ALLOGENEIC ISLET CELLS TRANSPLANTED ONTO THE OMENTUM

University of Miami IRB Protocol #: 20140144
University of Miami
Jackson Medical Towers East
1500 NW 12th Avenue, Ste. 1002
Miami, FL 33136

Study Sponsor
Alejandro, Rodolfo, MD
Diabetes Research Institute
1450 NW 10th Ave,
Miami, Florida 33136

Principal Investigators:
Alejandro, Rodolfo, MD
Diabetes Research Institute
1450 NW 10th Ave,
Miami, Florida 33136

Co- Investigator:
David Baidal, MD
Diabetes Research Institute
1450 NW 10th Ave,
Miami, Florida 33136

Dora Berman-Weinberg, PhD
Diabetes Research Institute
1450 NW 10th AVE R-134
Miami, Florida 33136 United States

Camillo Ricordi, M.D.
Diabetes Research Institute
1450 Northwest 10th Avenue R-134
Miami, Florida 33136 United States

Jason Salsamendi, M.D.
University of Miami, Vascular Interventional Radiology
1611 NW 12 Avenue
Miami, Florida 33136

George Burke, M.D.
UMH Medical Specialty Care (Transplant)
1321 NW 14th Street
Miami Florida 33136

Gaetano Ciancio, M.D.
UMH Medical Specialty Care (Transplant)
1321 NW 14th Street
Miami Florida 33136

Gennaro Selvaggi, M.D.
UMH Medical Specialty Care (Transplant)
1321 NW 14th Street
Miami Florida 33136

SITE(S):
University of Miami
Diabetes Research Institute
1450 Northwest 10th Avenue
Miami, Florida 33136
United States

Jackson Memorial Medical Center
1611 Northwest 12th Avenue
Miami, Florida 33136
United States

Clinical Research Center
University of Miami | Miller Office of Research
University of Miami Hospital
1400 NW 12th Avenue, 7th Floor South
Miami, FL 33136

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1 INTRODUCTION

1.1 Background

Transplantation of pancreatic islets represents a clinical therapeutic option to preserve and/or restore beta-cell function in patients with diabetes (1, 2). Since the 1970's islets are embolized into the hepatic portal system by a minimally invasive technique consisting of trans-hepatic cannulation of the portal vein under ultrasound and fluoroscopy guidance followed by sealing of the tract with thrombostatic treatment. Alternatively, in patients at risk of bleeding, the transplant is performed by cannulation of a tributary of the portal vein using open surgery (mini-laparotomy) or laparoscopic approach (3).

Several improvements in islet cell processing, immunotherapy and patient management have led to incremental advances in islet graft outcomes (4, 5). Progressive loss of islet graft function has been recognized after implantation in the intrahepatic site used clinically for the last three decades (2). This phenomenon seems to be common to autologous and allogeneic islets, and was described also in pre-clinical animal models of autologous islet transplantation (6).

An instant blood-mediated inflammatory reaction (IBMIR) occurring early after intra-portal islet infusion activates the coagulation cascade and the endothelium of the hepatic sinusoids, triggering adhesion of platelets and leukocytes, generation of thrombi and ischemia contributing to the loss of a conspicuous mass of transplanted tissue (7, 8). Nonspecific inflammation (innate immunity) generated at the time of transplant may heighten the intensity of subsequent adaptive immune responses that in organ transplantation are responsible for higher incidence of acute and chronic rejection episodes, and in type 1 diabetes mellitus (T1DM) promote also recurrence of autoimmunity. Accumulation of peri-insular fat in the liver of intrahepatic islet recipients has been described (9-14). Other disadvantages of the hepatic site include the relatively hyperglycemic environment and the elevated concentration of immunosuppressant (first-pass) that are toxic to islets.

Identification of a clinically relevant new 'home' for islet grafts will likely contribute achieving reproducibly successful biological replacement of beta-cell function in insulin-requiring diabetes. Definition of extra-hepatic transplantation sites has been recognized one of the current research priorities, and the focus of actively ongoing investigations (**Table 1**) aimed at identifying a microenvironment that could provide prompt engraftment, minimize early inflammation and islet cell death, while achieving sustained function.

Engraftment of islet grafts in several extra-hepatic sites and with or without bioengineering strategies has been demonstrated in experimental models (2, 15-17), although clinical translation for some remains arguable (**Table 1**)(18). An ideal new 'home' for islet grafts should accommodate relatively large volumes of tissue (*e.g.*, low purity, or pooled donor islet preparations, and/or re-transplantation), rely on minimally-invasive transplant procedures, allow for noninvasive longitudinal monitoring and easy access for biopsy. Portal blood drainage may be preferable to reproduce physiological metabolic responses. Confinement and irretrievability of the graft is desirable, particularly for bioengineering approaches to optimize the site.

Extra-hepatic sites already tested in humans include muscle (17, 19-21), and peritoneal cavity (to accommodate large microencapsulated islets) (22, 23). Clinical feasibility and

safety of intra-bone marrow (BM) islet transplantation (24) has also been recently proposed in patients with contraindications for intrahepatic islet transplantation.

Table 1. Islet transplantation sites

FEATURES	Tested clinically	Minimally-invasive implantation	Portal blood drainage	'Spacious'	Possibility of re-transplant	Site can be engineered	Easy graft implant procedure	Graft containment	Graft biopsy	Noninvasive monitoring	Immune privilege	Allows graft retrieval	Risk of islet exposure
Liver	✔	✔	✔	✔	✔	✗	✔	✗	Cumbersome (needle biopsy)	Metabolic	✗	✗	Portal hypertension, Portal thrombosis, Bleeding, IBMIR, Toxins, Drugs (first pass)
Omentum	✔	(Laparoscopy)	✔	✔	✔	✔	✔ (surgery)	✔	Invasive	Metabolic,Imaging (?)	✗	✔	
Peritoneum	✔	✔		✔	✔	Maybe	✔	✗	Cumbersome	Metabolic	✗	✗	Low oxygen (pelletting)
Muscle	✔	✔	✗	(Multiple sites)	(Multiple sites)	✔	✔	✔	Easy	Metabolic, Imaging	✗	✔	Temperature, Trauma
Subutaneous	✔	✔	✗	✔	(Multiple sites)	✔	✔	✔	Easy	Metabolic, Imaging	✗	✔	Temperature, Trauma
Bone Marrow	✔	✔	✗	✔	Maybe	Maybe	✔	✔	Easy	Metabolic, Imaging	✗	✗	
Pancreas		✔ (Laparoscopy)	✔	✗	✗	Maybe		✔	Cumbersome	Metabolic,Imaging (?)	✗	✗	Pancreatitis?
Gut submucosa	✔	Endoscopy, Laparoscopy	✔	✔	(Multiple sites)	Maybe	✔	✔	(Endoscopy)	Metabolic,Imaging (?)	✗	✔	
Gut segment		✗	✔	✔	(Multiple sites)	✔	✔ (surgery)	✔	Invasive	Metabolic,Imaging (?)	✗	✔	
Vascular segment		✗ Endovascular (?)	✗	Maybe	✗	✔	✔	✔	Invasive	Metabolic,Imaging (?)	✗	✔	
G.U. tract		✗ (Laparoscopy)	✗			Maybe		✔	Invasive	Metabolic,Imaging (?)	✗	✗	
Testis (male individuals)			✗					✔	✔	Metabolic,Imaging (?)	✔	✗	
Thymus		✗	✗					✔	Cumbersome	Metabolic,Imaging (?)	✔	✗	
Spinal fluid		✗	✗					✗	Cumbersome, Liquor tap	Metabolic	✔	✗	
Lungs	✔	✔	✗	Maybe				?	Cumbersome, Endoscopy	Metabolic,Imaging (?)	✗	✗	Pulmonary hypertension, Pulmonary thrombosis

Implantation of islets into the omentum is appealing because the site offers a large, well-vascularized surface that could be used to distribute an islet graft (18). Furthermore, the omentum has portal blood drainage, which may allow for the achievement of more physiological, metabolic responses when compared to other extra-hepatic sites shown to associate with hyperinsulinemia, insulin resistance and impairment of insulin action, none of which observed with intra-omental islet grafts (25, 26).

