consent forms, copies of correspondence with IRB and FDA, protocol and*** protocol amendments, revisions to the Informed Consent, documentation of the composition of the IRB, normal values with ranges for medical, laboratory, and technical procedures and tests included in the protocol, certification or accreditation of laboratories used, all monitoring reports, log of subject screening for eligibility, list of

participant names and codes, signature sheet for all persons authorized to make entries and/or corrections on the CRF, documentation of location and identification of any retained samples of body fluids.

6.12.5 Collaborative Islet Transplant Registry

Pre- and post-transplant data of consenting subjects will be entered in the Collaborative Islet Transplant Registry (CITR). CITR is a research effort funded by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) with supplemental funding from the Juvenile Diabetes Research Foundation International (JDRFI). The Registry is a collaborative effort between islet transplant centers in North America and other parts of the world to collect and analyze information to advance the science of islet transplants. Participation in the Registry is voluntary both by the islet transplant center and the islet transplant recipient. A coordinating center located at the EMMES Corporation, Rockville, MD, is responsible for the day-to-day activities of the Registry and data collection and analysis. Participating transplant centers code subjects' identities and enter data in a secure password-protected Internet Data Entry System according to the Registry protocol schedule in full compliance with HIPAA requirements.

6.13 Substudies

6.13.1 A Novel Beta Cell Specific Microfluidic Perfusion and Imaging Device for Islet Potency Testing Substudy

This substudy involves an innovative microfluidic device that will be optimized for human islet potency testing. If successful, the microfluidic-based assay could be used for islet product release testing, which represents an unmet regulatory requirement for FDA biologic licensure for islet transplant.

Islet transplantation is the only minimally invasive therapy for Type I diabetes that is able to achieve glycemic control without exogenous insulin. However, islet transplantation shows variable success rates, mainly due to the inconsistent quality of human islet preparations. For islet transplantation to become a FDA licensed biologic product, a well-established islet preparation process and product manufacturing consistency will need to be demonstrated. Federal regulations mandate that each biologic product lot be tested for potency before being
released for clinical use. At present, there is no reliable potency test available for human pancreatic islets. We hypothesize that an appropriate islet potency test must be beta-cell specific and simultaneously assess key factors associated with islet physiology, including glucose-stimulated changes in mitochondrial potentials, calcium influx and dynamic insulin secretion. To test this hypothesis, an innovative islet perifusion system with functional, live microscopy was developed using microfluidic chip technology to enable simultaneous measurement of glucose-induced changes in mitochondrial potentials, calcium influx and dynamic insulin secretion. Preliminary results indicate that this system can adequately distinguish low potency from high potency human islet preparations. This project will focus on the following aims: (1) To further improve the resolution of the microfluidic system through modification of the chip design. Specifically,

our proposal focuses on: a.) improving temporal resolution by reducing the volume of the chamber within the microfluidic chip, b.) improving flow dynamic control and increasing ease of use by adding a fluid mixer into the chip, c.) establishing glucose ramps to evaluate insulin kinetics, d.) integrating multiple perifusion chambers into the chip on a motorized platform to increase the sample size of human islets that can be evaluated and provide a better representation of the final islet product. e.) developing a rapid insulin secretion measurement. (2) To validate the microfluidic system in a pre-clinical nude mouse model using human islet cell grafts and develop an "Islet Potency Index" predictive of post-transplant islet graft function. Multivariable regression modeling will determine which islet cell characteristics are significantly associated with in vivo outcome, and these will be used to calculate the index. (3) To test the microfluidic system in the setting of a clinical human islet transplant trial and investigate the validity of the Islet Potency Index to predict islet graft function.

Subjects enrolled in the current Phase 3 clinical trial under IND BB-11807 will be involved in this research only to the extent that investigators will use data already required by the clinical trial protocol. No additional subject involvement will occur (see Appendix *A Novel Beta Cell Specific Microfluidic Perfusion and Imaging Device for Islet Potency Testing Substudy)*

7 Chemistry, Manufacturing and Control Information

7.1 General Information

7.1.1 Product Definition

The product consists of isolated allogenic human islets of Langerhans formulated in serum-free transplant media (indicator-free1066 with Hepes, without Bicarbonate and supplemented with Human Albumin).

7.1.2 Final Product Manufacturer

The product will be manufactured at the University of Illinois at Chicago. The University of Illinois Islet Processing Facility is a certified Class 1,000 clean room designed to provide a GMP environment appropriate for islet processing.

7.2 Product Manufacturing

7.2.1 Components Used in Manufacture

Cellular Components

a. Donor Screening

All potential donors will be screened at the time an offer is made by an Organ Procurement Organization via telephone. Only the principal investigator, Dr. Oberholzer, or a trained, qualified designee will screen potential donors. The medical-social history form must be reviewed for risk of disease transmission. All donors will be screened for safety and for organ quality. All offers will be logged and tracked using a Donor Screening Form and logbook. All screening criteria are outlined in standard operating procedure (SOP) form.

Safety screening will include criteria defined in the Suitability Determination for Donors of Human Cellular and Tissue-Based Products Final Rule, as well as current high-risk criteria for bloodborne pathogens defined by the Centers for Disease Control. All donors with risk of disease transmission according to these guidelines will be deferred. Safety screening exclusion criteria include the following:

- Men who have had sex with another man within the past five years
- Persons who have injected drugs for non-medical reasons within the past five years, including intravenous, intramuscular, or subcutaneous injections

- Persons with hemophilia or related clotting disorders who have received human-derived clotting factor concentrates

- Persons who have had sex for money or drugs within the past five years

- Persons who have had sex with any of the individuals described in the four items above within the past 12 months or with any individual suspected of having HIV or hepatitis B

- Persons who have been exposed within the past 12 months to known or suspected HIV, HBV, and/or HCV infected blood through percutaneous inoculation (e.g. needlestick) or through contact with an open wound, non-intact skin, or mucous membrane

- Persons whose history, physical exam, or medical records reveal other evidence of high risk behavior HIV, or hepatitis B or C infection, such as a diagnosis of AIDS or hepatitis B or C, unexplained weight loss, night sweats, blue or purple spots on the skin or mucous membranes typical of Kaposi's sarcoma, unexplained lymphadenopathy lasting > 1 month, unexplained temperature > 38.6 ° C for > 10 days, unexplained persistent cough and shortness of breath, opportunistic infections, unexplained persistent diarrhea, male-to-male sexual contact, sexually transmitted diseases, needle track marks or other signs of parenteral drug abuse, unexplained jaundice, or hepatomegaly

- Current inmates of correctional systems including jails and prisons and individuals who have been incarcerated for more than 72 consecutive hours during the previous 12 months

- Persons who have had close contact with another person having viral hepatitis within the past 12 months defined as contact which may allow exposure to blood or body fluids including sharing of kitchen and/or toilet facilities

- Persons who within 12 months prior to donation have undergone tattooing, acupuncture, or body piercing in which shared instruments are known to have been used

- Persons with history and/or symptoms of spongiform encephalopathy (CJD) or known family history of a blood relative with non-iatrogenic CJD

- Persons with dementia or degenerative neurologic disorders of viral or unknown etiology

- Persons who have received injections of human pituitary-derived growth hormone (pit-HGH)

- Persons with active viral encephalitis or encephalitis of unknown origin

- Persons with suspected rabies diagnosis
- Clinical history and/or laboratory suggestive of West Nile Virus, vaccine or SARS
- Persons known to have received transplants of human dura matter
- Persons who have had a xenotransplant of any type
- Septicemia

When the offer is made, blood type, consent, and serology results are verified and blood type is recorded. The informed consent for transplant and/or research is identified. Required serologies for HIV 1 and 2, Hepatitis B surface antigen, HCV, HTLV I and II, and syphilis are performed using FDA approved assays and must be negative or non-reactive. A sample which tests positive for CMV may be accepted if the donor was asymptomatic prior to death.

In addition, the blood sample used for serological testing must not be plasma diluted. A sample will be considered plasma diluted if: a.) the donor has received more than 2,000 mL of blood products or colloids during the 48 hours prior to the blood draw, b.) the donor has received more than 2,000 mL of crystalloids in the hour prior to the blood draw. In such cases, a Plasma Dilution Worksheet must be used to determine if the sample tested was hemodiluted. Only samples proven to be < 100% diluted will be acceptable.

Screening for organ quality will be done to help ensure an adequate amount of functional islets. Quality screening exclusion criteria include the following:

- Donor age < 15 or > 75 years
- Warm ischemia
- Cold ischemia > 12 hours
- Methanol toxicity
- History of diabetes (transplant team may consider donor HbA1C > 6.1% in the absence of transfusions in the week prior to death as an indication of exclusion with discretion for donors who have received transfusions)
- Pancreas is not preserved in UW, PF/UW, HTK or OF/HTK solutions
- BMI < 19

b. Pancreas Procurement and Preparation for Transfer

Pancreases are procured from multi-organ donors after informed consent has been obtained from the donor's relatives according to general UNOS policies. Donor selection criteria are derived from the results of multivariate analysis of factors that influence the success of islet isolation (32-35). In addition, donor screening will be performed using standard UNOS organ transplantation criteria. The pancreas is procured paying attention to preserve the integrity of the pancreatic capsule, by accessing directly the retrogastric space and mobilizing the pancreas, allowing placement of sufficient ice around and cooling appropriately the organ during the cold perfusion of the cadaver. The

duodenum will be transected using a mechanical stapler device. On the back table, the pancreas will be freed from the surrounding fat tissue, detached from the duodenum, and stored in UW (University of Wisconsin) solution (Barr Laboratories, Pomona, NY, and other sources) in sterile plastic bags or the equivalent and placed on ice. Cold preservation in the two-layer method with UW and Perfluorodecalin (Fluormed, Round Rock, TX) [5a] or with Histadine-Tryptophan-Ketoglutarate (HTK) solution (Odyssey Pharmaceuticals, Inc., Florham Park, NJ) is also acceptable. Packaging and labeling follow the general UNOS policies and the rules of the corresponding local organ procurement agencies assuring sterility of the contents and clear identification. The pancreas will be transported by courier via air or ground transport based upon distance to UIC from the site of procurement. Upon receipt of the pancreas at the Islet Processing Facility, the transport box will be opened and appropriate enclosed paperwork will be checked including ABO, serologies, donor medical-social history, consent form, and donor chart. Once all paperwork has been checked, the pancreas container will be removed from the surrounding ice. The exterior bag will be wiped thoroughly with 70% alcohol and the pancreas will be brought into the clean room through the appropriate entrance. The pancreas container will be kept in the 2°C cold-room until processing begins.

Critical Reagents

Reagents and sources of materials used in the islet preparation include components detailed in the standard operational procedures (SOPs) (see Table 7-1). Table 7-2 summarizes primary and alternative solutions. Source and lot number for reagents introduced into the islet preparation are recorded in the documentation of each islet isolation procedure. All reagents are handled using sterile techniques, and if they are not supplied sterile, they are filtered through 0.22 µm sterile filtration systems before use. A quarantine and release program for reagents is in place. Qualification of materials for release includes inspection of package integrity, review of the Certificate of Analysis against internal specifications, inspection of media for color, clarity, and consistency, and inspection of materials' labeling for identity, lot number, and expiration dating period. Certificates of Analysis for critical reagents are provided in Appendix *Certificate of Analysis*.

Table 7-1. Table for Critical Reagents

Reagent	Vendor	Source/Grade	Use
HTK	Essential Pharmaceuticals, LLD	Pharmaceutical	Decontamination and cannulation
Betadine (10% in HBSS)	Novaplus	Pharmaceutical	Decontamination of pancreas
Kefzol (cefazolin sodium, 1g in 150 mL HBSS,)	Glaxo SmithKline	Pharmaceutical	Decontamination of pancreas
Fungizone (amphotericin B, 100 mg in 150 mL HBSS)	Novaplus	Pharmaceutical	Decontamination of pancreas
Hanks Buffered Salt Solution (HBSS), No Phenol Red,	Cellgro	Research	Priming solution for digestion circuit and solvent for Serva, or Sigma, or Roche
Collagenase NB1, or V, or Liberase	Serva, Sigma, or Roche	Purified enzyme	Collagenase and proteinase for pancreas digestion
RPMI (dilution solution)	Cellgro	Research	Wash solution for dilution phase
M199 Media, No phenol red (wash solution)	Cellgro	Research	Wash solution for collection phase
DNase	Genentech	Pharmaceutical	Digestion supplement
Human Albumin 25%	Baxter	Human/ Pharmaceutical	For substrate inhibition of protease in Serva, or Sigma, or Roche used as supplement in wash, culture and transplant media
Biocoll 1.100	Biochrom	Research	Density gradient
UW	Barr	Pharmaceutical	Density gradient
CMRL 1066 Transplant Media with Hepes and w/o Bicarbonate (*)	Cellgro and Lonza	Research	Islet transplant media
CMRL 1066, Supplemented (*)	Cellgro	Research	For short term storage of isolated islets in the incubator
CMRL, 1066, No phenol red (culture media) Final Wash	Cellgro	Research	For short term storage of isolated islets in the incubator
ITS (10 mL)	Gibco BRL Life Technologies	Human/ Research	Supplement for CMRL
HEPES	Cellgro	Research	Supplement for CMRL
Glutamine	Gibco	Research	Supplement for CMRL
Ciprofloxacin	Bayer	Pharmaceutical	Supplement for CMRL
Sodium Bicarbonate	Sigma	Pharmaceutical	Supplement for CMRL

Table 7-1. Table for Critical Reagents (continuation)

Reagent	Vendor	Source/Grade	Use
Phase I solution (*)	Cellgro	Research	Priming
Trimming solution (*)	Cellgro	Research	Decontamination and cannulation
Heparin sodium, USP (*)	APP Pharmaceuticals	Pharmaceutical	Supplement for media
Insulin like growth factor 1 (IGF-1) (*)	Cell sciences	Research	Supplement for media
Hydrochloridic acid, 1N (*)	Sigma	Research	To reconstitute (IGF-1)
Calcium chloride (*)	Sigma	Research	Supplement for Serva and Roche Enzyme solution
Water for injection sterile (*)	Hospira	Pharmaceutical	Solvent for NP (Serva and Roche)
Cold Storage (*)	Cellgro	Research	Purification CIT
Gradient stock solution (*)	Cellgro	Research	Purification CIT
Optiprep (*)	Sigma	Research	Purification CIT

(*) Used and approved in BB IND9336 (CIT)

***Table 7-2**. Media comparison table*

Use	Primary Solution	Alternative solution
Priming circuit	Priming (Cellgro)	Phase I (Cellgro)
Trimming (during decontamination and cannulation)	UW (Barr) or HTK(Essential Pharmaceuticals, LLD)	Trimming solution (Cellgro)
Culture Media	CMRL 1066 Supplemented	CMRL 1066, final wash
Transplant Media	CMRL 1066 Transplant	CMRL 1066

7.2.2 Islet Manufacturing Process

Flow Chart of Manufacturing Process

The methods for islet cell preparation include procurement of the pancreas and mechanical and enzymatic dissociation of the organ, followed by a gradient density centrifugation of the tissue suspension obtained. The end product is an endotoxin-poor, sterile pancreatic islet suspension with a minimal amount of exocrine and non-endocrine cell contamination. The current manufacturing process is essentially the same as originally described by Camillo Ricordi, et al. (36), with modifications taking into account recent progress (37-40), facilities at the University of Illinois at Chicago, and the experience of the principal investigator in the clinical series at the University of Geneva, Switzerland (24, 35, 41, 42) from 1996 to 2002 and at the University of Alberta, Edmonton, Canada from 2002 to 2003 (43). A flow chart outlining the Islet isolation process is provided (see Figure 7-1 and 7-2).