The omentum develops as a loose mesothelial sheet of tissue from the yolk sac. The greater omentum develops in the eighth week of gestation from the dorsal mesogastrium (27). It is composed of two mesothelial sheets enclosing predominantly adipocytes embedded in a loose connective tissue, and also aggregates of mononuclear phagocytic cells. The omentum has a rich vascular supply with numerous characteristic capillary convolutions (*a.k.a*., omental glomeruli due to their similarity to renal glomeruli). These capillary beds lie directly under the mesothelium (28). The size of the human omentum varies from 300 to 2000 grams with a surface area of 300 to 1500 cm^2 (29).

The omentum has been utilized in different experimental models of islet transplantation over the last 20 years. Yasunami *et al*. first reported the successful reversal of experimental diabetes in rats after implantation of islet isografts into a peritoneal omental pouch constructed by encasing the omentum in a pouch formed from a strip of parietal peritoneum obtained from the recipient; normoglycemia was maintained in the recipients until removal of the pouch that resulted in a rapid return to hyperglycemia (30). The suitability of the omentum for islet transplantation in large animal model was first reported by Simeonovic *et al*. in pigs (31) and then extended by Ao *et al*. to the canine model with titration of mass in an allogeneic combination, though suggesting the need for higher numbers when compared to intrasplenic implantation (32, 33). More recently, the omental site has been explored as a site for bioengineering approaches aimed at enhancing islet transplant engraftment and survival (34, 35).

An interesting approach to intra-omental islet transplantation was presented by Hefty *et al*. in a canine model of total and partial pancreatectomy (36). The autologous grafts were prepared in a three-dimensional (3D) matrix consisting of autologous plasma supplemented with Vascular Endothelial Growth Factor (VEGF) that was placed in and rolled-up with the greater omentum to have omental layers on both sides of the matrix (36). Another recent study by Jacobs-Tulleneers-Thevissen *et al*. in immunodeficient rats (nude rats) showed that human islet cell implants survive better in the omentum than intra-hepatically, with positive influences of the number and purity of implanted beta cells (37). Similarly, Bartholomeus *et al*. demonstrated that perinatal porcine islet cell grafts display comparable growth of the β-cell volume over time when comparing the omentum and the kidney subcapsular space; but that the omentum leads to higher insulin reserves and an increased pool of proliferating cells, which might be related to a more extended vascular network (38).

Topical use of thrombin has become part of current medical practice as a means to attain hemostasis and prevent bleeding during various surgical procedures (39-41). The development of recombinant human thrombin lead to standardization of this product, overcoming limitations imposed by previous formulations of this product such as highly purified bovine thrombin and human plasma-derived thrombin, both of which were costly to manufacture. In addition, bovine thrombin carries the risk of immunogenicity,

potentially resulting in formation of cross-reactive antibodies to bovine thrombin, factor V, and other impurities that may be present in these formulations, and potential bleeding and thromboembolic events (40).

In this study we propose to use RECOTHROM ® (thrombin alfa, ZymoGenetics, Inc.), developed via recombinant DNA technology, identical in amino acid sequence and structurally similar to naturally occurring human thrombin. The cell line used to manufacture RECOTHROM® has been extensively tested and shown to be free of known infectious agents. It is not derived from animal or human blood, and contains no added human or animal components. Thus, RECOTHROM ® is inherently free from the risk of transmission of human- borne pathogens, such as HIV, hepatitis, parvovirus, and the infectious agents associated with Creutzfeldt-Jakob Disease (CJD) and variant CJD. RECOTHROM ® has low immunogenicity with a significantly lower rate of specific anti-product antibody formation than treatment with bovine thrombin. After surgery, only 1.5 per cent of RECOTHROM ® patients developed antibodies (42). It could be used safely in patients with pre-existing anti-thrombin antibodies to bovine thrombin preparations (43). RECOTHROM ® is easy to prepare and use. It is supplied as a lyophilized powder and sterile diluent for reconstitution in single use, preservative-free vials of 5,000 IU or 20,000 IU. It requires no refrigeration and can be stored in the operating room. When reconstituted, it can be kept up to 24 hours for lengthy surgical procedures. According to a recent clinical study, RECOTHROM ® was found to successfully stop 80 per cent of surgical bleeding within three minutes and 95 per cent of bleeding within 10 minutes (42). RECOTHROM ® is intended for topical use, and it is generally applied directly to the bleeding site, or used in conjunction with a compatible absorbable gelatin sponges to promote hemostasis, with clot formation occurring shortly after reconstituted RECOTHROM ® solution comes into contact with blood.

For the purposes of this study we propose to use RECOTHROM® to promote clot formation in autologous plasma used to re-suspend allogeneic islet cells for the implantation onto the omentum. Hence, we intend to use RECOTHROM® as for the approved clinical use. The data from the experimental studies conducted in small and large animals models confirm that the additional of RECOTHROM® to islet cells re--suspended in autologous plasma and placed in the omentum results in prompt gel (clot) formation and adherence of the islets to the surface of the omentum, desirable to promote engraftment. The latter is achieved through increased surface exposure and circumventing pelleting, which may result in reduced oxygen diffusion, and competition for nutrients and accumulation of metabolism bi-products.

1.2 Study Rationale

Current islet transplantation into the portal vein of the liver has shown the unique ability of islets to stabilize blood glucose levels and prevent severe hypoglycemia in a selected group of subjects with Type 1 diabetes. The main limitations of islet transplantation are the need for systemic immunosuppression to maintain function and the loss of islet function over time. Additionally, many studies have demonstrated that the current site of transplantation in the liver is not an ideal site due to several factors. These factors include (1) significant liver inflammation following islet infusion; (2) potential for life-threatening procedure-related complications such as bleeding and thrombosis; (3) high levels of

immunosuppressive drugs and GI toxins in the liver contributing to islet toxicity; (4) the inability to retrieve islets after infusion; and (5) development of graft dysfunction in a number of recipients of intrahepatic allogeneic and autologous islets.

Based on these premises, development of a clinical protocol for the implantation of islets into the omentum is a desirable goal. As an attempt to maximize the engraftment of islet cell clusters onto the omentum, implantation site should promote islet adherence to the omental peritoneal layer and avoid cell pelleting. We have recently performed a series of experiments in animal models of diabetes to assess the feasibility of transplanting pancreatic islets in the omentum using a plasma-thrombin gel. With our approach, the islets are re-suspended in either donor or autologous plasma and distributed onto the omentum. Cell adherence is achieved by addition of clinical-grade recombinant human thrombin that reacts with plasma to create a biocompatible, degradable gel containing the islet graft. Preliminary data obtained as a result of these studies demonstrate the feasibility of our approach, and are described in Section 8 of this document.

We have outlined our initial patient trial as 6 subjects, based on clinical judgment and extensive experience in clinical islet transplantation trials. If initial safety and efficacy is satisfactory (no adverse events related to the transplantation and efficacy in two of the three first transplanted subjects), we will transplant 3 additional subjects.

Following evaluation of the one year post transplant data for the first transplanted subject we have observed a decline in beta cell function compared to the 6 months data and similar to findings reported in recipients of intrahepatic islet allografts (114). Possible factors responsible for this loss/decline in graft function include immunological activation (i.e., allo- and/or autoimmune rejection), non-specific inflammatory responses and islet graft exhaustion due to metabolic demand in the setting of a marginal mass. In addition, factors inherent to this novel transplantation site may have also contributed to the observed decrease in beta-cell function.

In order to preserve long-term islet allograft function the introduction of adjuvant therapies starting in the peri-transplant period may be required to minimize islet graft loss, sustain beta-cell health and decrease burden of metabolic demand on islet allograft.

We have shown that in the intrahepatic site the use of GLP-1 RA therapies improve and prolong islet allograft function (115-118) for over 10 years. We have also recently reported that combination of GLP-1 RA therapies and granulocyte colony stimulation factor (G-CSF) leads to prolonged islet allograft survival (119). These therapies are aimed at improving metabolic function and allowing for an immunoregulatory repertoire that may promote a tolerogenic environment.