Figure 7-1. Preparation and Digestion of the Pancreas

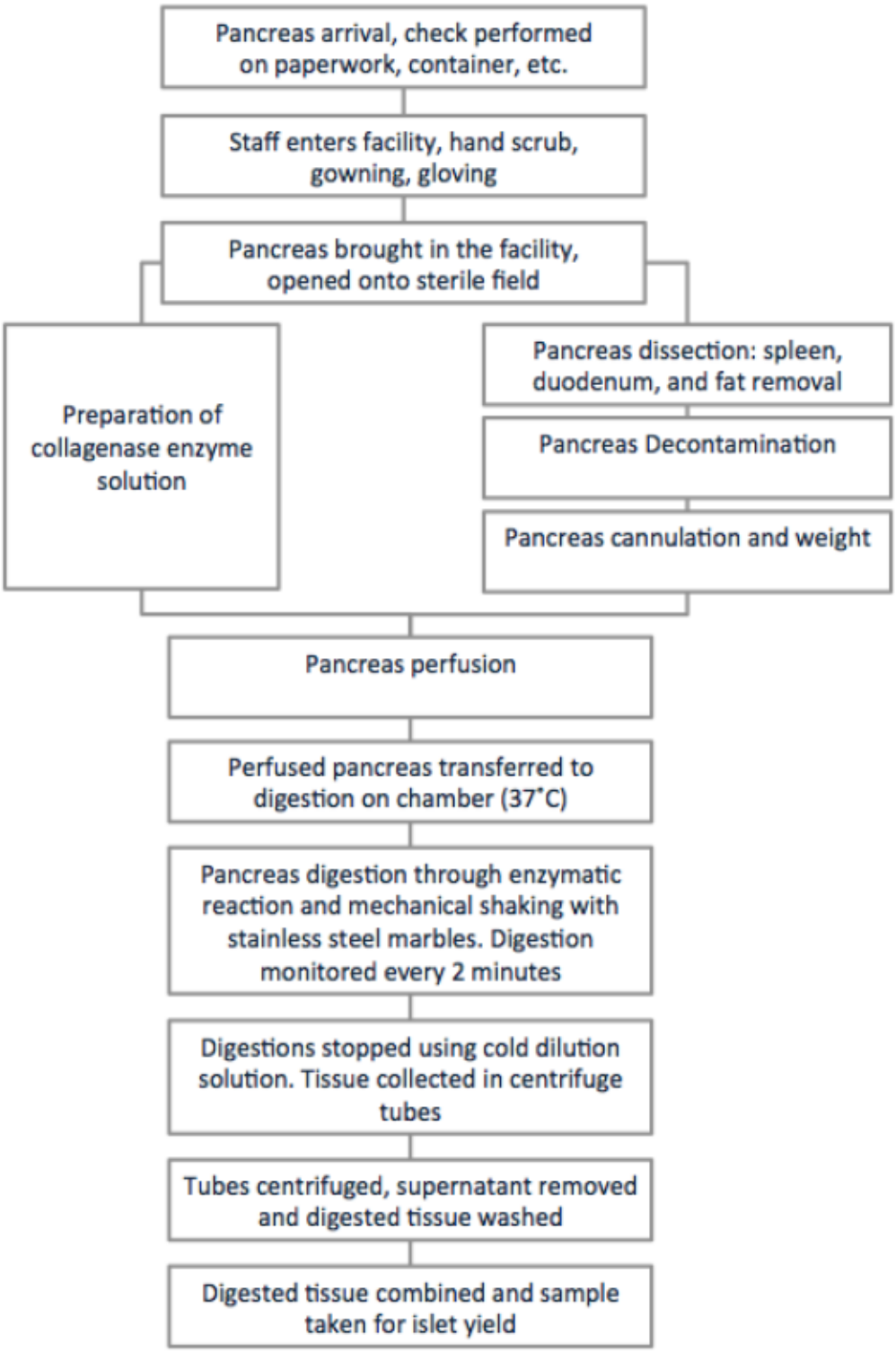
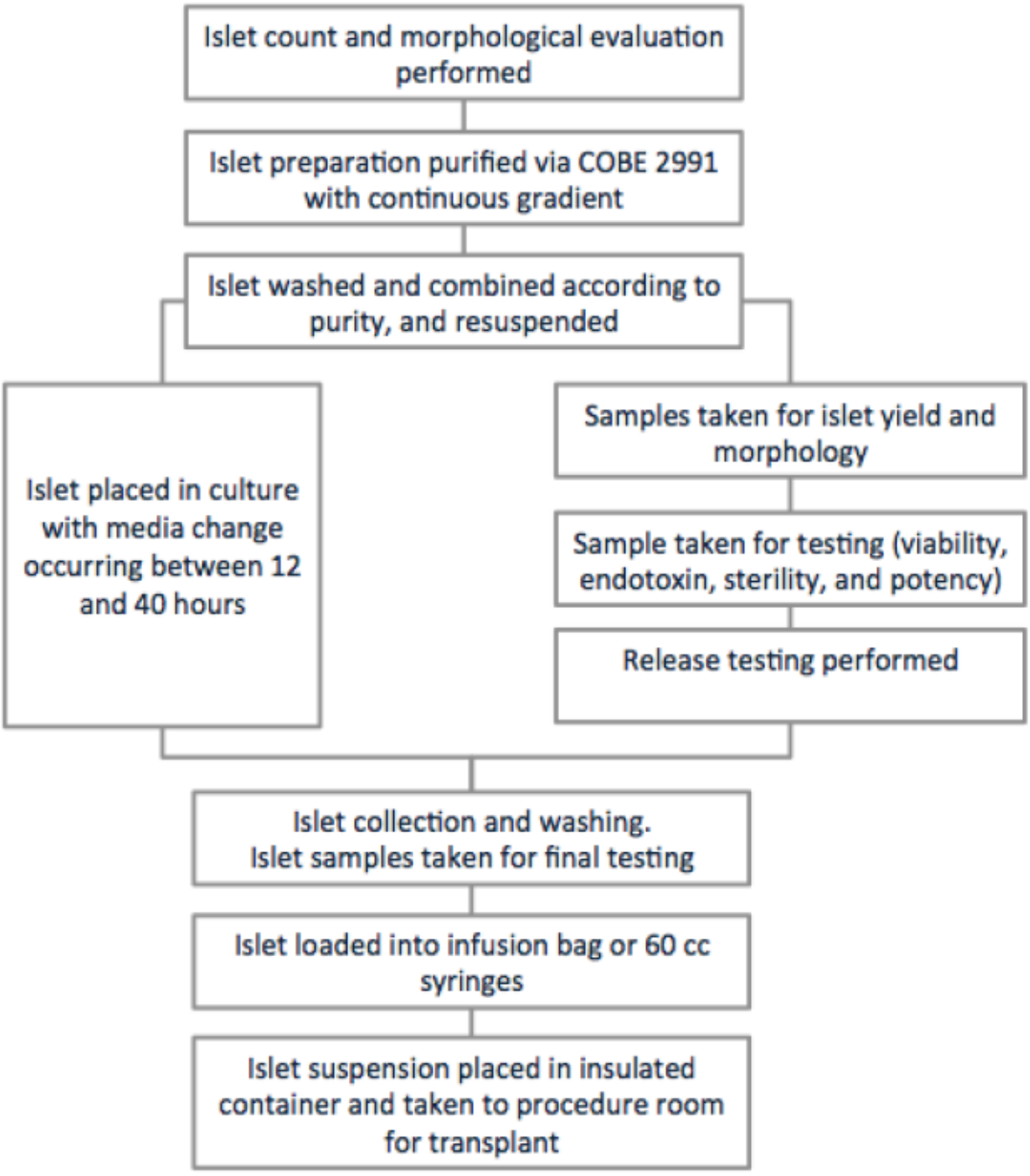


Figure 7-2. Purification of the Pancreatic Digest and Preparation of Islets



Detailed Description of Isolation Process

All steps are performed under aseptic conditions in clean rooms and Class II biohazard hoods with solutions comprised of sterile components.

a. Enzymatic Digestion of the Pancreas to Obtain Free Islet Cells

The pancreas is removed from the sterile interior of its transport jar by a sterile gowned team member. The spleen and duodenum are dissected away from the pancreas in a tray containing the same preservation solution used for shipping (either UW or HTK) or trimming solution. Then the pancreas is decontaminated by immersion into three solutions: 1) Betadine (5% in 300 mL HBSS, 2) Kefzol and Fungizone solution (1 gr Kefzol and 100 mg Fungizone re-suspended in 150 mL HBSS) and 3) HBSS (300 mL).

To isolate the islets, the pancreatic ducts are perfused in a controlled fashion with 350 mL of a cold enzyme solution (0.5 gr Collagenase NB1, Serva EU, or 1 gr Sigma V) in Hank's Balanced Salt Solution in HBSS, supplemented with HEPES and glutamine. A 60 mL syringe and two 16 gauge angiocatheters are used to infuse the enzyme.

After delivery of enzyme solution through the pancreatic ducts, the pancreas is divided into 6 to 10 sections and transferred to a sterile container designed to facilitate tissue digestion and containing 2 mL of DNase in a digestion solution. The islets are then separated by gentle mechanical dissociation provided by manually shaking the stainless-steel chamber with a closed-circulation flow-through system to bathe the tissue in the digestive enzymes at 37° C (Ricordi chamber, see Appendix *Ricordi Chamber*). At intervals during the digestion, samples are taken to assess the progress of the dissociation of islets from the acinar tissue. When adequate numbers of > 50% acinar-free islets are found, the enzymatic process is stopped by cooling and dilution with 6 to 10 liters of RPMI medium (Cellgro). The crude cellular fraction is initially collected in pre-filled 1L bottles containing RPMI, human albumin, heparin and insulin. Then the crude cellular fraction is collected in 250 mL conical tubes containing 15 mL of 25% human albumin (Grifols) and concentrated by centrifugation for 1 minute at 1,100 rpm. The number and size of the islets are determined by dithizone staining, measuring the volume of the pellet, and calculating the total number of islet equivalent (IE) units.

b. Purification of Islets by Centrifugation on Density Gradients and Culture of Purified Islets (Ficol-UW or Optiprep)

The resulting cooled crude extract, which contains a mix of exocrine tissue and islets, is purified with density gradients in an apheresis system (model 2991, COBE Laboratories). The COBE 2991 and the gradient mixer are placed in a refrigerated room to reduce the effects of temporary warming on the cells during the separation process, which occurs under hypoxic conditions. The gradient maker is located under the hood. The cold room temperature is 1-4° C resulting in a maximal gradient temperature of 8° C during gradient centrifugation as observed during the validation process. Two layers of solution at different density are slowly added to the COBE 2991 via a two-beaker system by using a peristaltic pump. While adding the layers to the COBE, the COBE spins at 1,800-2,000 rpm. Once the gradient has been completely pumped into the sterile COBE bag, the islet suspension is added in the same manner. The UW islet suspension

mixture is centrifuged at 1,800-2000 rpm for 3-5 minutes. The COBE is then used to pump the mixture, which is separated by density, into twelve 250 mL conical tubes located in the hood and partially filled with 230 mL of wash media. Each fraction is sampled (500 µl) with the sample being viewed under a microscope to determine which fraction(s) contain the islets of greatest purity, i.e., the greatest islet to exocrine tissue ratio. Fractions of the greatest purity are combined. Fractions of fair islet yield with fair purity are also combined with one another. Fractions that have poor purity of < 10% or fewer islets are discarded. In any case, the total volume of tissue recombined will not exceed 15 mL. The purified islet cells are then re-suspended in 250 mL of cold M199 media supplemented with 4.7% human albumin wash solution, and washed three times via centrifugation at 1,100 rpm for 1 minute. Samples of cells and supernatant are taken from the final product for quality control to define whether release criteria are met as addressed below. Supernatant sampling is performed after final centrifugation by drawing 50 mL of supernatant into a 50 mL conical tube.

Cell sampling is done by drawing 1 mL of the reconstituted islet preparation into 15 mL conical tubes and diluted 1:10 with wash solution. Two tubes are prepared using islets of higher purity fraction and one tube with islets of the lower purity fraction. Refer to section 7.6.3 for an explanation of islet enumeration, which is designated by EIN (Equivalent Islet Number). The samples are then distributed as follows:

- 2 x 1 mL for islet counting (EIN) and determination of islet purity for each fraction
- 9 mL for islet viability from a higher purity fraction tube
- 1 mL for endotoxin testing from the lower purity fraction
- 5 mL for microbial culture and direct Gram staining in duplicate

The cell sampling is based on the assumption of having a minimal islet mass of 250,000 EIN for clinically useful preparations (5,000 EIN/kg for a 50 kg patient). Thus, 1 mL of the final preparation contains a minimum of 1,000 EIN and 1 mL of the 1:10 diluted contains a minimum of 100 EIN, which is a representative sample for quality control.

For microbial and endotoxin testing and direct Gram staining, supernatant and cellular components are mixed 45 mL supernatant with 5 mL of cell suspension for microbial testing and Gram staining, and 1 mL supernatant and 1 mL cell suspension for endotoxin testing. In this way we avoid losing too many islets for quality control while assuring a relevant sample for microbial and endotoxin testing as well as the direct Gram staining. Results of all tests will be available prior to release of the product.

Once the count is available, 400-600EI are taken and cultured for Glucose static incubation (GSI).

Human islets are then spun and re-suspended in culture media containing indicator-free CMRL supplemented according to SOP.. After one washing the islet preparation is plated in a non-adherent culture T175 flask and incubated under standard cell culture conditions in an incubator at 22-37° C with 5% CO_2, in order to avoid loss of the product due to attachment to the culture flask. The time of culture will be kept as short as possible and will not exceed 40 hours.

c. Preparation of Purified Islet Cells for Transplantation

While the islets are in culture, all release testing is performed and documented in the Isolation Record and on the Transplant Release Form (see Appendix *Transplant Release Form*). The Transplant Release Form will be completed by a technician and will include all information relevant to release, i.e., Donor UNOS number, Batch Number, Donor ABO, Serologies, QC testing, etc. The Transplant Release Form will be reviewed and signed by the Medical Director or official designee prior to releasing product for transplant.

Islets are collected and washed with transplant media wash (indicator-free CMRL supplemented according to SOPs). The final islet preparation is maintained in transplant media (indicator-free CMRL supplemented according to SOPs) until the transplant team has prepared the subject for portal vein infusion of the preparation.

After the islet lot has been released by the PI, Dr. J. Oberholzer, or an official designee according to the release criteria (see Section 7.6 *Product Testing*), and once the surgeon and subject are ready for infusion, the suspension is drawn directly into sterile 60 mL syringes. The 30 mL of transplant media containing the islets will be aspirated into the 60 mL syringe and the flask will be rinsed with 20 mL of transplant media. The 20 mL of transplant media will be aspirated in the 60 mL syringe. One 60 mL syringe will contain a maximum of 1 mL total packed islet cells.

During cell re-collection, samples will be taken once again to test the batch for microbiology, Gram stain, viability, post-culture glucose static incubation, and islet count, and results will be compared to the post-isolation results. An additional sample will be taken to transplant into diabetic nude mice to further test islet potency. Then 2 -4 mice will be transplanted with 2,000 EIN per mouse.

As alternative to 60 mL syringes the isolated islet cells can be packaged in one or more sterile 250 mL infusion bags, each with 250 mL CMRL 1066. Non-sealed ports will be clamped as necessary via sterile methods.

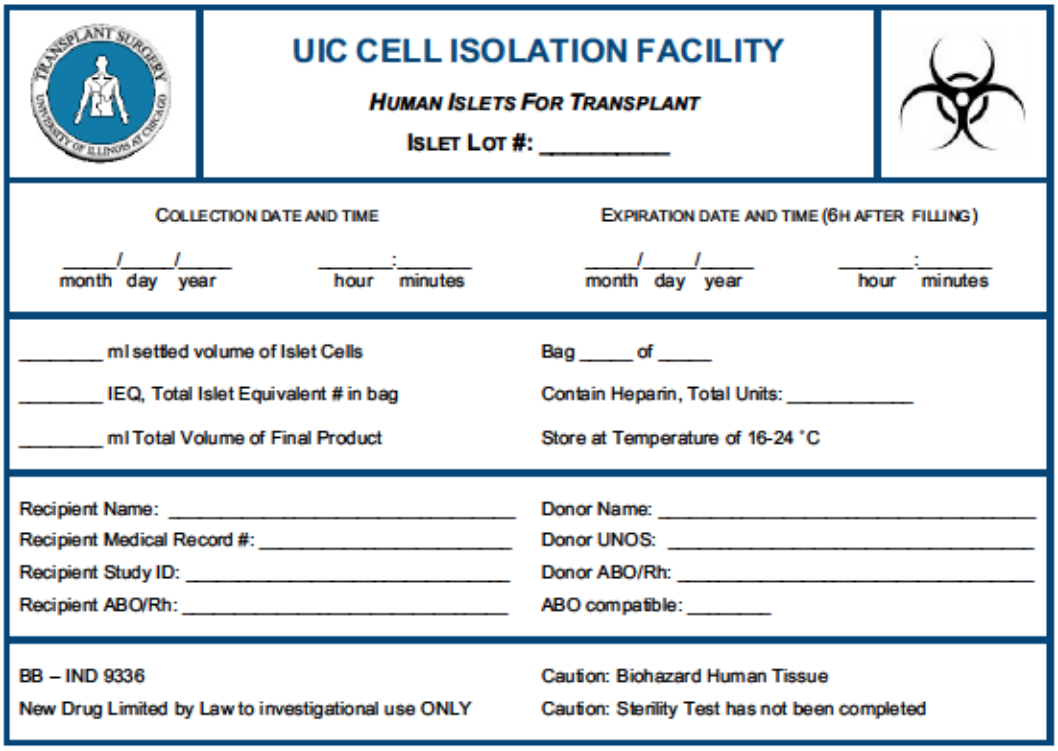
The preparation will be labeled, packed into sterile bags and brought to the operating theatre via a hand carried insulated container at room temperature. The surgeon will have the recipient ready for the infusion via a pre-placed intraportal catheter.

7.3 Product Label

A label will identify the batch, blood group and purity of the corresponding fraction collected (see Figure 7-3). The label will indicate the date and end time of processing, the unique isolation process number, the date and time of expiration of the preparation for transplant use, the donor identification (UNOS #), the recipient identification (UIC patient's personal medical record number and study ID if applicable), donor and recipient name and blood type. The label will further indicate the specifications *UNOS donor*, *for use by intended recipient only*, *human islets for transplant*, *biohazard* and

Caution: Biohazard Human Tissue, Sterility Testing has not been completed, heparin content and For Investigational Use Only.

Figure 7-3. Islet Transplant Label at UIC



UIC CELL ISOLATION FACILITY

HUMAN ISLETS FOR TRANSPLANT

ISLET LOT #: _____

COLLECTION DATE AND TIME		EXPIRATION DATE AND TIME (6H AFTER FILLING)	
_____/_____/_____	_____:_____	_____/_____/_____	_____:_____
month day year	hour minutes	month day year	hour minutes

_____ ml settled volume of Islet Cells

_____ IEQ, Total Islet Equivalent # in bag

_____ ml Total Volume of Final Product

Bag _____ of _____

Contain Heparin, Total Units: _____

Store at Temperature of 16-24 ˚C

Recipient Name: _____

Recipient Medical Record #: _____

Recipient Study ID: _____

Recipient ABO/Rh: _____

Donor Name: _____

Donor UNOS: _____

Donor ABO/Rh: _____

ABO compatible: _____

BB – IND 9336

New Drug Limited by Law to investigational use ONLY

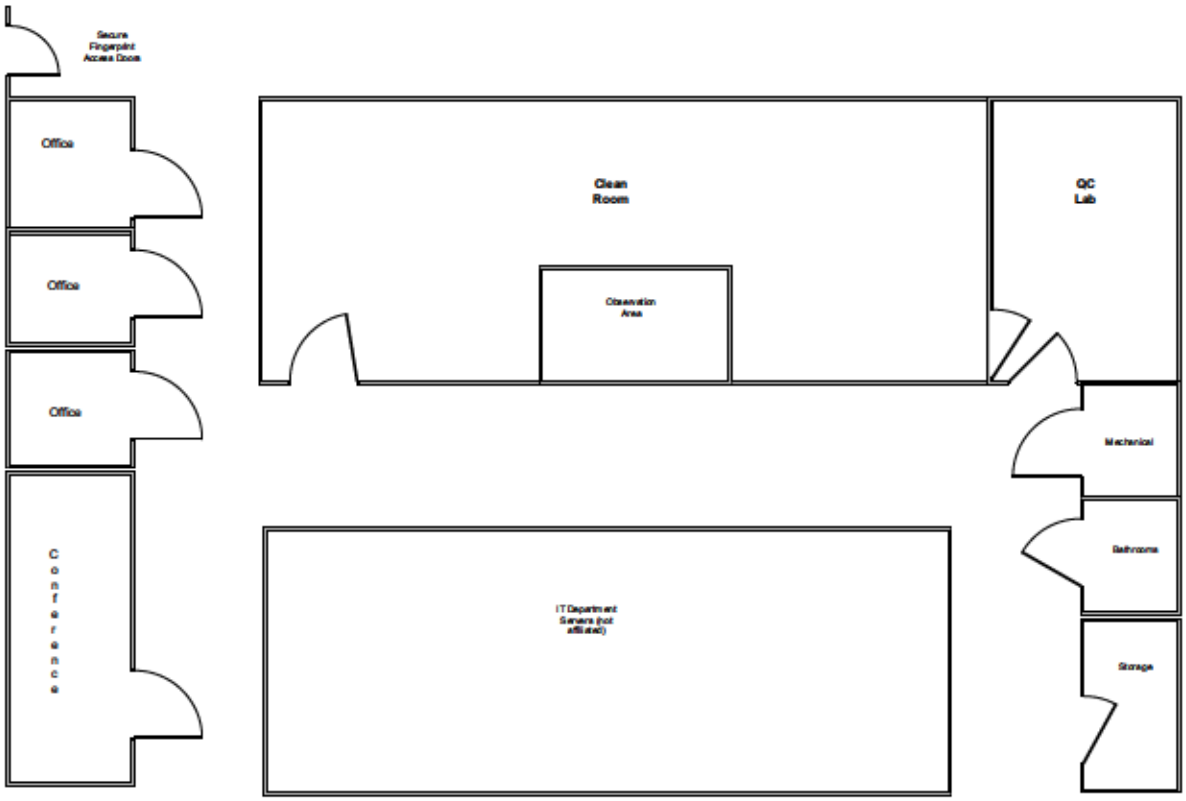
Caution: Biohazard Human Tissue

Caution: Sterility Test has not been completed

7.4 University of Illinois at Chicago Islet Processing Facility

The University of Illinois at Chicago has designed and constructed a certified and validated 1,000 clean room to allow for the production of allogeneic human islets for transplant. The Islet Processing Facility is located in the basement of the UIC Medical Center at 1740 West Taylor Street, Chicago IL.

Figure 7-4. Overall Plan of the UIC Islet Processing Facility



The clean room is 800 square feet in size and serviced by two dedicated air handling units and two refrigeration condensing units supplied by Trane Company. All air is 100% single pass and filtered via ducted filters, filtra LF panel and HEPA filters. The clean room is designed to adhere to Good Manufacturing Practice (GMP) regulations.

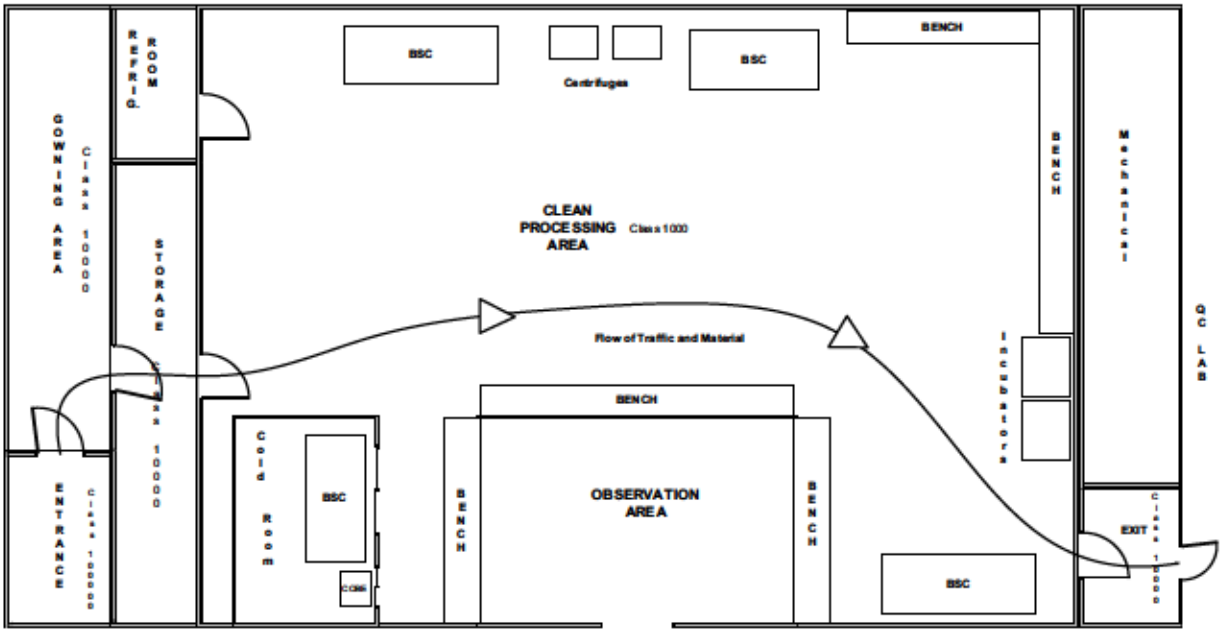
Processing is performed in four class 100 Nuaire laminar flow bio-safety cabinets, two inside the main processing area and one located within the cold room (mentioned below). The clean room is positively pressurized with the processing room being the area of greatest pressure. Room pressures are monitored via a bank of Magnehelic differential pressure gauges. Rooms are air-locked and doors are equipped with magnetic locks to ensure only one door may open at a given time within the same space.

The clean room and hoods are certified bi-annually. Maintenance checks of lighting, air handlers, and Magnehelic gauges are also performed at this time. In addition, a thorough, intensive cleaning is performed on defined quarterly and annual schedules to supplement the routine day-to-day cleaning of the room. Routine environmental monitoring for viable and non-viable particles is performed under both static and dynamic conditions according to SOP.

Installation qualification (IQ), operation qualification (OQ), and performance qualification (PQ) have been performed during qualification runs to validate all critical equipment including hoods, centrifuges, and incubators, and are documented in individual equipment logs. All equipment is regularly checked and calibrated by a professional. Weekly equipment checks are performed on critical equipment to ensure consistent performance. All critical systems are constantly monitored for temperature and humidity by a central computer. Facility users are paged by the computer if values go outside of expected parameters.

Traffic of materials and personnel is carefully restricted in a clean in, dirty out manner. Gear will be wiped down and staff will don clean room gowning material consisting of mask, Tyvek suit, gloves, hairnet, shoe covers, and eye protection upon entering the facility through the defined entrance. After islet processing, gear and staff will exit via the defined exit only.

Figure 7-5. UIC Islet Processing Facility



Located within the clean room is a refrigerated cold room. This space is designated for use in the purification of islet cells. Islets will be separated from unwanted exocrine tissue through use of a COBE 2991 cell processor which must be refrigerated while

islets are inside. The cold room is used to accomplish this. The cold room is equipped with two refrigeration units operating independently of the clean room. The air within the cold room is HEPA filtered.

7.5 Quality Program

7.5.1 Facility Controls

Cleaning

The Islet Processing Facility manager is responsible for ensuring that personnel responsible for cleaning and sanitizing the Islet Processing Facility clean room are appropriately trained, and that there is compliance with the cleaning protocol. Cleaning is performed according to controlled SOPs. Routine cleaning is carried out following each islet isolation or at a minimum weekly. More thorough cleaning is performed on defined quarterly and annual schedules. All cleaning and/or sanitizing for each clean room facility or clean room equipment is documented on the appropriate log sheet.

Environmental Monitoring

Environmental monitoring is performed weekly under static conditions. In addition, dynamic environment testing is performed weekly with the first pancreas processed in a given month.

Environmental monitoring includes particle counts, airborne microbe sampling, and contact plate cultures in the facility, as well as monitoring sterile gloves worn by personnel in contact with the pancreas.

Equipment

All equipment is operated according to SOPs describing operation, cleaning, maintenance, and calibration. Installation qualification where possible, operation qualification, and performance qualification have been performed on all critical equipment. Lab staff makes weekly checks of all equipment used in isolation and/or storage. Checks are recorded along with associated charts, printouts, etc. in equipment log books. All thermometers and mechanical equipment are calibrated and/or certified annually. Calibrations and certifications are documented in individual equipment logbooks. BSC and air handling units are recertified semi-annually by an outside vendor, including HEPA filter integrity testing.

Documentation

All activities required for production and maintenance and control of the facility occur according to written procedures and systems and are documented in related logs. SOPs are organized into the following categories: Contracted Company SOPs, Program Definitions, Quality Assurance, Documentation, Isolation Procedure, Quality Control Batch Release, and Archived SOPs. All documentation related to islet isolation occurs on the Isolation Record. This involves records relating to traceability of product from receipt of pancreas to final distribution of tissue, including donor information, media

tracking information, processing information, notes during processing, QC sampling and results, verification of final product acceptability before release. The final distribution of tissue is recorded. When tissue is transplanted, the recipient's medical record number is logged in the Isolation Record under distribution. The isolation batch number is also recorded in the recipient's medical record. All records will be kept for 10 years in a locked file cabinet in the Islet Processing Facility manager's office.

Gowning

Prior to entry to the facility all staff will gown in Tyvek suits, hairnets, gloves, masks, eye protection, and shoe covers. All gowning occurs in a clearly defined area in the entrance vestibule. This area is clearly marked on the ground with white tape. Only fully gowned persons are permitted across the tape.