It is known that inflammatory responses account for substantial beta-cell loss early after transplantation. Notably, TNF-alpha has been associated with islet graft loss. Farney *et al.* described a beneficial role of etanercept, a TNF-alpha blocker, in promoting engraftment of marginal mass islet grafts in mice (91). Hering *et al.* used etanercept in a recent trial of 8 type 1 diabetic patients receiving single donor islet transplant, and all 8 achieved insulin independence suggesting a beneficial role for anti-TNF therapy in

clinical islet transplantation (92). Although experience with anti-TNF alpha therapies in clinical and experimental islet transplantation has been limited, the introduction of TNF-alpha blocking therapies has led to improved long term outcomes likely related to improvement in engraftment due to minimization of early graft loss.

Traditionally we have used etanercept (Enbrel®) as a TNF-alpha blocker in islet transplantation (5). Recently, it has been shown that adalimumab (Humira®) in addition to blocking TNF-alpha promotes the development of T-regulatory cells (128, 129). Adalimumab has been shown to be clinically comparable and with a similar side effect profile to Enbrel as tumor necrosis factor blocker. Since adalimumab may also promote a tolerogenic environment we will use adalimumab instead of Enbrel as a TNF-alpha blocker.

However, currently there are no clinical trials that incorporate the use of anti-inflammatory therapies chronically in islet transplantation.

A safe, orally administered, anti-inflammatory intervention that has been extensively evaluated in the medical literature is the use of omega 3 fatty acids (120). The use of omega 3 fatty acids has been shown to reduce pro-inflammatory metabolites and increase anti-inflammatory cytokines. The omega 3 fatty acids derived bioactive mediators, resolvins and protectins, have been postulated as the molecular basis for the anti-inflammatory properties of omega 3 fatty acids (121).

Derivatives from omega 3 fatty acids [eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] have been associated with marked anti-inflammatory effects whereas arachidonic acid (AA), an omega 6 fatty acid, is the precursor of eicosanoids associated with pro-inflammatory properties. Thus, a high AA/EPA ratio would suggest that the balance is shifted towards a pro-inflammatory state. Treatment with omega-3 fatty acids aimed at lowering the AA/EPA ratio may then prove to be beneficial particularly in diseases associated with a high inflammatory state.

Taking into account the first transplant experience under this protocol as well as additional pre-clinical data in small and large animal models, it is clear that adjuvant therapies are likely required to improve long-term islet allograft survival and thus we will incorporate the following interventions to be started in the peri-transplant period:

a. Pegylated G-CSF (Neulasta®; Pegfilgrastim)
b. Exenatide extended-release (Bydureon®)
c. Ultra-refined omega-3 EPA/DHA concentrate (ZoneLabs® OmegaRx®2)
d. Supplemental oxygen therapy via nasal cannula

Pegylated G-CSF (Neulasta®; Pegfilgrastim)

G-CSF, an agent well known to help mobilize hematopoietic precursors from the bone marrow, has been used clinically for nearly 20 years to help repopulate peripheral cell

counts in patients undergoing cancer therapy. However, a rapidly growing body of experimental and clinical evidence suggests that G-CSF has the potential to be used in the treatment of autoimmune diseases. G-CSF has been shown to favor the differentiation and mobilization of Treg cells, induce tolerogenic DC, and alter the balance between proinflammatory and anti-inflammatory soluble mediators both animals and in humans Specifically, G-CSF can mobilize functional bone marrow CD4+CD25+FoxP3+ Treg cells. Preclinical models of G-CSF-induced inhibition of autoimmune and allogeneic T cell responses have demonstrated the potential efficacy of G-CSF in treating Crohn's disease, myasthenia gravis, and T1D (122).

Pegylated G-CSF is a covalent conjugate of recombinant methionyl human granulocyte colony stimulating factor (filgrastim) and monomethoxypolyethylene glycol. Neulasta® is a Colony Stimulating Factor that acts on hematopoietic cells by binding to specific cell surface receptors, thereby stimulating proliferation, differentiation, commitment, and end cell functional activation. Neulasta® is supplied in 0.6 mL prefilled syringes for subcutaneous injection. Each syringe contains 6 mg pegfilgrastim (based on protein weight), in a sterile, clear, colorless, preservative-free solution (pH 4.0) containing acetate (0.35 mg), sorbitol (30.0 mg), polysorbate 20 (0.02 mg), and sodium (0.02 mg) in water for injection, USP.

Exenatide extended-release (Bydureon®)

The naturally occurring form of exenatide (exendin-4) was originally isolated from the salivary secretions of the lizard Heloderma Suspectum (Gila monster). Exendin-4 has a 53% amino acid sequence overlap with mammalian glucagon-like peptide-1 (GLP-1). In mammals, GLP-1 is processed from the proglucagon gene in L-cells in the small intestine. Exendin-4 is transcribed from a distinct gene, not from the Gila monster homologue of the mammalian proglucagon gene, from which GLP-1 is expressed. In mammals, exendin-4 is resistant to degradation by dipeptidyl peptidase-IV (DPP-IV) and has a much longer plasma half-life than GLP-1, which is degraded by DPP-IV with a half-life of less than 2 min. Exendin-4 is not an analogue of GLP-1. In other words, the structure of the synthetic exendin-4 peptide (exenatide) was not created by sequential modification of the structure of GLP-1. However, exendin-4 and GLP-1 share many glucoregulatory actions, which may be mediated by the known pancreatic GLP-1 receptor; they enhance glucose-stimulated insulin secretion and inhibit glucagon secretion. (123-125).

A summary of the effects of GLP-1 are:

- Insulinotropic effects, which are glucose dependent (potentiates glucose action with no effect on insulin secretion at glucose <4.5mM); this contrasts with the action of other insulin secretagogues agents, such as sulfonylureas, which increase insulin secretion regardless of glucose concentrations; GLP-1 is therefore unlikely to cause lasting and profound hypoglycemia. GLP-1 enhances insulin secretion through the regulation of ion channels (including ATP-sensitive K+ channels, voltage-dependent Ca^{2}+ channels, voltage-dependent K+ channels, and nonselective cation channels), of intracellular energy homeostasis, and exocytosis Moreover, the expression of genes that are essential for beta cell function, such as glucokinase and GLUT 2, are up- regulated following GLP-1 treatment

• Stimulation of all steps of insulin biosynthesis, as well as insulin gene transcription, thereby providing continued supplies of insulin for secretion. Effects on insulin gene transcription are proposed to occur via the up-regulation of the transcription factor pancreatic duodenal homeobox-1 (PDX-1)
• Improvement of insulin sensitivity and glucose uptake, which was reported in human and rat adipose tissue and skeletal muscle. Several studies suggest that GLP-1 may directly enhance glucose disposal in an insulin-independent fashion, although this may also result from the overall inhibition of glucagon secretion
• Stimulation of beta-cell proliferation, and enhancement of the differentiation of new beta cells from progenitor cells in the pancreatic duct epithelium. A proliferative action of GLP-1 on insulin secreting cells has been shown both in vitro and in vivo models.
• Inhibition of apoptosis induced by cytokines or free fatty acids.). Studies have shown that GLP-1 added to freshly isolated human islets preserves morphology, function and inhibits cell apoptosis. (126) Furthermore, the ability of GLP-1 to inhibit apoptosis is relevant to the islet transplantation procedure. Treatment of islets with GLP-1 or its analog could potentially enhance islet graft survival through inhibition of apoptosis and stimulation of proliferation, thus decreasing the requirements for a large islet cell mass
• Anti-inflammatory properties, which have been documented in several studies (). Our own studies showed that exenatide has anti- inflammatory properties that protect human islets in vitro (127). Exenatide treatment improved islet function, significantly reduced content of inflammation-related molecules (tissue factor, IFN-γ, IL-17, IL-1β, and IL-2) and caspase 3 activation, whereas increased phosphorylation of ERK1/2, STAT3, and Akt in vitro. Immunostaining showed expression of GLP-1R in β-cells but not in α-cells. IL-1β colocalized with GLP-1R in β-cells. Induction of serine proteinase inhibitor 9 (PI-9) was detected after exposure of human islets to exenatide in vitro, and after transplantation into immunodeficient mice. These anti- inflammatory and cytoprotective properties of exenatide could be beneficial to protect the transplanted pancreatic human islets.