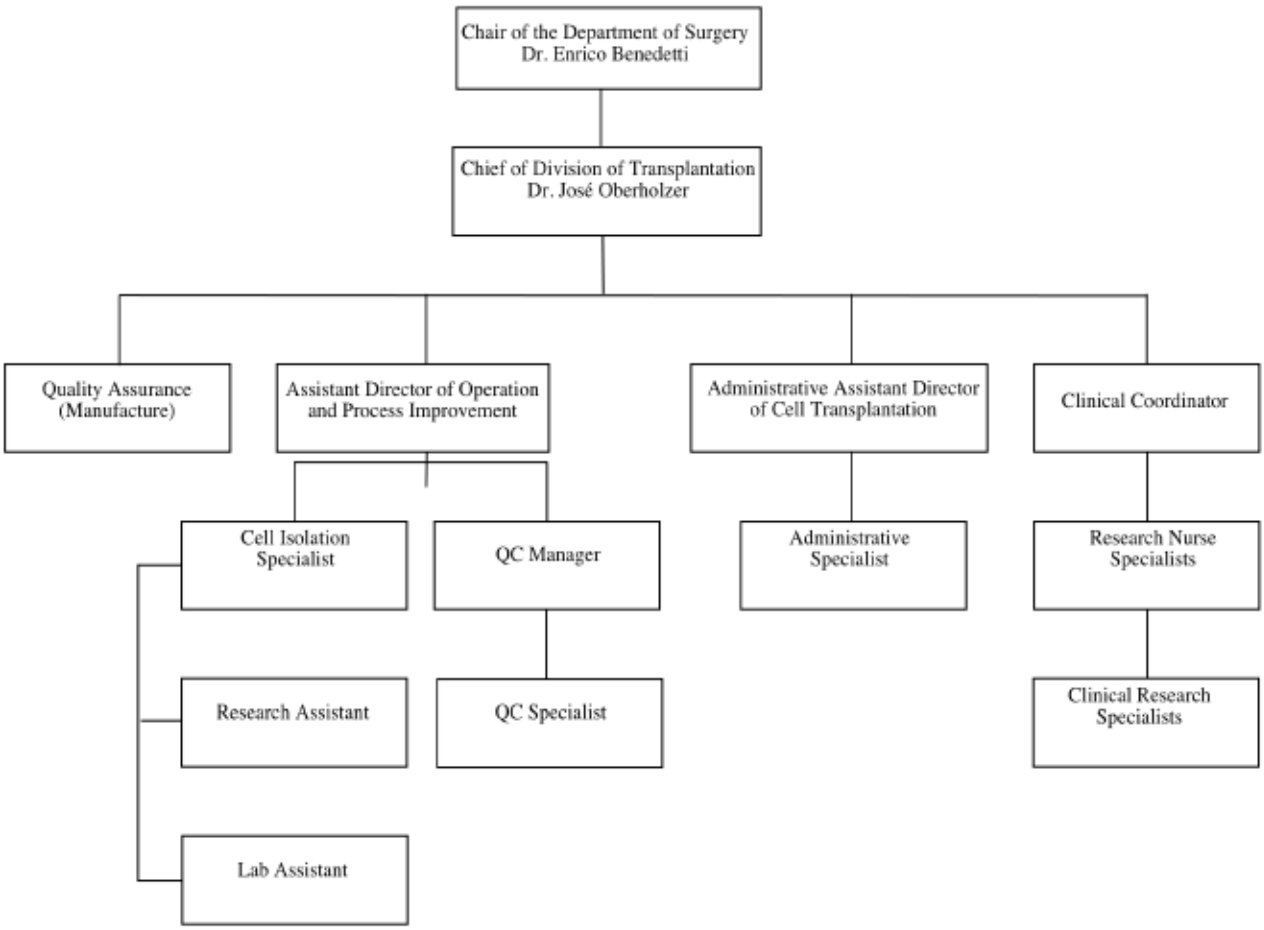
7.5.2 Quality Control Testing

The UIC Quality Control Laboratory (QC Lab) is located directly outside of the clean room exit. All QC testing for release is done in this lab. Appropriate protocols are in place for each test performed including glucose static incubation, islet quantification, viability staining, and endotoxin. *In vivo* quality testing of islet function by transplanting human islets into diabetic nude mice is performed in the UIC animal facility, Results are available only retrospectively. Quality Assurance, such as quarantine and/or release, equipment checks, critical equipment monitoring, etc., is performed in much the same manner as in the clean room. The QA specialist oversees the QC Lab and coordinates QC and QA for the Quality Program. All QC results are reviewed by Dr. Oberholzer or an official designee prior to release of islets for transplant and are audited by the QA Specialist weekly.

7.5.3 Quality Assurance

Personnel Organization

Figure 7-6. The Organizational Chart for the Islet Processing Facility



The Cell Isolation Program is attached to the Department of Surgery and the Division of Transplantation at the University of Illinois Medical Center at Chicago. Dr. José Oberholzer is the Chief if Division of Transplantation and the Director of the Cell Isolation Program. He will direct all matters involving the program. The Quality Assurance Manager is responsible for regulation of processing general in-house QA. His/her responsibilities include but are not limited to ensure proper documentation, sign off on SOPs, Transplant Release Checklists, QC reports and row material release form, Quality Assurance and environmental monitoring. The Assistant Director of Operation and Process Improvement direct all operational matters in both research and processing labs. His/her responsibilities include but are not limited to coordination, scheduling, staff training and maintaining the compliance to federal regulations. The Quality Control Laboratory is overseen by the Assistant Director of Operation and Process Improvement. All QC required for release will be performed in this area.

The Assistant Director of Operation and Process Improvement is responsible for training and directing technical and support staff. Technical Staff perform technical assignments as needed by following established protocols, and report to the Assistant Director of Operation and Process Improvement for all day-to-day operations of the facility. These operations include working in isolations on an on-call basis, performing quality assessment on the isolated islets, and filing proper documentation during and after each isolation. A minimum of four staff members are involved in an isolation. Dr. Oberholzer or an official designee will supervise all activities during an isolation.

The Quality Assurance Manager will conduct a weekly review of the Isolation Records from the previous week. All records will be checked for completion, consistency, legibility, and accuracy. Specific attention will be paid to release testing. The Quality Assurance Manager will also conduct a quarterly audit of all Islet Processing Facility practices. Logbooks, protocols, and other documents will be reviewed at this time. The Quality Assurance Manager will submit all weekly and quarterly observations to the Program Director. The Quality Assurance Manager does not participate in any processing, and reports to an outside independent supervisor in order to avoid conflict of interest.

The facility Support Staff will assist in day-to-day operation of the facility. Secretarial, cleaning, and other support staff will perform their duties as assigned according to their job descriptions.

Training

Training is conducted by Dr. Oberholzer, the Chief of Operations, the Quality Assurance Manager, or a qualified, experienced staff member. All staff receive mandatory GMP clean room training at a minimum once per year. Staff must complete this training prior to participating in an islet isolation. Ongoing training will be conducted weekly at staff meetings. All participants in training sessions will sign in on a Training Form that will be kept in a training log in the Quality Assurance Manager's office.

Audits

Internal audits of the entire Quality Program will be conducted annually by an outside agency. These audits will be a system review of facilities, production, preventative and corrective action, compliance with SOPs, record keeping, and QC, training. Audits will be documented in a quarterly report that will be submitted directly to the Director of the Islet Processing Facility.

Ongoing review of the Quality Program will be conducted by the Quality Assurance Manager. There will be weekly checks of: preventative and corrective action, charting, QC, training, etc. Results will be documented in appropriate logs, and Discrepancy Reports will be generated when needed. Results of these checks will be communicated at weekly meetings with processing staff.

Additional audits may occur on an event-driven basis. In cases of adverse outcomes after transplant, unscheduled internal audits may occur at the discretion of the Director. Additional audits may be conducted by external federal and/or state agencies. Results of audits will be reviewed by the Director of the Islet Processing Facility, and changes will be made at his discretion and only with his approval. Occurrences of audits will be documented in the QA- Audit Log along with any notes or documentation associated with any audit.

SOPs are in place to support all QA activities. These SOPs are: Complaint Files, Corrective Action, Receipt of Supplies and Quarantine - Release of Media, Reporting Requirements, Training Program, QA Program Preventative Action, and Equipment Validation and Certification.

Deviation Control

Unexpected deviations from SOPs will be recorded in the Isolation Record. Equipment malfunctions will be noted in the logbook of the individual piece of equipment. Each deviation will also be recorded on a Discrepancy Form to be maintained in the Discrepancy Logbook, which will track the incident through its resolution. Corrective action(s) will be logged on the same form as the individual deviation. All deviations will be reviewed by Dr. Oberholzer and the Quality Assurance Manager. Retraining of staff will be conducted when appropriate.

A planned deviation must be approved by Dr. Oberholzer in advance of implementation and must be tracked in the Isolation Record. Planned deviations will also be reviewed by the Quality Assurance Manager.

Document Control

All processes and procedures are described in SOPs. SOP copies are located in the processing area and the Director's office, and one signed original is maintained in the Quality Assurance Manager's office. All staff will be trained in all SOPs. Changes to procedures will only be made by the Director or an official designee and must be signed and approved by the Quality Assurance Manager and Director. When a change occurs, outdated SOPs will be collected and staff will be re-trained. A copy of all outdated SOPs will be kept in the SOP file in the Quality Assurance Manager's office under Archived SOPs. SOPs are reviewed annually.

SOPs are given a unique identifying number consisting of the year separated by a hyphen from a three digit number and a version number. SOPs will be recorded in the SOP log in sequential number according to the three digit unique number between hyphens. For example, the first SOP to appear in the SOP log for 2003 will be SOP number 03-001-01. This means that the SOP was written in 2003, is the first SOP in the log, and is the first version of that SOP. In addition, all SOPs will have a suffix to designate the category of the protocol. This suffix will appear after a forward slash (/) in the SOP number section, e.g., 04-001-01/1.

All product batch documentation will occur in the Isolation Record. This record will trace the product from receipt through transplant and record all necessary data (dates, times, volumes, media information, etc.) in between. Each batch will be assigned an individual, unique number for traceability. All paperwork and testing results for each batch will be kept in an individual chart, numbered with the batch number. All charts will be kept in the locked office of the Quality Assurance Manager in a secure file cabinet. All charts will be kept for a minimum of ten years.

Additionally, all logbooks and associated paperwork will be located within the Quality Assurance Manager's office or within the facility when appropriate.

7.6 Product Testing

Samples of both product and transport media are tested at various points to monitor the process and provide information on the purity and safety of the product. Testing will be performed during and after islet production Quality Control 1 (QC1) , and on the final product prior transplantation Quality Control 2 (QC2), See Table 7-3 for a complete list of product quality testing.

7.6.1 Quality Control Testing during and after Islet Cell Production (QC1)

During and after the islet isolation process multiple samples for QC are taken and tests are performed in the Quality Control Laboratory, LABS Inc. and the UIC Pathology Laboratory (see Table 7-3):

- Purity
- Yield
- Viability
- Endotoxin
- Bacteriology
- Glucose Static Incubation and Stimulation Index calculation. The result of this quality test will be determined retrospectively (post-transplantation) and will not be used as a quality release test. Rather, the result of this test will be correlated with the clinical outcomes.

7.6.2 Quality Control Testing of Final Product after Culture and before Transplantation (QC2)

After a minimum of 12h of culture and before transplantation multiple samples for QC are taken and tests are performed in the Quality Control lab, LABS Inc. and in UIC pathology laboratories (see Table 7-3):

- Purity
- Yield
- Tissue Volume
- Viability
- Endotoxin
- Bacteriology

- Bioassay by transplant into nude mice and Post-culture Glucose Static Incubation and Stimulation Index calculation. These quality tests will be performed retrospectively (post-transplantation) and will not be used as a quality release test. Data from this test will be collected and correlated with the clinical outcomes.

Table 7-3. Product Quality Testing

Test	Parameter and Test Method	QC Time Point
Purity	Dithizone-staining and determination of acinar-free islets using visual examination under microscope by qualified personnel	QC1 QC2
Islet Yield	Dithizone-staining cell size and enumeration evaluation using light microscopy with visual examination by qualified personnel	QC1 QC2
Tissue Volume	Tissue volume is estimated using a glass pipette or by visualization of centrifuged pellet.	QC2
Viability	Fluorescent dye method using fluorescein diacetate and propidium iodide with visual examination by qualified personnel	QC1 QC2
Endotoxin	Endotoxin unit (EU) content by Endosafe® Portable Test System with disposable cartridges, version 7.09, Charles River Laboratories	QC1 QC2
Bacteriology	Anaerobic, aerobic, and fungal growth as well as Gram staining are performed by qualified personnel in the UIC Pathology Laboratory	QC1 QC2
Glucose Static Incubation and Stimulation Index calculation	In vitro insulin secretion under low and high glucose stimulation. Insulin concentration evaluated by ELISA	QC1 QC2
Bioassay	Islets are transplanted into diabetic nude mice to determine function. Glucose and human c-peptide will be measured.	QC2

7.6.3 Assay Methods

Purity

Islets will be stained with dithizone. Dithizone bonds with the zinc contained in beta and alpha cells, stains islets red, and allows investigators to differentiate islets from other tissue. Microscopy is used to gauge the ratio of islets to other tissue in a given sample within a visualized field.

Islet Yield (Islet Enumeration)

Islets will be stained with dithizone as described above. Every visualized islet identified within a sample will be recorded and sorted according to its Equivalent Islet Number (EIN). That is, based upon the observed islet diameter at a given magnification via

microscopy, islets are assigned values according to size. A larger islet, therefore, counts more than a smaller islet in terms of its islet equivalent. The Equivalent Islet Numbers are totaled and multiplied by a dilution factor (weight of the islet suspension within a conical tube). The result is the total islet yield (EIN) for the isolation.

Settled Volume Assessment

Tissue volume is evaluated by visualization of pellet immediately after centrifugation. An alternative method to assess tissue volume is by aspirating the tissue into a glass pipette and allows the tissue to settle in the pipette.

Viability

Fluorescent dyes fluorescent diacetate (FDA) and propidium iodide (PI) or sitogreen and ethidium bromide are applied to an islet sample. The sample is observed using a fluorescent microscope with appropriate filters. Viable islets will stain a bright green and dead islets will stain red. An investigator will record the ratio of viable islets to non-viable islets based upon an observation of the sample.

Tissue Transplant Volume

The final islet suspension is centrifuged within a 250 mL conical tube. The packed cell volume at the bottom of the conical tube is measured using the calibrations on the tube. This amount represents the volume of tissue to be transplanted.

Microbiological Assessment

Upon receipt of the pancreas, a 10 mL sample of the pancreas transport solution is taken. This sample is used as a baseline and is sent for 14-day aerobic, anaerobic, and fungal cultures. A sample is also taken from the cells and supernatant of the final product as described earlier. Samples for aerobic and anaerobic tests are taken in duplicate and in tested by LABS Inc. and UIC Pathology Laboratory. LABS Inc. performs microbiology testing to according to 21 CFR 610.1 while UIC Pathology Laboratory uses BacT/ALERT, a new system that is more specific and less operator dependent. A stat Gram stain is performed on the sample containing supernatant and cells by qualified technicians in the Microbiology Laboratory prior to transplant, and the sample is cultured for 14 days. An additional sample taken at the end of the culture period is cultured for 14 days using anaerobic, aerobic, and fungal cultures.

Microbiology testing will be done at UIC Pathology Laboratory via use of BacT/ALERT SN anaerobic and BacT/ALERT SA aerobic culture bottles (Biomerieux). The BacT/ALERT SN culture bottle contains media consisting of the following: pancreatic digest of casein (1.7%
w/v), papaic digest of soybean meal (0.3% w/v), sodium polyanetholesulfonate (0.035% w/v), pyridoxine HCL (0.001% w/v) and other complex amino acid and carbohydrate substrates in purified water. The BacT/ALERT SA culture bottle contains media consisting of the following: pancreatic digest of casein (1.36% w/v), papaic digest of soybean meal (0.24% w/v), sodium polyanetholesulfonate (0.035% w/v), hemin (0.0005% w/v), menadione (0.00005%% w/v), yeast extract (0,376% w/v), pyridoxine

HCL (0.0008% w/v), pyruvic acid (sodium salt, 0.08% w/v), reducing agents, and other complex amino acid and carbohydrate substrates in purified water.

The culture bottles are placed into the BacT/ALERT Microbial Detection System for culture and microbial detection. This machine utilizes a colorimetric sensor and reflected light to monitor the presence and production of CO2 that is dissolved in the culture medium. When growth of microorganisms produces CO2, the color of the gas-permeable sensor installed at the bottom of each culture bottle changes to yellow. In case of a positive test, samples from the tube are taken and stained to identify the species. Positive results are always reported directly to Dr. Oberholzer or the Quality Assurance Manager.

Endotoxin Content

A sample of 2mL from the pre-culture and final product suspension is collected in endotoxin free testing tube. At the UIC Quality Control Laboratory, this supernatant sample is diluted with equal amount of endotoxin free distilled water and read using Endosafe®-PTSTM system.

The Endosafe®-PTSTM is a handheld spectrophotometer that utilizes FDA-licensed disposable cartridges for accurate, convenient endotoxin testing. 25µl of well-mixed sample are loaded into each well of the disposable cartridge. Quantitative endotoxin results are available in 15 minutes.

Potency Assay

Glucose Static Incubation and Stimulation Index Calculation

The function of the islets is evaluated by glucose static incubation (GSI) assay in which insulin concentration of the incubation media is measured after exposure of islets to low and high concentrations of glucose.