Ultra-refined omega-3 EPA/DHA concentrate (ZoneLabs® OmegaRx®2)

A safe, orally administered, anti-inflammatory intervention that has been extensively evaluated in the medical literature is the use of omega 3 fatty acids (120). The use of omega 3 fatty acids has been shown to reduce pro-inflammatory metabolites and increase anti-inflammatory cytokines. The omega 3 fatty acids derived bioactive mediators, resolvins and protectins, have been postulated as the molecular basis for the anti-inflammatory properties of omega 3 fatty acids (121).

Derivatives from omega 3 fatty acids [eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] have been associated with marked anti-inflammatory effects whereas arachidonic acid (AA), an omega 6 fatty acid, is the precursor of eicosanoids associated with pro-inflammatory properties. Thus, a high AA/EPA ratio would suggest that the balance is shifted towards a pro-inflammatory state. Treatment with omega-3 fatty acids aimed at lowering the AA/EPA ratio may then prove to be beneficial particularly in diseases associated with a high inflammatory state.

Some fish may contain potentially harmful contaminants, such as heavy metals (including mercury), dioxins, and polychlorinated biphenyls (PCBs). In order to minimize contaminats we will use ultra-refined Omega-3 ***(ZoneLabs® OmegaRx®2***).

Supplemental Oxygen Therapy.

a. Adequate oxygenation at implantation of the islet allograft is critical for beta cell survival. We hypothesize that oxygen supplementation in the peri-transplant period may result in increased oxygen delivery at the transplant site allowing for improved engraftment and allograft survival. Subjects will be started after islet transplantation on supplemental oxygen by nasal cannula at 4L/min (to provide FiO2 of 36%) which will be continued throughout the hospitalization, and up to 7 days post hospital discharge.

2 Study Objectives and Endpoints

2.1 Primary Objectives

Safety: To demonstrate the safety of islet transplantation onto the omentum for the treatment of subjects with Type 1 Diabetes (T1D).

2.2 Secondary Objectives Efficacy: To demonstrate the efficacy of islet transplantation onto the omentum for the treatment of T1D in subjects with hypoglycemia unawareness and a history of **severe hypoglycemic** episodes.

2.3 Primary endpoints

The primary safety endpoint is to demonstrate patient safety throughout all stages of the trial.

The primary efficacy endpoint is the proportion of subjects with HbA1c ≤6.5% at 1 year AND free of severe hypoglycemic events from Day 28 to Day 365, inclusive, after the islet transplant.

2.4 Secondary endpoints

Secondary Efficacy Endpoints

At 75±7, 365 ± 14 ,and 730 ± 14 days following the islet transplant(s): the percent reduction in insulin requirements; HbA1c; LI; Ryan hypoglycemia severity (HYPO) score; Clarke score; number of severe hypoglycemic episodes; basal (fasting) and 90-min glucose and c-peptide derived from the mixed-meal tolerance test (MMTT); β-score; C-

peptide/glucose creatinine ratio; glucose variability and hypoglycemia duration derived from the continuous glucose monitoring (CGM); and Quality of life (QOL) measures (EQ-5D, Hypoglycemia Fear Survey-HFS, SF-36v2, Diabetes Distress scale).

Secondary Safety Endpoints

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

At 75 ± 7 and 365 ± 14 days following the islet transplant, and at two years following the final islet transplant: the incidence and severity of AEs related to the islet transplant procedure including: bleeding (>2 g/dL decrease in hemoglobin concentration); wound complication (infection or subsequent hernia); torsion of omentum; gastrointestinal obstruction; abscess; cysts; need for surgical intervention. The incidence and severity of AEs related to the immunosuppression including: allergy; reduction in GFR; addition or intensification of antihyperlipidemic therapy; gastrointestinal toxicity; neutropenia, anemia, or thrombocytopenia; viral, bacterial, or fungal infections; and benign or malignant neoplasms. The incidence of immune sensitization defined by presence of anti-HLA antibodies absent prior to transplantation. The incidence of discontinuation of immunosuppression.

2.5 Statistical considerations and analytical plan

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

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

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

Incidence of serious adverse experiences will be tabulated by body system and graded by Terminology Criteria for Adverse Events (TCAE).

Life table methods will be used to estimate mortality rates.

2.5.1 Baseline Characteristics and Demographics

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

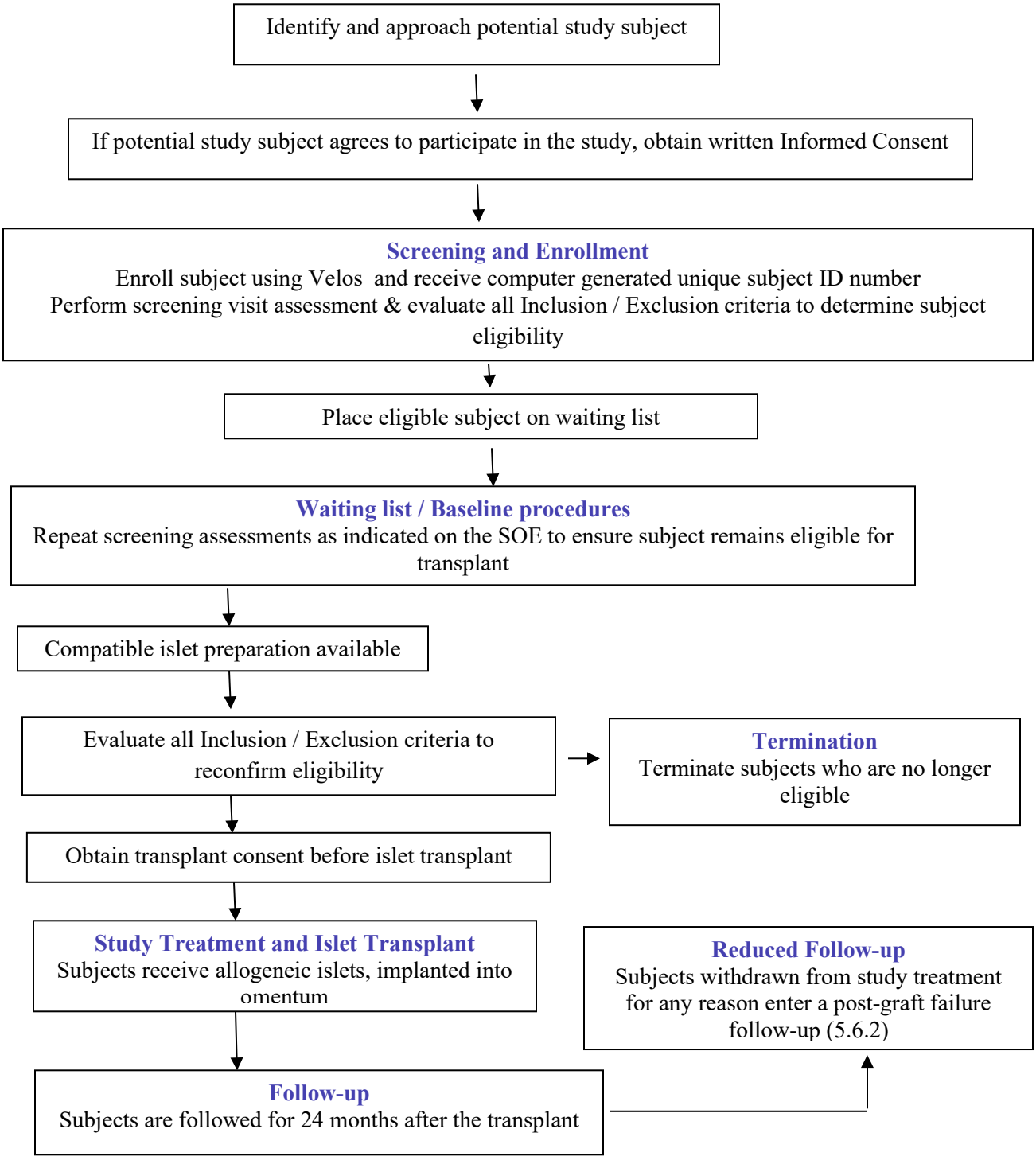
- Continuous data (i.e., age, body weight, and height) will be summarized descriptively by mean, standard deviation, median, and range.

- Categorical data (i.e., sex and race) will be presented as enumerations and percentages.

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

3 STUDY DESIGN

Figure 1: Study Design Diagram



3.1 Patient Population

This trial is an open label, single-arm, Phase I/II, single center trial.

Potential participants will be initially identified using pre-screening tools as approved by the IRB. Candidates who meet the general inclusion/exclusion criteria will be consented, enrolled, and assigned a unique subject identification number. Subjects will then be more thoroughly evaluated for eligibility as part of the protocol-specific screening visit procedure. Recruitment at the University of Miami will continue until all subjects [N=6] needed for the trial are identified, appropriately consented, screened and transplanted.

3.1.1 Placement of Islets into the Omentum

The central hypothesis of this proposal is that the transplantation of islets in a more favorable alternative site, *i.e*. omentum, will result in similar islet engraftment (and therefore similar or higher rates of insulin dose decrease and similar or better metabolic outcomes) than conventional islet transplants in the liver. In the last 7 intraportal islet infusions, there was an insulin reduction of 57 ± 16% reduction of insulin use (range 32 to78%), basal c-peptide 0.85 ± 0.35 ng/ml (range 0.4 to 1.48 ng/ml), and 90 minute C-peptide 1.89 ±1.19 ng/ml (range 0.8 to 4.3 ng/ml) at 75 days after a single islet infusion. Immunosuppression regimen will be similar to that used in the CIT consortium trials (44). In order to avoid side effects related to Sirolimus (e.g. impairment of wound healing), we will use Tacrolimus and Mycophenolate Mofetil (MMF) as maintenance immunosuppression. This protocol has proven to be successful in recent islet clinical trials conducted by the Edmonton group (45, 46).

3.2 Inclusion Criteria

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

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

7. At least one episode of severe hypoglycemia in the 12 months prior to study enrollment.
8. Reduced awareness of hypoglycemia as defined by a Clarke score of 4 or more

OR

A HYPO score greater than or equal to the 90th percentile (1047) during the screening period;

OR

Marked glycemic lability characterized by wide swings in BG despite optimal diabetes therapy and defined by a lability index (LI) score greater than or equal to the 90th percentile (433 mmol/L2/h·wk-1) during the screening period;

OR

A composite of a Clarke score of3 or less and a HYPO score greater than or equal to the 75th percentile (423) and a LI greater than or equal to the 75th percentile (329) during the screening period.

3.3 Exclusion Criteria

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

1. Body Mass Index (BMI) >30 kg/m^2 or patient weight ≤50 kg.
2. Insulin requirement of >1.0 IU/kg/day or <15 U/day.
3. HbA1c >10%.
4. Untreated proliferative diabetic retinopathy.
5. Blood Pressure: SBP >160 mmHg or DBP >100 mmHg.
6. Measured glomerular filtration rate <80 mL/min/1.73 m^2 calculated using the subject's measured serum creatinine and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation1 or Modification of Diet in Renal Disease [MDRD] study estimation formula). Strict vegetarians (vegans) with a calculated GFR <70 mL/min/1.73m^2 are excluded. The absolute (raw) GFR value will be used for subjects with body surface areas >1.73m^2
7. Presence or history of macroalbuminuria (>300mg/g creatinine).
8. Presence or history of panel-reactive anti-HLA antibodies above background by flow cytometry.
9. For female subjects: Serum or urine Positive pregnancy test, presently breast-feeding, or unwillingness to use effective contraceptive measures for the duration of the study and 4 months after discontinuation. For male subjects: intent to procreate during the duration of the study or within 4 months after discontinuation or unwillingness to use effective measures of contraception. If sexually active, subject must use at least two medically accepted methods of birth control from the following list: oral contraceptives, Norplant®, Depo-Provera®, intrauterine device (IUD), barrier devices with spermicide. Condoms used alone are not acceptable. Instead of the male condom, it is acceptable to use a female condom with one of the other methods for women listed above.

10. Presence or history of active infection including hepatitis B, hepatitis C, HIV, or tuberculosis (TB) Subjects with laboratory evidence of active infection are excluded even in the absence of clinical evidence of active infection.
11. Negative screen for Epstein-Barr Virus (EBV) by IgG determination.
12. Invasive aspergillus, histoplasmosis, and coccidioidomycosis infection within one year prior to study enrollment.
13. Any history of malignancy except for completely resected squamous or basal cell carcinoma of the skin.
14. Known active alcohol or substance abuse.
15. Baseline Hb below the lower limits of normal at the local laboratory; lymphopenia (<1,000/µL), neutropenia (<1,500/µL), or thrombocytopenia (platelets <100,000/µL). Participants with lymphopenia are allowed if the investigator determines there is no additional risk and obtain clearance from a hematologist.
16. A history of Factor V deficiency.
17. Any coagulopathy or medical condition requiring long-term anticoagulant therapy (*e.g*., warfarin) after transplantation (low-dose aspirin treatment is allowed) or patients with an international normalized ratio (INR) >1.5.
18. Severe co-existing cardiac disease, characterized by ***any one*** of these conditions:

a.) recent myocardial infarction (within past 6 months).
b.) evidence of ischemia on functional cardiac exam within the last year.
c.) left ventricular ejection fraction <30%.

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

28. A previous pancreas transplant, unless the graft failed within the first week due to thrombosis, followed by pancreatectomy and the transplant occurred more than 6 months prior to enrollment.
29. Inflammatory bowel disease.
30. History of intestinal obstructions.
31. Previous major abdominal surgery.
32. History of peritonitis.

4 TREATMENT OF PATIENTS

4.1 Study Therapy and Dosages

4.1.1 Study Investigational Therapy: Isolation of Allogeneic Islet Cells

Pancreas is removed from the shipping container with UW solution and placed in a tray with Trimming Solution with Cefazolin (a sample of UW is collected for microbiological analysis), placed in a larger tray with 1 L of cold sterile ice slush. The organ is trimmed of fat and non-pancreatic tissue, and weighed.

Trimming Solution is poured off, the tray with pancreas is removed from ice, and the organ is rinsed with HBSS. The pancreatic duct is cannulated with a 16-18 gauge angiocatheter, and the pancreas is distended with cold collagenase solution (Liberase MTF and Thermolysin dissolved in water, supplemented with HEPES, Calcium Chloride, NaOH, DNAse). The distended organ is placed inside a digestion chamber (47-49); additional collagenase solution is added, re-circulated and brought to 37ºC, while chamber is agitated (47-49). During the digestion samples are collected at regular intervals to monitor the breakdown of the pancreas, via microscopy. When sufficient number of free islets are detected by DTZ staining, digestion is stopped, the heat is switched off, and digest is collected (while collagenase is diluted) in cold RPMI-1640 (with 5-1.5% HSA, DNAse, 25 mM HEPES, 10 U/ml Heparin, 0.2 U/ml insulin) to quickly cool the digest. The digest is centrifuged and washed in Wash Solution (Cold Storage / Purification Stock Solution, 10% PentaStarch, 0.625% HSA, 10 U/ml Heparin, 0.2 U/ml Insulin). Digest volume is recorded, and re-suspended in the final volume of 200 ml of CIT Purification Solution (Cold Storage / Purification Stock Solution, 10% PentaStarch, 10 U/ml Heparin). Islets are separated from exocrine tissue using continuous and modified continuous density gradient centrifugation, in a COBE 2991 blood cell processor (47-51).