400-600 EIN will be collected from the pre-culture and final preparation. The islets are washed and re-suspended in 0.5% BSA-Krebs solution. The cells are evenly distributed in 3 wells of a 24well plate containing cell insert (Millipore). Each well is prefilled with 1 mL of 1.6 or 2.8mM glucose solution (low concentration), 16.7 or 28mM of glucose solution (high concentration) and acid alcohol. The plate is then placed into a 37°C incubator for 30 minutes to pre-incubate in a low glucose stimulation condition. After the 30-minute incubation time, the inserts bearing islets are moved into wells containing low glucose solution for an incubation of 60 minutes. The supernatant is collected and stored at –20˚C. .

The inserts now are moved into the high glucose solution wells and the low glucose solution is collected and stored at -20˚C. Islets are incubated in high glucose solution for 60 minutes, and supernatant is collected and incubated with acid alcohol for a total insulin extraction.

Each tube is labeled with the date, glucose concentration, Isolation Number and technician's initials, capped, and placed into a -20° C freezer. Insulin concentration is

measured using a standard ELISA assay (Mercodia, Sweden). The Stimulation Index is calculated as the ratio between insulin produced under high glucose stimulation and low glucose stimulation.

Bioassay
Human islet function will be evaluated *in vivo* by transplanting under kidney capsule of three separate streptozotocin (STZ) induced diabetic nude mice. The nude mice will be rendered diabetic by a single injection of STZ (200 mg/kg). Diabetic status is defined as blood glucose levels above 350 mg/dL on 2 consecutive days, which normally occurs between 72 to 96 hours after STZ injection.

Approximately 2,000 EIN will be collected from the final preparation and washed twice with HBSS and then pelleted in PE 50 tubing connected with a 1 mL syringe. The mice will be anesthetized by inhalation of isoflurane using an isoflurane vaporizer with anesthetic system (Viking Medical, Medford Lakes, NJ). The left kidney will be exposed through a lateral incision and islets will be gently injected. After transplantation, the glycemic profile of the mice will be monitored every second day by collecting a blood sample from a tail vein using a glucometer (Lifescan). An intraperitoneal glucose tolerance test (IPGTT) will be performed at day 28 after transplantation as follows: 2 gr/kg of glucose will be injected intraperitoneal using 20% glucose. Glycemia will be measured at minutes 0, 5, 15, 30, 45, 60 and 120. Human C-peptide will be measured before and at 60 minutes after the glucose injection. In particular, we will be looking at the area under the Glycemia-Time curve and recording any delay occurring in this response curve. Human C-peptide levels will also be determined by ELISA (Mercodia) at weeks 2, 4, and 6 after transplantation by sampling 150 µL of blood by micro-incision on the tail vein. Blood will be centrifuged in order to collect the serum, which will be stored at –20° C until analysis. If at 28 days diabetes has been reversed following human islet transplantation, nephrectomy is performed to assure that the restoration of euglycemia it is to attribute solely to the transplanted islets. Nephrectomy is performed toward the end-of bioassay time point.

7.6.4 Criteria for Quality Control Testing

Islet purity, yield, tissue volume, viability, direct Gram staining, and endotoxin levels will be analyzed prospectively and will determine the decision whether a preparation can be released for transplantation. Bacterial and fungal growth, islet potency determined by glucose static incubation, and transplantation into nude mice are performed retrospectively. Acceptable parameters of quality control testing of the final product are summarized in Table 7-4.

Table 7-4 Acceptable Parameters of Quality Control Testing

QC test	***Acceptable Parameters***
Purity	≥ 50%
Yield	≥ 5,000 EIN/Kg
Viability	≥ 70% for high purity layer and >29% for combined preparation
Tissue Volume	≤ 15mL
Endotoxin	≤ 5 EU/Kg bodyweight of recipient
Bacteriology	Gram staining negative Aerobic growth negative Anaerobic growth negative Fungal growth negative
GSI and Bioassay	To be determined during the study, based on correlation with observed clinical outcomes

7.6.5 Action Plan in the Case of a Positive Sterility Result after Product Release

Since results of sterility testing are not available until after administration of the islets, a plan of action for patient notification and treatment in case of positive results in the sterility culture is in place. Any organism detected from the final implanted islet cell preparation will be further evaluated for species identification and antibiotic sensitivity and resistance, and the recipient of the preparation will be treated as medically indicated.

The PI will evaluate the recipient for signs and symptoms of infection. The PI will use vital signs, WBC, and blood cultures to screen for infection. If evidence of infection is found, the patient will be treated according to sensitivity of identified micro-organism(s) and monitored as required. Infection of the recipient by an islet preparation will be considered a serious adverse event, and the necessary action will be taken as described in Section 6.10.5 *Monitoring and Reporting of Adverse Events*.

7.7 Manufacture Comparison Data between CIT (BB IND9336) and UIC human islet isolation

Manufacture Comparison Data between CIT (BB IND9336) and UIC human islet isolation (BB IND11807).

7.7.1 Media Comparison Data

Media utilized of very similar composition have shown to be equivalent in our operation.

7.7.2 Islet Purification Comparison Data

UIC and CIT islet purification are technically very similar, as centrifuge speed, number of fractions and fraction volumes are almost identical. The main difference between the two protocols is the tissue volume that can be purified in once. UIC method allows to purify up to 45ml of tissue per COBE run while CIT method can purify no more than 25ml per COBE run. This divergence is due to a different density range of the gradients.
The UIC gradient is made using Ficol solution while the CIT gradient is made using optiprep.

The purification outcome and islet quality are comparable and the results are summarized in table 7-5.

Table 7-5 Comparison of UIC (H###) and CIT (C###) isolation of clinical grade. **Top Purity**: purity of top layer islet product in percentage; **Islet Yield**: Post purification islet yield in islet equivalent; **Recovery Rate**: islet recovery rate from a purification process expressed in percentage; **Tissue Vol**ume: settled tissue volume of islet product in millimeter.

ID	Top Purity	Islet Yield	Recovery Rate	Tissue Vol
H038	85.0	652375.0	100.0	1.1
H041	87.0	404928.0	71.6	1.4
H045	85.0	627731.0	100.0	11
H048	80.0	378414.0	58.2	2.5
H050	87.0	569702.0	100.0	4.2
H051	85.0	716434.0	100.0	4.3
H056	85.0	1094297.0	100.0	6.4
H065	87.0	921426.0	91.8	4.8
H077	88.0	417736.0	54.8	7.2
H079	75.0	372839.0	99.3	8.2
H080	90.0	386091.0	100.0	3.1
H084	85.0	377895.0	100.0	2.9
H085	90.0	635958.0	100.0	14
H098	85.0	501400.0	100.0	1.7
H132	95.0	642469.0	81.8	3.1
H139	80.0	462933.0	77.4	3.1
H141	85.0	877177.0	100.0	3.5
H192	88.0	490580.0	100.0	1.4
H245	87.0	315268.0	77.0	2
H282	90.0	574023.0	100.0	2.9
H289	93.0	376758.0	100.0	4.6
H309	82.0	521903.0	100.0	4.6
H318	90.0	609221.0	100.0	4.25
H319	90.0	474444.0	50.4	10.6
H321	85.0	480117.0	79.2	4.1
H331	85.0	394386.0	83.4	3.2
H355	85.0	435186.0	64.2	7.3
H368	91.0	797357.0	78.8	10.7
H370	92.0	495655.0	81.8	3.6
H379	85.0	1300903.0	100.0	3.9
H380	93.0	535618.0	82.3	1.7
Mean	86.8	575523.4	85.29	4.75
SD	4.2	224879.3	15.50	3.22

ID	Top Purity	Islet Yield	Recovery Rate	Tissue Vol
C012	95.0	439218.0	89.0	3.5
C023	90.0	479009.0	66.0	5.9
C026	90.0	391760.0	83.3	3.25
C032	90.0	640450.0	74.5	1.9
C031	95.0	399527.0	66.6	5.5
C035	93.0	719243.0	70.4	6.4
C036	93.0	493075.0	94.3	6.5
C038	95.0	588601.0	100.0	12.7
C045	92.0	647404.0	100.0	3
Mean	92.6	533143.0	82.68	5.41
SD	2.2	119100.5	13.83	3.20

As shown in Table 7-5, clinically equivalent purification outcomes were observed between two purification protocols, including top-layer islet purity, islet yield, islet recovery rate, and tissue volume.

7.7.3 Islet Isolation Outcome Comparison Data

Isolation outcome of CIT and UIC manufacture are compared in table 7-6.

Table 7-6. Comparison of Isolation outcome of CIT (C###) and UIC (H###) manufacture. **EIN/kg**: islet equivalent transplanted per kilogram body weight of recipient; **VB**: viability of final islet product in percentage; **Pt:** purity of final islet product in percentage. **TV**: transplanted Settled tissue volume in millimeter; **Ex**: endotoxin level in final islet product expressed as Eu/kg of body weight of recipient; **GS**: gram staining; **GSI**: glucose stimulated insulin release index; **MB**: microbiology test result of final islet product

ID	EIN/ KG	VB	PT	TV	EX	GS	GSI	MB
H038	11883	90.0	>35	7.9	0.910	N	8.6	NG
H041	6135	95.0	>35	6.8	0.260	N	2.7	NG
H045	9352	87.0	>35	5.0	0.740	N	4.9	NG
H048	6905	80.0	>35	8.0	2.090	N	3.9	NG
H050	8645	85.0	>35	4.2	0.560	N	1.4	NG
H051	11612	80.0	>35	8.8	0.440	N	2.3	NG
H056	8253	95.0	>35	4.7	0.460	N	2.4	NG
H065	15383	90.0	>35	4.1	0.420	N	9.5	NG
H077	5851	80.0	>35	7.8	0.350	N	5.5	NG
H079	6142	90.0	>35	8.4	0.410	N	1.5	NG
H080	7526	90.0	>35	2.0	1.750	N	8.5	NG
H084	6108	90.0	>35	6.1	0.400	N	5.0	NG
H085	9937	95.0	>35	9.0	0.390	N	3.1	NG
H098	7274	90.0	>35	2.0	0.400	N	5.1	NG
H132	9904	98.0	>35	1.5	0.470	N	1.3	NG
H139	7900	90.0	>35	5.5	0.430	N	3.5	NG
H141	13230	90.0	>35	1.3	0.380	N	10.0	NG
H192	8394	90.0	>35	1.8	0.430	N	2.1	NG
H245	6326	95.0	>35	1.8	0.260	N	2.4	NG
H282	8547	97.0	>35	2.3	0.190	N	1.3	NG
H289	6799	94.0	>35	8.0	0.250	N	1.6	NG
H309	5124	97.0	>35	1.7	0.640	N	5.0	NG
H318	5962	95.0	>35	2.0	0.770	N	2.1	NG
H319	8412	95.0	>35	4.6	2.230	N	4.7	NG
H321	7651	94.0	>35	2.0	0.790	N	3.2	NG
H331	6455	94.0	>35	6.0	0.420	N	1.9	NG
H355	6891	98.2	>35	7.0	1.240	N	1.9	NG
H368	10363	97.8	>35	1.9	0.050	N	1.4	NG
H370	4627	97.0	>35	2.8	0.050	N	1.4	NG
H379	13632	96.1	>35	6.2	0.071	N	2.4	NG
H380	5909	93.6	>35	5.1	0.020	N	2.1	NG
Mean	***8295***	***91.9***		***4.7***	***0.589***		***3.6***	
SD	***2634***	***5.2***		***2.6***	***0.547***		***2.5***	

ID	EIN/ KG	VB	PT	TV	EX	GS	GSI	MB
C012	9942	89.2	>35	2.1	0.037	N	1.6	NG
C023	5924	98.3	>35	2.9	0.246	N	1.3	NG
C026	6883	98.7	>35	3.3	0.126	N	1.6	NG
C031	4119	99.0	>35	3.9	0.080	N	1.2	NG
C032	5574	98.6	>35	0.8	0.017	N	3.6	NG
C035	6673	91.5	>35	4.5	0.050	N	1.1	NG
C036	5140	95.0	>35	1.9	0.018	N	2.8	NG
C038	5176	96.2	>35	3.1	0.075	N	1.2	NG
C045	7644	96.8	>35	4.1	0.014	N	2.9	NG
C055	5521	98.3	>35	1.1	0.010	N	1.5	NG
C056	5377	96.0	>35	4.1	0.027	N	4.3	NG
Mean	***6179.4***	***96.2***		***2.9***	***0.1***		***2.1***	
SD	***1577.4***	***3.2***		***1.3***	***0.1***		***1.1***	

As shown in table 7-6 all final islet products for transplantation in both CIT and UIC manufactory processes meet the criteria of quality control testing and have comparable results.

7.7.4 Comparison of Viability and Potency Over Extended Culture

The viability and potency of UIC and CIT manufacture over an extended culture are compared in table 7-7.

Table 7-7. Comparison of viability and potency of CIT (C###) and UIC (H###) manufacture over culture. **VB pre:** viability of pre-culture islet product in percentage; **VB post:** viability of post-culture islet product in percentage; **SI pre:** glucose stimulated insulin release index for pre-culture islets; **SI post:** glucose stimulated insulin release index for post-culture islets; **EIN pre:** pre-culture islet equivalent; **EIN post:** post-culture islet equivalent; **RV %:** percentage value of islets recovered post-culture; **CT:** culture time in hours

ID	VB pre	VB post	SI pre	SI post	EIN pre	EIN post	RV %	CT		ID	VB pre	VB post	SI pre	SI post	EIN pre	EIN post	RV %	CT
H309	96.76	97.30	5.02	---	521,903	328,954	63	12.5		C023	99.60	98.34	1.06	1.31	479,009	390,958	82	37.3
H318	98.22	94.88	2.12	---	609,221	365,496	60	12.9		C026	98.80	98.68	1.56	8.95	391,760	402,410	103	38.3
H319	94.94	95.22	4.69	---	472,899	474,444	100	11.8		C031	99.04	96.00	1.28	1.22	399,527	240,541	60	36.3
H321	92.42	93.90	3.2	---	480,117	474,376	99	14.0		C032	98.72	98.64	3.63	N/A	646,450	402,470	62	52.3
H331	95.50	94.12	2.45	---	394,386	385,412	98	11.7		C035	95.00	91.50	1.05	1.17	719,243	394,024	55	42.4
H355	98.02	99.00	4.89	---	435,186	398,745	92	9.7		C036	95.50	94.96	2.82	1.38	493,075	375,718	76	21.0
H368	95.96	97.88	1.44	---	797,357	539,285	68	18.0		C038	97.66	96.24	8.29	1.2	588,601	341,590	58	37.7
H370	95.46	97.00	---	---	495,653	312,179	63	23.0		C045	97.88	96.76	2.86	2.64	647,404	508,335	79	27.5
H379	96.16	96.14	2.42	---	1,300,903	858,856	66	8.5		C055	98.32	96.04	1.18	1.45	468,965	396,926	85	26.0
H380	96.90	93.06	2.14	---	535,618	376,999	70	14.2		C056	96.64	95.66	4.84	4.29	365,506	322,091	88	24.0
H386	94.06	95.58	1.95	---	673,261	505,748	75	9.3		C058	96.48	95.92	3.181	3.664	807,095	448,114	56	36.0
Av	***95.85***	***95.83***	***2.73***	***---***	***610,591***	***456,408***	***75***	***13.2***		***Av***	***97.60***	***96.25***	***2.89***	***2.73***	***546,057***	***383,925***	***73***	***34.4***
Sdv	1.69	1.83	1.21	---	256,033	152,282	16	4.2		*Sdv*	*1.51*	*2.03*	*2.18*	*2.46*	*145,401*	*684,44*	16	9.1

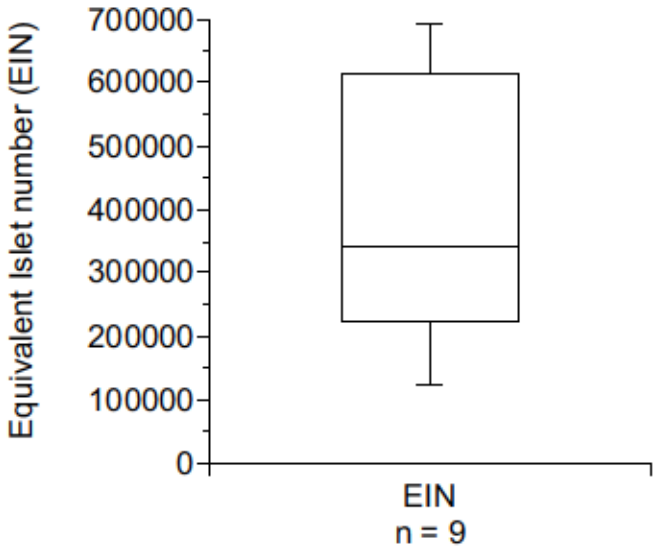
7.8 Process Qualification Data

7.8.1 Collection of Process Qualification Data

UIC team has extensive previous training in human islet isolation and transplantation. Since January 2004, the Islet Isolation Facility at the University of Illinois at Chicago has processed over 450 human pancreata. Many of the pancreata processed were of research quality, meaning that these were organs refused by clinical centers for whole pancreas grafts or for clinical islet isolation and transplantation. The low quality of the organs must be taken into account when analyzing the process qualification data.