Continuous gradients are composed of Purification Solution mixed with OptiPrep, 10% PentaStarch and gradient Stock Solution to achieve densities of 1.100 gm/ml and 1.065gm/ml. 20-30 ml of pancreatic digest is re-suspended in 100 ml total volume of Purification Solution, and incubated in the cold, for 30-45 minutes. High density gradient is topped off with a continuous density gradient made from the 1.100 and 1.077 or 1.6065 density gradients, loaded while the COBE 2991 is spinning at a speed of 2,000 rpm. At this point, the digest is loaded and centrifuged 3-5 minutes. Purified islets are collected in fractions, the purity of which is assessed with DTZ. Islet fractions with purities >30% are saved and washed with CMRL-1066. Similar purity fractions are then combined for culture.

Modified continuous purification is used as a rescue purification method. Light (1.062 gm/ml) and heavy (1.073 gm/ml) modified continuous density gradients are achieved by adding different volumes of 1.1gm/ml Biocoll to UW. Up to ~45 ml of digest is re-suspended in 150 ml of UW and incubated for ~30 minutes. Biocoll 1.1 gm/ml is loaded first, followed by heavy, 1.073 gm/ml, and light, 1.062 gm/ml, density gradients, followed by the digest. Digest is centrifuged for 3 minutes at 3000 rpm, islets are collected in the CMRL-1066, and their purity is assessed. Fractions of similar purity are cultured together.

Islets are cultured 36-72 hours, in 95% air, and 5% CO_2, at 37°C, in CMRL 1066, Supplemented (0.5% HSA, 10 U/ml Heparin and IGF-1), at a concentration of ~20,000 IEQ/30 ml media, in 175cm^2 tissue culture flasks. Islets with purity ≥70% are cultured at 37°C for the first 12-24 hours, and at 22°C thereafter; islets with purity ≤70% are cultured at 22°C. In preparation for implantation onto the Omentum, islets are collected from the tissue culture flasks, re-suspended in Tx media (CMRL 1066, 2.5% HSA, 25 mM Hepes), washed and settled by gravity to remove cellular debris. Final assessment samples are collected at this point. The final settled islet cell pellet is transferred to a 10 or 20 ml luer-lock syringe, depending on the settled tissue volume.

4.1.2 Study Investigational Therapy: Administration

This is a single procedure protocol. Only a single islet transplant will be performed in the patient. Islets are isolated from a single pancreas donor. A minimum of 5000 IEQ/KG will be transplanted. A single dose of at least 5000 IEQ/KG should be able to achieve a meaningful metabolic improvement and prevention of severe hypoglycemia, as previously seen in our experience with intraportal islet transplants.

Allogeneic islet cells will be isolated as described in section 4.1.1of this document (see above) and cultured for 36-72 hours. At the time of transplant, islets will be collected in a sterile conical tube, and settled by gravity. The final settled islet cell pellet in Tx media (CMRL 1066, 2.5% HSA, 25 mM Hepes) will be transferred to a 10 or 20 ml luer-lock syringe, depending on the settled tissue volume. The final settled islet cell pellet is transported to the Operating Room (OR) in the final container (10-20 ml luer-lock syringe), washed in autologous plasma, settled again, and re-suspended in 2:1 ratio of settled islet cell pellet to autologous plasma for implantation in the omentum. The final islet product is a sterile suspension of ≥70% viable, ≥30% pure, allogeneic islets. The final product contains a single dose of 5,000 or more Islet Equivalents (IEQ)/kg recipient body weight (BW).

At the time of transplant and under general anesthesia, through a laparoscopic approach and/or mini laparotomy the islets will be delivered and distributed on the surface of the omentum using a 12 Fr. Gastrostomy catheter (Cook Medical). RECOTHROM® will be added to promote formation of a gel clot (as needed to cover the graft surface, approximately in a volume equal to that of the graft implanted) to promote islet cells adhering to the surface of the omentum, and avoid cell pelleting.

An omental pouch will be created by wrapping the islets with an omental flap. Each procedure is projected to take approximately 1-2 hours. Recombinant human thrombin

is added to the islets placed on the omentum to promote formation of a gel clot and facilitate adherence to the surface of the omentum. Only in the case of a surgical complication a secondary procedure will be performed if needed. We have included in the exclusion criteria possible scenarios that may require future surgeries or subjects with previous abdominal surgeries, inflammatory bowel disease, and history of bowel obstruction.

4.1.3 Dose Rationale

This study is not a dose escalation study. A single dose of 5,000 or more IEQ/kg recipient body weight (BW) will be transplanted.

There is no method to assess islet survival as a fraction of surviving cells/total islets transplanted. Once the patient is stabilized (in 6 – 8 weeks), however, it is possible to monitor islet mass via FSIGT. FSIGT allows for measurement of acute insulin release in response to the injected glucose (38, 48).

Our extensive past experience with islet transplantation has proven that a dose of ≥5,000 IEQ/kg recipient body weight (BW) has proven to be safe, and demonstrated to result in long term improvement of metabolic control, and reduction of hypoglycemic unawareness associated with intensive insulin therapy (52-63).

Although this study is a single dose protocol, islet transplant recipients with ***partial islet graft function*** will be considered for a second islet transplant (intra-hepatic administration) if they:

a. do not achieve primary efficacy endpoint criteria at 1 year, or
b. have evidence of a progressive decline in stimulated C-peptide during a MMTT resulting in either reintroduction of insulin therapy or increase in insulin requirements, or
c. experience severe hypoglycemia from Day 28 to Day 365.

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

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

7. Absence of any medical condition that, in the opinion of the investigator, will interfere with a safe and successful second islet transplant.

If a participant is deemed eligible for an intrahepatic islet infusion, the participant will continue to be followed according to the 2nd year schedule of events until intrahepatic islet transplantation is performed at which time follow up will reset and continue for 2 years as per protocol.

D-dimer testing and samples for unmethylated insulin DNA (Benaroya collaboration) are not required to be obtained for intrahepatic islet transplants under this protocol.

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

Islet transplant recipients with ***partial islet graft function*****,** i.e. subjects who do not achieve primary endpoint criteria at 1 year follow-up, will be eligible to receive a second islet transplant via intra-hepatic administration. With the exception of performing induction with basiliximab (Simulect®) instead of anti-thymocyte globulin (ATG, Thymoglobulin®), the regimen will be identical to the initial islet transplant. The transplant will be done by a radiologist in the Special Procedures section of the Radiology department, under local anesthesia and conscious sedation. Islets will be infused under sterile conditions over 20-30 min. by gravity drainage. Gel foam plugs and/or collagen/thrombin paste will be used to embolize the entire peripheral catheter tract immediately before the catheter is withdrawn, to reduce the chances of bleeding. A minimum of 5,000 IEQ/kg recipient body weight (BW) will be transplanted to maximize possibility of insulin independence.

In case the PI decides it is not safe for the subject to have the second transplant through intrahepatic infusion (in radiology), he can offer a second infusion through a mini-laparotomy (in the operating room).

The mini-laparotomy is done under general anesthesia. A small incision (about 2-3 inches) is done on the abdominal wall. The surgeon will do a catheterization of the mesenteric vein toward the portal vein. The study team will then infuse the islets into the portal system. Islets will be delivered from the Ricordi bag through catheter into your liver over about 30±25 minutes. The portal vein blood pressure will be checked often to make sure it does not critically increase. After the islets are injected, the catheter will be removed and the vein it was in will be tied off so it does not bleed. The incision will be closed with stitches, and a sterile dressing will be put over the incision.

4.1.4 Dosage and Dosing

The study team will maintain detailed records as to the cell dose each patient enrolled in this study will receive. Allogeneic islet cells will be placed in the omentum, under general anesthesia, by a mini laparotomy and/or laparoscopic approach. During the procedure, patients will be monitored followed established standard of care. The

procedure will be terminated if the patient becomes unstable.