The first 12 organs were used to establish the methods and train the personnel in human islet isolations. Nine of the first 17 procedures were completed until final purification for process validation. The islet yields obtained are illustrated in Figure 7-7 and Table 7-4.

Figure 7-7



Number of human pancreatic islet equivalents isolated during the process qualification at the University of Illinois at Chicago. The top, bottom, and line through the middle of the box correspond to the 75th percentile, 25th percentile, and 50th percentile respectively. The whiskers on the bottom extend from the 10th percentile and the top to the 90th percentile. ■ represents the arithmetic mean.

Table 7-7

Procedures	EIN
1	340,314
2	371,154
3	727,461
4	644,393
5	251,387
6	336,349
7	606,551
8	138,488
9	112,002
Mean	392,011

The next five consecutive human islet isolations were performed as part of the qualification of the manufacturing process. The final product quality control testing is summarized in Table 7-8.

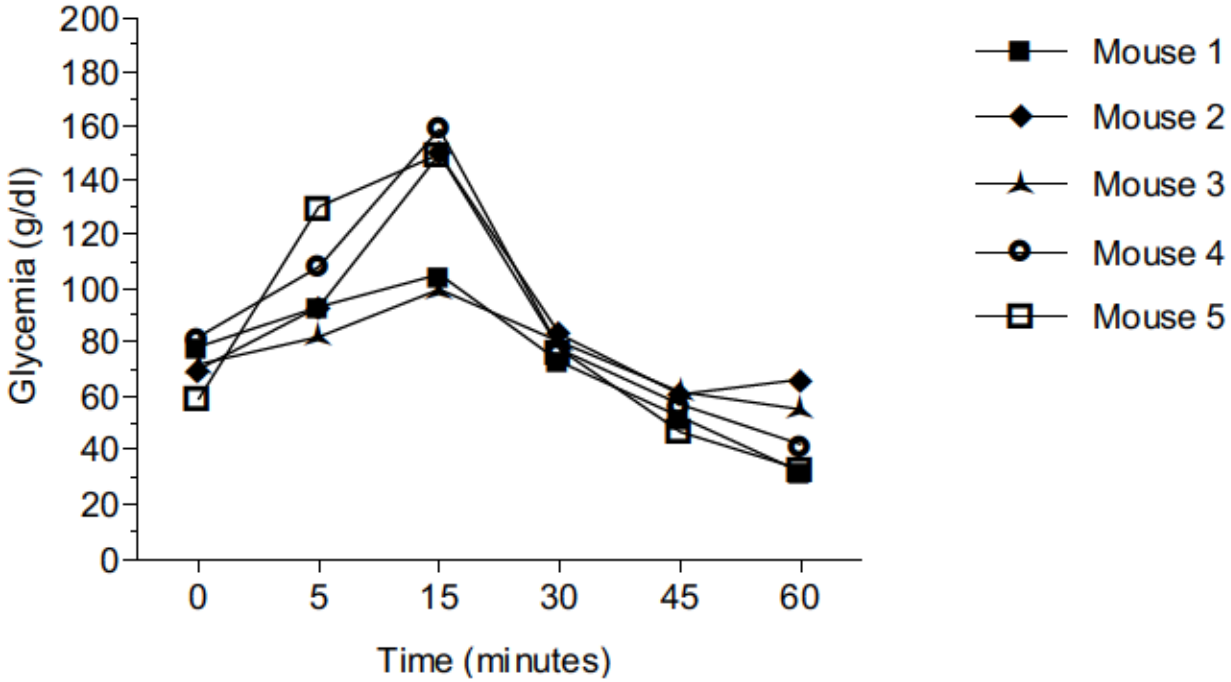
Table 7-8. Process Qualification Data

	H013	H014	H015	H016	H017
Donor age	75	59	76	60	31
Cause of death	IC Bleed	IC Bleed	IC Bleed	Anoxia	Blunt Injury
Body weight	112 kilo	72 kilo	105 kilo	61 kilo	97 kilo
Ischemia time (cold)	7 hr.	3.5 hr.	9 hr.	9 hr.	12 hr
Final EIN	251,387	336,349	606,551	138,488	112,002
Final Purity	70%	70%	90%	80%	50%
Final Volume	4.5 mL	3 mL	4.5 mL	4 mL	5 mL
Final Viability	90%	85%	95%	78%	80%
Sterility of final islet preparation	yes	yes	yes	yes	yes
Endotoxin Content of final islet preparation	0.38 EU/mL	0.56 EU/mL	0.51 EU/mL	0.36 EU/mL	0.29 EU/mL
Quality Assessment 12 hours post-isolation @ 37°C					
EIN	194,582	186,299	404,270	158,565	98,813
Delta-EIN (%)	-56,805 (-23%)	-150,050 (-44%)	-202,281 (-33%)	+20,077 (+13%)	-13,187 (-12%)
Purity	80%	90%	95%	90%	60%
Delta-Purity	+10%	+20%	+5%	+10%	+10%
Viability	85%	88%	95%	90%	95%
Delta-Viability	5%	+3%	0	+12%	+15%
Endotoxin	0.36 EU/mL	0.51 EU/mL	0.50 EU/mL	0.38 EU/mL	0.33 EU/mL
Stimulation Index	2.02	3.62	7.59	11.80	7.10
Cured diabetic mice	yes	yes	yes	yes	yes

As part of the process qualification, islets obtained during the process qualification were tested in diabetic nude mice (n = 3 per islet isolation procedure) for their ability to reverse diabetes. For this purpose 8-12 week old nude mice were rendered diabetic by injection of 200 mg/kg of strepozotocin. After 72 to 96 hours, mice became hyperglycemic (glycemia > 360 gr/dL). 2000 EIN were transplanted beneath the left kidney capsule. All the tested islet preparations (H013-H017) reversed diabetes in the nude mice after storage at 37º C for 12 hours.

To assure proper function of the transplanted islets, an intraperitoneal glucose tolerance test was performed in the transplanted animals (1 representative mouse of the 3 mice transplanted per islet isolation) by injecting 10% glucose 2 gr/kg ip. Glycemia was measured before glucose injection and at minutes 5, 15, 30, 45 and 60 after glucose injection. All mice tested (n = 5) normalized glucose within the observation period of 60 minutes (see Figure 7-8).

Figure 7-8. Glycemia during Intraperitoneal Glucose Tolerance Tests in Diabetic Nude Mice Transplanted with Human Islets 30 to 60 Days after Transplantation. One representative animal per group is shown.



7.8.2 Interpretation of Process Qualification Data

Safety of the Islet Preparations

All the microbial cultures of final islet preparations for process qualification have been negative, i.e., no microorganism could be detected, validating the isolation team's ability to perform aseptic processing in the facility.

The endotoxin content of the final islet preparation for process qualification has been low and within the specified release criteria for future islet graft preparations.
The viability, purity, and final volume of all final islet preparations for process qualification were within the range defined for product release criteria.

Islet Yield

Seven of the nine completed isolation procedures delivered islet yields superior to 250,000 EIN. For the required minimal transplant mass of 5,000 EIN/kg, 250,000 EIN would be the minimal islet yield to transplant a 50 kg patient.

Again, considering that we used only research pancreata not accepted by other centers for transplantation, these are excellent islet yields that show the proficiency of the UIC islet isolation team.

Function of the Islet Preparations

All islet preparations tested after 12 hours of storage responded with an increase in insulin secretion upon exposure to high glucose levels and reversed diabetes in streptozotocin induced diabetic nude mice. Transplanted islets achieved a normal glucose tolerance in all the transplanted diabetic mice.

Conclusion

The team at the Islet Isolation Facility at the University of Illinois at Chicago has demonstrated competence in producing viable and functional sterile human pancreatic islets in sufficient quality and quantity for use in human transplantation.

7.8.3 Stability Data

For this investigational protocol, the islet preparation will be stored for a maximum of 12 hours under standard culture conditions in an incubator with 5% CO2 at 37°C.
We investigated, as indicated in Table 7-5, the effect of 12 hours of culture on the EIN, viability, purity, and islet function of the islet preparation used for process qualification.

The number of islets (EIN) decreased by a mean of 20% (range from increase of 13% to decrease of 44%), whereas the viability increased by 7% (range from 0 to 15%), indicating that the mean net loss of viable islets during the storage period was 13%.
All glucose static incubation assays and mouse transplants were performed after a minimal storage period of 12 hours. The preparations remained sterile and the levels of endotoxins did not increase during the period of storage. The presented data indicates that the islets are stable during the 12-hour period of storage. During the proposed clinical trial, additional stability data will be collected from post-transplant samples including assessment of sterility, islet function, islet purity, and islet viability.

7.9 Claim for Categorical Exclusion

In conformance with the National Environmental Policy Act, The University of Illinois, Chicago claims exclusion from the requirement to prepare an environmental assessment

for the processing of pancreatic islet cells, based on the categorical exclusion of 21 CFR 25.31(e) in that this application constitutes action on an IND.

7.9.1 Islet Isolation and Islet Quality Assessment of Transplanted Preparations at UIC for Phase 1/ Phase 2 Trial

Table 7-9 summarizes the quality testing results for each islet batch used for transplant at UIC.

Table 7-9. Quality Testing Results for Each Islet Batch Used for Transplant at UIC
Tx = Transplant

Isolation Number	EIN/Kg of Recipient	Viability	Purity	Tx. Tissue Volume	Endotoxin (Eu/Kg)	Gram Stain	GSI	Final Microbiology	Subject Transplanted
H038	11,882.96	90%	>50%	7.9cc	0.91	Neg.	8.60	No growth	CAS004
H041	6,135.27	95%	>60%	6.8cc	0.26	Neg.	2.67	No growth	TRG005
H045	9,352.40	87%	>70%	5cc	0.74	Neg.	4.85	No growth	LRD003
H048	6,905.36	80%	>30%	8cc	2.09	Neg.	3.90	No growth	CAS004
H050	8,644.94	85%	>85%	4.2cc	0.56	Neg.	0.40	No growth	TRG005
H051	11,611.57	80%	>50%	8.8cc	0.44	Neg.	2.26	No growth	DLP010
H056	8,252.68	95%	>70%	4.7cc	0.46	Neg.	2.38	No growth	DLP010
H065	15,382.73	90%	>50%	4.1cc	0.42	Neg.	9.50	No growth	DLP010
H077	5,850.64	80%	>30%	7.8cc	0.35	Neg.	5.49	No growth	KAP011
H079	6,142.32	90%	>30%	8.4cc	0.41	Neg.	1.52	No growth	LRD003
H080	7,526.14	90%	>70%	2cc	1.75	Neg.	8.53	No growth	CAS004
H084	6,107.88	90%	>70%	6.1cc	0.40	Neg.	4.96	No growth	KGM008
H085	9,936.84	95%	>60%	9cc	0.39	Neg.	3.10	No growth	KDC012
H098	7,273.67	90%	>90%	2cc	0.40	Neg.	5.05	No growth	SRP013
H132	9,903.93	98%	>95%	1.5cc	0.47	Neg.	1.28	No growth	KDC012
H139	7,899.88	90%	>50%	5.5cc	0.43	Neg.	3.45	No growth	ATC016
H141	13,230.42	90%	85%	1.3cc	0.38	Neg.	10.00	No growth	SKA018
H192	8,394.23	90%	>50%	1.8cc	0.43	Neg.	2.09	No growth	SKA018
H245	6,325.82	95%	75%	1.8cc	0.26	Neg.	2.44	No growth	SEJ102
H282	8,547.42	97%	64%	2.3cc	0.19	Neg.	1.34	No growth	PJJ108
H289	6,798.56	94%	80%	8cc	0.25	Neg.	1.59	No growth	KDC012
H309	5,123.89	97%	65%	1.7cc	0.64	Neg.	5.02	No growth	DKW106
H318	5,962.41	95%	>45%	2cc	0.77	Neg.	2.12	No growth	DKW106
H319	8,412.12	95%	45%	4.6cc	2.23	Neg.	4.70	No growth	SKA018
H321	7,651.20	94%	65%	2cc	0.79	Neg.	3.20	No growth	EGS117

This table represents the quality testing performed on each islet batch used for transplant by UIC. Endotoxin, Gram stain, purity, viability, and final count were performed prior to transplant and were within the parameters for release. GSI and microbiology results were obtained post-transplant, and results were recorded in the batch record.

8 Pharmacology and Toxicology Information

8.1 Islet Transplantation

There is large accumulated data that islet transplantation can reverse diabetes in a variety of diabetes models in rodents and larger animals. The minimal invasive nature of islet transplantation makes this procedure safer than whole pancreas transplantation requiring complex surgery.

8.2 Immunosuppression – the Edmonton Protocol

In non-obese diabetic mice, a rodent model of Type 1 diabetes, the combination of daclizumab, sirolimus and tacrolimus allows for prolonged islet graft survival and maintenance of normoglycemia (44). The Edmonton protocol has been investigated in a number of clinical trials and extended number of patients as discussed in Section *9*. Recently, basiliximab was introduced in place of daclizumab because Roche withdrew the latter from production.

In addition to the Edmonton protocol, the drug etanercept, a TNF alpha receptor antagonist, will be used in immunosuppression. Tumor necrosis factor alpha is cytotoxic to human islet beta cells (45). In murine models, selective inhibition of tumor necrosis factor alpha in the peritransplant period has promoted reversal of diabetes after marginal-mass islet transplants (46) The use of etanercept in this trial is based on the recent publication in JAMA by the University of Minneapolis group indicating that this drug improves the islet graft function and engraftment (27).

Another addition to the Edmonton protocol, is the drug exenatide. Exenatide displays biological properties similar to human glucagon-like peptide-1 (GLP-1), a regulator of glucose metabolism and insulin secretion. It has shown in several clinical studies that it can improve glycemic control in Type 2 diabetic patients in a dose dependent manner (47-49). In experimental models, exenatide has a number of effects such as protection against apoptosis and induction of beta-cell proliferation and increase glucose-dependent synthesis of insulin from pancreatic beta cells in the presence of elevated glucose concentrations (50-53). In rodent models, exenatide can improve glycemic control after transplantation of sub-optimal islet mass (54). The success of islet transplantation depends largely on the amount of islets engrafting. Based on findings from pre-clinical and clinical studies there is sufficient data to support the hypothesis that exenatide will exhibit beneficial effects on the engraftment of islets transplanted to diabetic patients and improve glycemic control. Given the low side effect profile and safety of the product, and the potential improvement in the outcome of islet transplantation, exenatide should be evaluated in the clinical setting of allogeneic islet transplantation in Type 1 diabetic patients.