4.1.5 Storage and Handling of Study Investigational Therapy

Study therapy (allogeneic islet cells) will be dispensed only after a patient has provided a written informed consent, met all eligibility criteria for entry into the study, and completed all the necessary evaluations. As the cells are intended for administration immediately following preparation, they will be destroyed should they're not used right away.

4.1.6 Study Investigational Therapy Accountability Procedures

The Principle Investigator (PI) is responsible for the study investigational therapy accountability, reconciliation, and record maintenance. In accordance with all applicable regulatory requirements, the PI or the cGMP Cell Processing Facility (CPF) responsible for cell manufacture will maintain accountability records throughout the length of the study.

5 STUDY PROCEDURES

5.1 Study outline

- Prior to transplantation, the patient is screened, qualified, listed for transplanted, and signs the informed consent form.

- Subjects screening data from 20053135 protocol will be accepted for subjects eligible for this study. If 20053135 visit was 12 months prior to enrollment, Visit 2 laboratory should be repeated.

- At the time a suitable islet preparation becomes available, the patient will receive allogeneic islet cells transplanted onto the omentum. Islet transplant will be performed under rabbit Anti-Thymocyte Globulin (ATG, Thymoglobulin®) induction immunosuppression (5 doses, day -2 prior to transplant to day 2 post-transplant). Maintenance mycophenolate mofetil (MMF) therapy (1-2 g/day as BID dosing) will be started on Day –1 pre-transplant. Tacrolimus will be administered orally twice daily starting on Day 1 post-transplant to target a trough level of 10-12 ng/mL for the first 3 months, and 6-10 ng/mL thereafter. However, target drug levels will ultimately depend on clinical status, tolerability, and biochemical/immunological profiles as determined by the principal investigator.

  Adalimumab (Humira) will be given IV before the islet transplant (80 mg) and then 40 mg subcutaneously every 2 weeks after islet transplant for 2 doses.

5.2 Islet Transplant: Immunosuppression, Adjuvant Therapies and Concomitant Medications

The immunosuppressive agents used in this study are being administered for an indication other than the one for which they are currently approved and for use in combinations, which have not been formally studied. These medications have only been used for a short period of time and in a small number of subjects. In the islet transplant setting, they have been used in several islet alone and islet after kidney transplant trials. A number of known side effects can be experienced when taking these drugs. The study medications will be provided by study team upon to 2 years after the last islet infusion.

IMMUNOSUPPRESSION REGIMEN

- Induction immunosuppression will consist of five doses IV infusion of rabbit Anti-thymocyte Globulin (ATG, Thymoglobulin®), starting two days prior to the islet transplant. The doses will be administered as directed on the package insert.

  Considering that the presence of pre-formed rabbit antibodies may predict the occurrence of an immunologic response against Thymoglobulin®, participants will be tested during the screening visit for presence of antibodies to rabbit serum proteins and epithelium.

  Patients who have: a) a positive history of rabbit allergy/anaphylaxis, or b) positive IgE to rabbit serum proteins and/or epithelium, will not be eligible to undergo induction with Thymoglobulin® due to the increased risk for an anaphylactic reaction. These patients will be considered for induction with alemtuzumab (see below) once this drug becomes available for utilization under this protocol.

- Maintenance mycophenolate mofetil (MMF) therapy (1-2 g/day as BID dosing) will be started on Day –1 pre-transplant. Tacrolimus will be administered orally twice daily starting on Day 1 post-transplant to target a trough level of 10-12 ng/mL for the first 3 months, and 6-10 ng/mL thereafter. However, target drug levels will ultimately depend on clinical status, tolerability, and biochemical/immunological profiles as determined by the principal investigator.

Rabbit Anti-Thymocyte Globulin (ATG, Thymoglobulin®)

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

Pre-medications will be used as follows:

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

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

#3: Methylprednisolone (Solu-Medrol®) 1 mg/kg IV one hour prior to ATG infusion and as needed to prevent cytokine release syndrome.

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

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

- Dilute the Thymoglobulin® in 500 cc Normal Saline (not D5W)
- Combine with Heparin 1000 units and Hydrocortisone 20 mg.

If a significant adverse reaction to Thymoglobulin® occurs, the patient will be treated accordingly, the transplant will be cancelled and no further doses of Thymoglobulin® will be administered. The patient will undergo islet transplantation at a later time under induction with basiliximab which will be administered as described for participants who are eligible for a second islet transplant (see section 5.3).

Alemtuzumab (Lemtrada®):
Alemtuzumab induction, instead of Thymglobulin®, will be performed in the event of high risk for anaphylactic reaction to Thymoglobulin® (i.e. patients with history of rabbit allergy/anaphylaxis or having positive IgE to rabbit serum protein and/or epithelium).

Induction with alemtuzumab will consist of an initial dose of 20mg IV over 3 hours given on Day –1 and a second dose of 20mg IV administered on the day of transplant.
Patients will pre-medicated 30 minutes before the first dose of alemtuzumab with Benadryl (50mg IV), acetaminophen (650mg PO), ibuprofen (400mg PO) and (Solumedrol 125mg iv). Pre-medication, WITHOUT Solumedrol, will also be given before the second dose of alemtuzumab.

We will monitor and perform Risk Evaluation and Mitigation Strategy (REMS) for Alentuzumab (Lemtrada®) due to serious risks of autoimmune conditions, infusion reactions and malignancy.

Mycophenolate mofetil (CELLCEPT®)
Maintenance mycophenolate mofetil (MMF) therapy (1-2 g/day as BID dosing) will be started on Day–1 pre-transplant.
The MMF treatment regimen may be modified if clinically required, (e.g. in times of inter-current illness, suspected graft dysfunction, or other clinical circumstances including intolerable or undesirable side effects) at the discretion of the Principal Investigator. Enteric-coated mycophenolate sodium (Myfortic®) may be substituted for MMF, at the discretion of the Principal Investigator and as clinically indicated. Standard mycophenolate sodium dosing is 720 mg PO BID (or as tolerated).
Mycophenolate-containing medicines are associated with an increased risk of first trimester pregnancy loss (miscarriage) and congenital malformation (birth defects) if taken during pregnancy. Subjects will be informed on these risks and must practice two methods of contraception while taking MMF.

If a subject experiences severe neutropenia (absolute neutrophil count <$1x10^9$/L) while taking mycophenolate mofetil), mycophenolate mofetil exposure will be reviewed and mycophenolate mofetil administration will be adjusted as part of the study protocol's neutropenia management plan.

The protocol and informed consent will be consistent with MMF's Risk Evaluation and Mitigation Strategy (REMS). "Mycophenolate can cause fetal harm when administered to a pregnant female. Exposure to mycophenolate during pregnancy is associated with increased risks of: First-trimester pregnancy loss, congenital malformations (especially external ear and abnormalities such as a cleft lip and palate), anomalies in babies of the distal limbs, heart, esophagus, and kidney" . We will follow the following steps to help ensure the successful implementation of Mycophenolate REMS with females with reproductive potential:

1. Enroll in Mycophenolate REMS
2. Check Pregnancy Status
3. Educate Females of Reproductive Potential
4. Obtain a Signed Patient-Prescriber Acknowledgment Form
5. Report Any Mycophenolate-Exposed Pregnancies.

Tacrolimus (PROGRAF®)

Tacrolimus will be administered orally twice daily starting on Day 1 post-transplant to target a trough level of 10-12 ng/mL for the first 3 months, and 6-10 ng/mL thereafter. However, target drug levels will ultimately depend on clinical status, tolerability, and biochemical/immunological profiles as determined by the principal investigator.
Should subjects experience a sustained increase in serum creatinine ≥ 2 times pre-transplant baseline or decrease in their eGFR of ≥33% compared with baseline, a nephrology consult will be obtained, and Tacrolimus target trough levels will be reduced by 25% should CNI toxicity be suspected as the primary cause for the decline in renal function.

Sirolimus (RAPAMUNE®)

Sirolimus will be used in case of Tacrolimus or/and mycophenolate mofetil intolerance.