Previous Human Experience with the Investigational Drugs
Sirolimus, tacrolimus, mycophenolate mofetil, basiliximab, etanercept, and exenatide have been thoroughly investigated in small and large animals and their safety and potential risks are well documented. Each of the immunosuppressive drugs is FDA approved and widely used in clinical practice. Therefore the pharmacology and toxicology will be discussed in Section 9.

9 Previous Human Experience with the Investigational Drugs

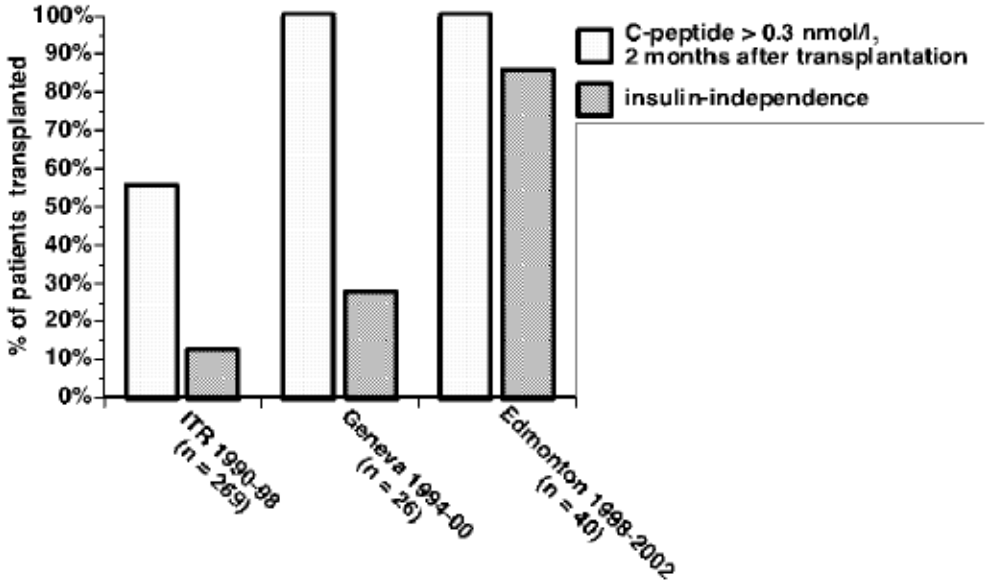
9.1 Islet Transplantation

9.1.1 The Early Clinical Trials of Allogeneic Islet Transplantation in Uremic Type 1 Diabetic Patients

Thus far, islet allotransplantation has been prescribed mostly for Type 1 diabetic patients with a functional solid organ graft, or for patients on the waiting list for an organ transplantation. This group of worst-case diabetic patients has a long history of unstable diabetes and presents many chronic diabetic complications. These patients require immunosuppression to prevent rejection of the transplanted organ (usually a kidney graft because of end-stage diabetic nephropathy), and the islet graft was not thought to present a relevant additional risk.

More than 400 islet allotransplantations have been reported to the International Islet Transplantation Registry in Giessen (ITR). Islet transplantation in Type 1 diabetic patients with end-stage kidney failure has had some important (*Figure for Islet Transplant Success Rate*), though still unreliable, success (19, 21-24). The data from the University of Geneva showed that with improved islet isolation techniques, the rate of primary islet function could be increased from 55% (11) to 100% (24) (*Figure for Islet Transplant Success Rate*).

Figure 9.1 Islet Transplant Success Rate

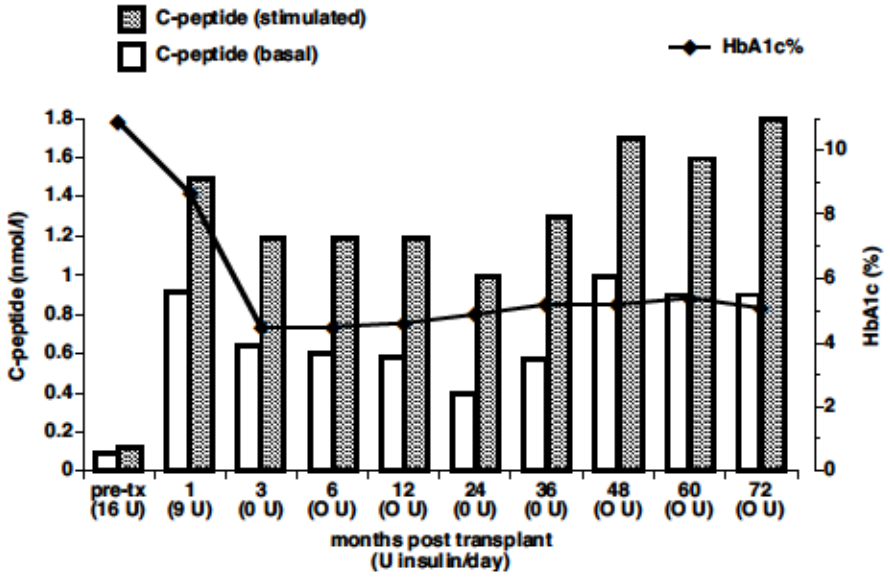


However, the applied immunosuppression including a combination of steroids and high dose calcineurin-inhibitors (either cyclosporin or tacrolimus) did not consistently prevent

rejection or recurrence of autoimmune-diabetes, although in some patients long-term insulin-independence could be observed (see *Figure for Long Term Function*).

Figure 9.2 Long Term Function

Long term follow-up in a Type 1 diabetic patient transplanted under conventional immunosuppression, several years after kidney transplantation, at the University of Geneva in Switzerland.

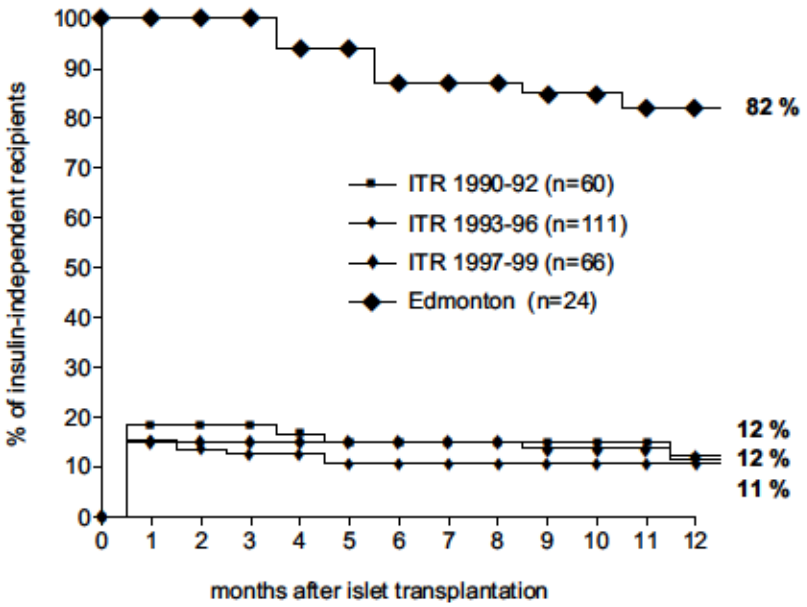


In addition, the combination of steroids and calcineurin-inhibitors induced marked insulin resistance and direct beta cell toxicity. While in whole pancreas transplantation this insulin resistance can be counterbalanced by a sufficient transplanted islet mass, the endocrine mass in an islet graft is often insufficient to overcome the increased metabolic demand induced by the diabetogenic impact of immunosuppression on a background of pre-existing insulin resistance. Even though few patients became insulin-independent with this conventional immunosuppression, the restoration of an endogenous insulin-production stabilized glucose homeostasis, reduced hypoglycemic events (55), normalized glycosylated hemoglobin (24), and may have had a beneficial effect on long term diabetic complications (56).

9.1.2 Islet Allotransplantation Alone in Non-Uremic Type 1 Diabetic Patients

Shapiro, et al reported a series of successful islet allografts in non-uremic Type 1 diabetic patients (patients with normal kidney function and thus not requiring a kidney transplantation) using a sirolimus based, steroid-free immunosuppressive regimen and repeated islet injections until transplantation of a sufficient islet mass achieved insulin independence (10, 14, 57). With this regimen, insulin independence after allogeneic islet transplantation in Type 1 diabetic patients was obtained consistently and persisted in 82% of patients one year post transplant (*Figure for One-Year Graft Function*).

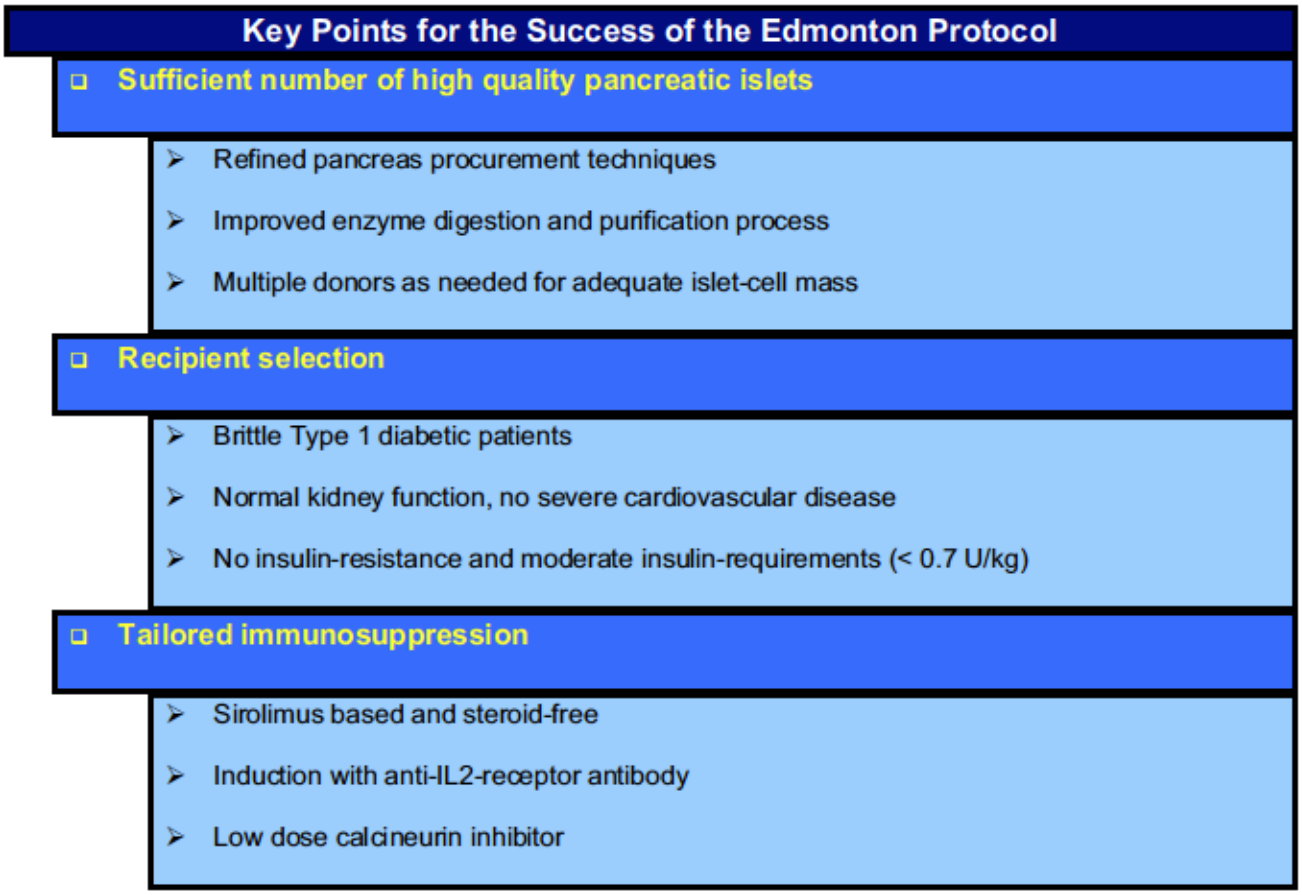
Figure 9.3 One-Year Graft Function



Cumulative one-year insulin independence after allogeneic islet transplantation in Type 1 diabetic patients as reported to the International Islet Transplant Registry and as observed in the Edmonton series.

This remarkable breakthrough in the field of islet transplantation provides insight into the reasons for the previous frequent graft failures. The major changes involved in the development of the Edmonton protocol are summarized in *Figure for Reasons for Success*.

Figure 9.4 Reasons for Success



The amount of islets injected and engrafting therefore are of utmost importance. In the Edmonton series, at least 9,000 islet equivalents per kilogram of body weight of the recipient were needed to achieve insulin independence. Therefore, a patient with a body weight of 70 kg would require at least 630,000 islet equivalents to achieve insulin-independence. This number corresponds to approximate 50% of the islet content of a normal adult pancreas. In previous series, most patients received a lower islet mass. Even with improved islet isolation techniques, 9000 islet equivalents per kilogram bodyweight can be rarely isolated from a single pancreas. In particular, patients with a normal bodyweight still require more than one donor, while light patients may receive a sufficient islet mass with one donor. Thus, strategies are required to lower the islet mass needed to achieve insulin independence in order to make islet transplantation accessible to more patients, and future studies will address this.

Immunosuppression with sirolimus and low dose tacrolimus, without steroids, is also one of the keys for the success of the Edmonton trial. Most previous islet transplantation series used a combination of high doses of calcineurin inhibitors and steroids, which is diabetogenic as describe previously (58, 59). In contrast to standard immunosuppression, the Edmonton protocol has much less diabetogenic impact. Moreover, considering the relative low acute insulin response to intravenous glucose, and the excellent metabolic results described in the Edmonton series (57), the question

can be raised, whether sirolimus may increase the effect of insulin in humans, as previously suggested in animal studies (60).

While balancing the risk and benefit of islet transplantation in non-uremic patients, we must consider some rare complications that occurred in the patients transplanted in Edmonton (10, 57). Complications related to the transhepatic portal injection are either bleeding or portal vein thrombosis. Multiple attempts to reach the portal vein and prophylactic anticoagulation with heparin increase the risk of bleeding. In the initial experience, to prevent bleeding after islet transplantation, the catheter was removed and the puncture tract embolized with gelatin sponge plugs (14). Prophylactic anticoagulation is required to prevent portal vein thrombosis after intraportal injection of the islet preparation, as has been occasionally described (61-63). Improved islet isolation techniques allowed for purer islet preparations, which reduced the risk of thrombosis and consequently permitted lower doses of heparin.

In addition to the risks of the injection (bleeding, partial transitory portal vein thrombosis), many patients evidenced side effects related to the immunosuppression. Increased cholesterol levels were found in 15 of the first 17 recipients (10), most likely as a side effect of sirolimus (64). The mean serum creatinine for the first 17 recipients, after a median follow up of 20 months, rose slightly from 89 ± 8 pre-transplant to 104 ± 14 µmol/l post-transplant ($P = 0.047$). However, when two patients who had abnormal kidney function pre-transplant and serious deterioration in renal function post transplant, are removed from analysis, there was no difference pre- and post-transplant (79 ± 5 vs. 85 ± 16 µmol/l, respectively; $P = 0.117$) (10). The markedly decreased kidney function found in these two patients was likely related to the side effects of the calcineurin-inhibitor tacrolimus. Both patients had pre-existing impaired kidney function consequent to diabetic nephropathy (10). In these patients, tacrolimus was discontinued and replaced by the non-nephrotoxic immunosuppressant mycophenolate mofetil, resulting in stabilization of creatinine. During one-year follow-up, three of 12 patients also developed post-islet transplant diabetes (57).

Thus, as long as long-term immunosuppression is required, it may be appropriate to restrict islet transplantation only to patients with unstable diabetes at risk for severe hypoglycemia and long-term complications. This particular category of patients will benefit by a better glucose regulation achieved after islet transplantation, even if low doses of exogenous insulin and life-long immunosuppression may be needed.

9.2 Immunosuppression

9.2.1 Sirolimus (Rapamycin, Rapamune®)

The FDA approved sirolimus (Rapamycin, Rapamune®) as an immunosuppressive agent in 1999. Also see package insert (Appendix *Package Insert Sirolimus*). Phase III studies of sirolimus in cyclosporine-based regimens revealed mild dose-related thrombocytopenia and increases of serum triglycerides and cholesterol. This latter finding is probably its most disturbing effect and was seen in 40-50% of subjects. Hypertriglyceridemia is the predominant sign. HMG-CoA reductase inhibitors and fibric

acid have been used and well tolerated. Additionally, the unexpected finding of nephrotoxicity has been encountered but it is not clear whether it is directly attributable to sirolimus or to a potentiation of cyclosporine's nephrotoxicity. Other common events reported in the Phase III trials include leukopenia, hypertension, anemia, nausea, vomiting, diarrhea, elevated liver enzymes, rash and acne at the 2 mg maintenance dose. At the 5 mg dose, more marrow suppression and hyperlipidemia were observed. A European protocol compared sirolimus therapy with azathioprine (Imuran®) and prednisone to cyclosporine, azathioprine and prednisone in kidney transplant recipients. The sirolimus treated group experienced more hypercholesterolemia, hypertriglyceridemia, thrombocytopenia, and leukopenia. Side effects were related to drug concentration and were improved with maintenance of the sirolimus level between 10-20 ng/mL. Sirolimus' effect on the developing fetus is not known and is not recommended for administration to nursing mothers. The agent is not recommended for nursing mothers, and it is recommended that women of childbearing potential use effective contraception before, during, and for at least 4 months following sirolimus administration.

The combination of low-dose tacrolimus with sirolimus has not led to nephrotoxicity in the Edmonton trial or in other preliminary data from over 100 subjects treated with this combination to date (65-67).

Cure et al. (68) reported that female recipients of allogeneic islets for Type 1 diabetes under immunosuppression therapy based on daclizumab induction and tacrolimus/sirolimus maintenance frequently develop menstrual cycle alterations and clinically significant benign ovarian cysts, some requiring medical or surgical intervention. Impaired spermatogenesis has been observed in sirolimus-treated patients with though levels of 10–15 ng/mL that was reversible after discontinuation of the drug.

9.2.2 Tacrolimus (Prograf®, FK506)

Tacrolimus (Prograf ®, FK506) has been in wide clinical use for the prevention of allograft rejection since 1994 when the FDA approved it after several years of testing. Also see the package insert (Appendix *Package Insert Prograf®).* The agent is almost invariably administered with other immunosuppressive agents but is known to be associated with several side effects including hypertension, diabetes (due to diminished beta cell function), nephrotoxicity, hyperkalemia, pruritis, and neurologic sequelae (i.e., tremor, ataxia, and extremely rarely central pontine myelinolysis), nausea, vomiting, and diarrhea. Most of these complications are dose-related, and are expected to be minimized with target serum levels in the range of 3-5 ng/mL as proposed in the current trial. As with all immunosuppressive agents, the risks for opportunistic infections and certain malignancies are increased.

9.2.3 Mycophenolic Acid (CellCept®)

Mycophenolic acid (Cellcept®) is widely used concomitantly with other immunosuppressive agents to prolong the survival of allogeneic organ transplants, and is used for islet transplant patients who do not tolerate sirolimus. It is contraindicated for pregnant women because of pregnancy loss and fetal defects, and must not be used by

women who are breast feeding. The principal adverse events reported in > 20% of patients include diarrhea, leukopenia, vomiting, opportunistic infections, hypertension, anemia, leukopenia, and peripheral edema. Non-melanoma skin carcinomas and other malignancies have been reported.

9.2.4 Azathioprine (Imuran®)

Imuran has been widely used in patients with renal transplants. Hematologic toxicities may occur; including leukopenia, thrombocytopenia, macrocytic anemia, and pancytopenia. Monitoring is recommended, and dose adjustment or treatment interruption may be required. If azathioprine is planned, the subject may be tested for the presence of thiopurine methyltransferase (TPMT), the main enzyme responsible for inactivating toxic products of azathioprine metabolism. Patients with homozygous deficiency of TPMT have no enzyme activity and have a high risk of pancytopenia (28, 29). Patients with reduced TMPT activity may require alternate therapy, dose reduction, or discontinuation. As with other immunosuppressive medications, malignancy may occur, including skin cancer. Gastrointestinal hypersensitivity reaction, characterized by severe nausea and vomiting and possibly diarrhea, rash, fever, malaise, myalgias, liver enzyme elevations, and hypotension, has been reported, usually during initial therapy, and may recur on rechallenge. Infections that are serious and including opportunistic, new, or reactivated latent infections, may occur. Progressive multifocal leukoencephalopathy (PML) associated with JC virus infection, including fatalities, has been reported. Azathioprine is a known teratogen; women of childbearing potential must avoid pregnancy (69).

9.2.5 Basiliximab (Simulect®)

Basiliximab (Simulect®) is a chimeric (murine/human) monoclonal antibody that functions as an immunosuppressant agent by binding to and blocking the interleukin-2 receptor α-chain, also known as CD25 antigen, on the surface of activated T-lymphocytes while the drug is in circulation. Severe acute hypersensitivity reactions including anaphylaxis occurring within 24 hours of administration have been observed. Women of childbearing potential should use effective contraception, and nursing mothers should discontinue nursing before taking the drug. In clinical trials, > 10% of patients experienced constipation, nausea, abdominal pain, vomiting, diarrhea, peripheral edema, viral infections, hyperkalemia, hypokalemia, hypercholesterolemia, hypophosphatemia, hypertension, anemia, headache, tremor and insomnia.

9.2.6 Rabbit anti-human thymocyte immunoglobulin (ATG) (Thymoglobulin®)

Rabbit Thymoglobulin® is a purified pasteurized gamma immune globulin obtained by immunizing rabbits with human thymocytes. This immunosuppressive product contains cytotoxic antibodies directed against antigens expressed by human T-lymphocytes. The FDA approved Thymoglobulin for treatment of renal transplant acute rejection in conjunction with concomitant immunosuppression, and FDA also approved its use in islet transplant clinical trials (e.g. CIT under NIH). The mechanisms by which polyclonal antilymphocyte preparations suppress immune response are not fully understood. Possible mechanisms include T-cell clearance from the circulation and modulation of T-

cell activation, homing, and cytotoxic activities. Thymoglobulin includes antibodies against CD8, CD11a, CD18, CD25, CD44, CD45, HLA-DR, HLA Class I heavy chains, and β2 micro-globulin. Thymoglobulin has not been shown to be effective for treating antibody (humeral) mediated rejections.

Thymoglobulin has an established safety record. Adverse events are generally manageable or reversible with a reduction in infusion rates and/or with medications. Thymoglobulin is contraindicated in patients with a history of allergy to rabbit proteins or to any product excipients, or who have active acute or chronic infections which contraindicate any additional immunosuppression. Thymoglobulin should be administered under close monitoring in a hospital setting by a physician experienced in immunosuppressive therapy, and medical personnel should be available to treat patients who experience anaphylaxis. The recommended dosage for treatment of acute renal graft rejection is 1.5 mg/kg of body weight daily for 7-14 days. The first dose should be infused over a minimum of six hours in a high-flow vein. Overdosage may result in leukopenia including lymphopenia, neutropenia, and/or thrombocytopenia. The dose should be reduced by one-half if the WBC is between 2,000 and 3,000 cells/mm3 or if the platelet count is between 50,000 and 75,000 cells/mm3. Stopping thymoglobulin should be considered if the WBC counts falls below 2,000 cells/mm3 or platelets below 50,000 cells/mm3. Thymoglobulin may cause cytokine release-related reactions including fever, chills and rigors. To minimize infusion-associated reactions, patients will receive premedication with corticosteroida, acetaminophen, and diphendramine.

9.2.7 Etanercept (Enbrel®)

Etanercept (Enbrel®) is a tumor necrosis factor receptor p75 Fc fusion protein; TNFR:Fc which acts as a TNF alpha receptor antagonist. Etanercept was approved by FDA for use in Rheumatoid arthritis in 1998. Also see the package insert (Appendix *Package Insert Etanercept*). The drug will be given one hour pre-transplant intravenously and on the 3rd, 7th and 10th day post-transplant for a total of 4 doses (including the pre-transplant dose) and this dose regimen is repeated for subsequent islet transplants. If a subsequent transplant is done within this treatment period, the regimen is re-started upon each transplant.) This drug has been used widely in the treatment of rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis. Tumor necrosis factor alpha is cytotoxic to human islet beta cells (45). In murine models, selective inhibition of tumor necrosis factor alpha in the peritransplant period has promoted reversal of diabetes after marginal-mass islet transplants (46). In humans the use of etanercept in islet transplantation is documented in the article published in JAMA by the University of Minneapolis Group indicating that this drug by acting as TNF alpha receptor antagonist improves islet graft function and engraftment (27). Although the drug is in general well tolerated, the use of this drug is also associated with various side effects like abdominal pain, vomiting, injection site reactions (37%), new onset/exacerbation of CNS demyelinating disorders, cough, rhinitis, and some serious side effects like anemia, aplastic anemia, leukopenia, neutropenia, pancytopenia, thrombocytopenia, allergic reactions (< 2%), infections including sepsis, optic neuritis (rare), tuberculosis (very rare) and malignancy (very rare). The use of drug in pregnant women is not well established and it is in US FDA Pregnancy Category B.

9.3 Additional Medication

9.3.1 Valganciclovir (Valcyte®)

Valganciclovir is FDA approved for the prevention of cytomegalovirus (CMV) infection after kidney, heart, and kidney-pancreas transplantation in patients at high risk , e.g., donor CMV seropositive and recipient CMV seronegative. In the proposed study, valganciclovir will be used for the prevention of CMV infection during a period of 3 months after islet transplantation. Valganciclovir is a prodrug of ganciclovir that exists as a mixture of two diastereomers. After oral administration, both diastereomers are rapidly converted to ganciclovir by intestinal and hepatic esterases. Ganciclovir is a synthetic analogue of 2'-deoxguanosin, which inhibits replication of human CMV. The main side effects encountered in the clinical use is myelosuppression as cited in the package insert (see Appendix *Package Insert Valcyte®)* with granulocytopenia, anemia, and thrombocytopenia. In animal studies ganciclovir was carcinogenic, teratogenic, and caused aspermatogenesis. Therefore, women in child-bearing potential should be advised to use effective contraception during treatment. Similarly, men should be advised to practice barrier contraception during, and for at least 90 days following treatment with Valcyte. See Appendix *Package Insert Valcyte®.*

9.3.2 Trimethoprim-Sulfamethoxazole (Septra® or Equivalent)

Trimethoprim-sulfamethoxazole is an FDA approved antibiotic and has been used for decades in daily clinical practice. In the herein described investigation, it will be prescribed twice per week at 80 mg trimethoprim and 400 mg sulfamethoxazole (Septra® single strength, 2 tablets per week), as pneumocystis carinii prophylaxis. The known adverse effects of trimethoprim-sulfamethoxazole include gastro-intestinal side effects such as nausea and diarrhea, myelosuppression with leuco- and thrombopenia, and rarely agranulocytosis and nephrotoxicity. See Appendix *Package Insert Septra®.*

9.3.3 Cefazolin (Kefzol®)

Cefazolin is an FDA approved first generation cephalosporin that will be given during the first 24 hours after islet transplantation for antibiotic prophylaxis. Allergic reaction can be encountered. In general, adverse reactions are uncommon and include pseudomembranous colitis, increase in liver enzymes, cholestatic hepatitis, and rarely nephrotoxicity. See Appendix *Package Insert Kefzol®.*

9.3.4 Enoxaparin (Lovenox®)

Enoxaparin is an FDA approved low molecular weight heparin that will be given for the prophylaxis of portal vein thrombosis for a duration of seven days after islet transplantation. Known adverse effects include bleeding and thrombocytopenia. See Appendix *Package Insert Lovenox®.*

9.3.5 Exenatide (Byetta®)

Exenatide is an FDA approved incretin mimetic agent that enhances glucose-dependent insulin secretion. Exenatide has also several other antihyperglycemic effects (70). Exenatide will be given to enhance glucose-dependent synthesis of insulin and promote

insulin release from beta cells in the presence of elevated glucose concentrations. Exenatide may also promote islet engraftment through its anti-apoptotic action (53). The known adverse effects of exenatide are mild to moderate hypoglycemia, reduction in appetite, food intake, and/or body weight, headache, and mild to moderate nausea, vomiting, and diarrhea. A gradual titration regimen has been shown to reduce the frequency of nausea. See Appendix *Package Insert Exenatide*

Since October 2007, FDA received reports of six cases of hemorrhagic or necrotizing pancreatitis in patients taking Byetta. All patients required hospitalization, two patients died, and four patients were recovering at time of reporting. Byetta was discontinued in all 6 cases. Byetta and other potentially suspect drugs should be promptly discontinued if pancreatitis is suspected. There are no signs or symptoms that distinguish acute hemorrhagic or necrotizing pancreatitis associated with Byetta from the less severe form of pancreatitis. If pancreatitis is confirmed, the physician must initiate appropriate treatment and carefully monitor the patient until recovery. Byetta should not be restarted. Rodent studies have linked some forms of GLP-1 mimetic therapy with thyroid tumors, but no causal relationship is established in humans (71, 72). Therefore, as a precaution, subjects in this clinical trial will be monitored by periodic thyroid ultrasound scans.

9.3.6 Immunizations

Influenza Vaccine

Subjects will be asked to obtain influenza virus vaccine from a primary care provider or community flu vaccine program annually in the fall or winter in accord with current CDC recommendations for patients with diabetes or immunocompromising conditions caused by medications, and also in accord with the American Diabetes Association Standards of Medical Care in Diabetes - 2008.

Pneumococcal Vaccine

Subjects will be asked to obtain at least one pneumococcal vaccine in accord with current CDC recommendations for patients with diabetes or immunocompromising conditions, and also in accord with the American Diabetes Association Standards of Medical Care in Diabetes - 2008. CDC recommends a one-time revaccination if the vaccine was administered > 5 years ago.

Hepatitis Vaccine

Subjects will be asked to obtain a series of hepatitis B vaccine in advance of transplant in accord with current Centers for Disease Control (CDC) recommendations for patients with diabetes or immunocompromising conditions.

10 Additional Information

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10.2 Appendices

10.2.1 Appendix"Letter of confirmation by collaborating organ procurement agencies"

10.2.2 Appendix "Pre-Screening"

10.2.3 Appendix "Detailed Visit Schedule"

10.2.4 Appendix "Transplant Order" and "Post-Transplant Orders"

10.2.5 Appendix "HYPO Score sheets"

10.2.6 Appendix "Letter of consent"

10.2.7 Appendix "Certificate of Analysis"

10.2.8 Appendix "Ricordi Chamber"

10.2.9 Appendix..“Transplant Release Form”

10.2.10 Appendix.. "Package Insert Sirolimus"

10.2.11 Appendix .."Package Insert Prograf®"

10.2.12 Appendix "Package Insert Simulect®"

10.2.13 Appendix "Package Insert Valcyte®"

10.2.14 Appendix "Package Insert Septra®"

10.2.15 Appendix "Package Insert Kefzol®"

10.2.16 Appendix "Package Insert Lovenox®"

10.2.17 Appendix "Package Insert Etanercept"

10.2.18 Appendix "Package Insert Mycophenolate Mofetil"

10.2.19 Appendix "Package Insert Thymoglobulin"

10.2.20 Appendix "Package Insert Byetta®"

10.2.21 Appendix "Product Monograph Imuran®"

10.2.22 Appendix "Collaborative Islet Transplant Registry"

10.2.23 Appendix "A Novel Beta Cell Specific Microfluidic Perfusion and Imaging Device for Islet Potency Testing Substudy"

Selected Scientific Papers