ADJUVANT INTERVENTIONS

b. **Pegylated GCSF (Neulasta®)** will be administered at a dose of 6mg SC every two weeks for a total of 6 doses (post-operative day [POD] 2, 14, 28, 42, 56, and 70).
c. **Exenatide extend-release (Bydureon®)** will be administered subcutaneously at a dose of 2 mg SC weekly for as long as there is islet allograft function (POD -2 and weekly thereafter). If not tolerated or at the discretion of the PI we may consider an alternative GLP-1 RA or a DPP-4 inhibitor.
d. **Ultra-refined omega-3 EPA/DHA concentrate (ZoneLabs® OmegaRx®2 Fish Oil - Liquid).** Patients will be started at a dose of one teaspoon (i.e., 4500 mg fish oil concentrate containing 2250 mg EPA and 1125 mg DHA in 5 mL) of Omega 3 fish oil

for the first two days (starting on POD -2) and then increasing to one tablespoon (i.e., 13500 mg fish oil concentrate [6750 mg EPA, 3375 mg DHA]) by the third day and continuing daily administration thereafter. If liquid form is not tolerated, subject will be started on OmegaRx®2 capsules (containing 500 mg EPA and 250 mg DHA per capsule). The starting dose will be 1 capsule twice a day and increased up to 4 capsules three times a day as tolerated. Dose will be adjusted to maintain a target AA/EPA ratio of 1.0-2.0. AA/EPA ratios will be monitored monthly until achieving target levels and as clinically indicated thereafter.

e. **Supplemental oxygen therapy.** Supplemental oxygen will be delivered to subjects via nasal cannula at 4L/min continuously starting after islet transplantation, throughout the hospitalization, and up to 7 days post hospital discharge.

CONCOMITANT MEDICATIONS

Concomitant medications may be administered, as per standard clinical practice. Listed below are the expected concomitant medications to be used after islet transplant. A detailed list will be kept of all medications a subject takes from the time of admission for transplantation. However, a number of drug interactions with the three immunosuppressive agents are known to exist. Concomitant administration of any drug known to interact with the study drugs should be carefully considered and monitored as appropriate. Please refer to package inserts and investigational brochures of these drugs. It will be suggested that the participant refrain from using any herbal remedies.

- **Immunosuppressive / Anti-Inflammatory Therapy:** Adalimumab (Humira) will be given IV before the islet transplant (80 mg) and then 40 mg subcutaneously every 2 weeks after islet transplant for 2 doses.
  - **Antibacterial, Antifungal, and Antiviral Prophylaxis:** Broad-spectrum antimicrobial prophylaxis should be administered preoperatively according to site-specific standards, or as the Transplant Infectious Disease consultant recommends.

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

  - **Clotrimazole (Mycelex Troche®)** Clotrimazole will be administered as 1 troche PO QID starting on day –2 relative to transplant, to be continued for 3 months after transplantation. Alternatively, antifungal prophylaxis per standard practice at each site may be administered instead of Clotrimazole.

  - **Valganciclovir (Valcyte®)** Valganciclovir will be administered starting on day -2 at a dose of 450 mg PO QD, increasing to 900 mg QD by day 12 and continuing for 14 weeks post-transplantation. If the CMV status of the donor and recipient are both negative, then Valganciclovir administration can be adjusted or eliminated.

INSULIN THERAPY

Glucose levels will be targeted to 80-120 mg/dL. Insulin (*e.g*., Regular, Lispro, NPH, Glargine) will be administered, as needed, to maintain glucose levels in the target range. The subject will test BG five times per day (AM fasting, before lunch, 2 hours after lunch, before supper, and at bedtime). For subjects not on CGM, a CGM device will be placed on day of admission to facilitate adjustments to insulin therapy for as long as clinical indicated. The subject's daily BG levels will be reviewed by a study nurse and/or one of the investigators three times per week during the first two weeks after discharge, and then weekly during the next month. Exogenous insulin will be withdrawn or adjusted as needed. Patients able to maintain fasting BG levels below 140 mg/dL and 2-hour postprandial levels below 180 mg/dL after insulin discontinuation will be considered insulin independent.

OTHER STANDARD THERAPIES

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

RESCUE MEDICATIONS

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

PROHIBITED MEDICATIONS

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

- steroid medication (except topical and prednisone at a dose of ≤5 mg daily, or an equivalent dose of hydrocortisone, for physiological replacement)
- any medications in the macrolide antibiotic class other than Zithromax.
- other investigational products
- other immunosuppressive therapies
- immunomodulatory agents
- other anti-diabetic agents
- Dapsone

5.3 Second Islet Transplant (re-transplant, intra-hepatic administration): Immunosuppression

INDUCTION IMMUNOSUPPRESSION FOR SECOND ISLET INFUSION

The immunosuppressive regimen for subsequent islet transplants will be identical to the regimen for the initial islet transplant with the exception of Thymoglobulin.

Basiliximab will be used instead of Thymoglobulin for all subsequent islet transplants. The follow-up will be per SOE in appendix A. (Same as first infusion).

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

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

Administration:
The islet mixture is delivered slowly via gravity drainage from a bag attached to the catheter in the portal vein or portal vein tributary. Access to the portal vein is achieved by percutaneous transhepatic access under fluoroscopic, ultrasonographic, or real-time CT guidance. Alternatively, access to a mesenteric or omental venous tributary of the portal vein can be obtained by mini-laparotomy under general anesthesia (transplant site preference or in the rare circumstance that percutaneous access cannot be achieved).
At a minimum, portal pressure will be monitored before and after infusion of each bag of the islet product, as well as after the final wash. Portal pressure measurements will be documented in the medical record.

CONCOMITANT MEDICATIONS

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

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

OTHER STANDARD THERAPIES

ASPIRIN

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

5.4 Known and Potential Risks and Benefits to Human Subjects

5.5.4.1 Risks of Use of Investigational Agent: Transplant of Allogeneic Islets

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

5.4.2 Transmission of Disease from Donor to Recipient

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

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

Donors are excluded if: a) there is known pre-existing metabolic disease including T1 or Type 2 diabetes, or if the HbA1c is elevated above 6.1% in the absence of transfusions in the week prior to death, b) if there is malignancy other than primary brain tumors, c) septicemia is present or suspected at the time of death, d) there is evidence of clinical or active viral hepatitis (A, B or C), acquired immunodeficiency syndrome (AIDS), syphilis, active viral encephalitis of unknown origin, Creutzfeldt-Jacob disease, rabies, treated or active tuberculosis, septicemia, dementia, individuals that have received pituitary growth hormone (pit-hGH), or serious illness of unknown etiology.

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

The administration of valganciclovir routinely post-transplant may minimize risk for certain viral pathogens. The risk of transmission of CMV disease from donor to recipient has been surprisingly low in recipients of islet allografts to date, particularly in the most recent era with routine use of purified islet preparations. For instance, there have been no episodes of CMV disease in 77 consecutive islet recipients transplanted at the University of Alberta. In the international Immune Tolerance Network

(ITN)/NIAID multi-center islet trial, there was no CMV disease in any of the 36 patients transplanted at the nine different sites. Sixteen of 36 (44%) subjects were CMV positive initially. Two initially negative subjects became CMV IgG positive without any apparent clinical sequelae. The University of Miami recently presented data on three islet recipients that became CMV positive and one did develop CMV disease occurring late, after discontinuation of anti-viral prophylactic therapy.

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

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

5.4.3 Risk of Microbial Contamination of Islet Preparations

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

In 152 islet preparations transplanted consecutively at the University of Alberta since 1999, there have been no cases of transmission of bacterial or fungal disease through islet transplantation, when islets are prepared under cGMP conditions. In 74 islet preparations transplanted consecutively at the University of Miami since 1999, there have been no cases of transmission of bacterial or fungal disease through islet transplantation, when islets are prepared under cGMP conditions.

There have been previous reports of two cases of islet transplantation-related septicemia (Enterobacter cloacae) due to transplantation of contaminated cryopreserved pancreatic islets (64). Additionally, the University of Minnesota investigators have previously reported on the incidence and significance of contaminated islet preparations in clinical islet auto- and allotransplantation (65). Positive cultures from islet tissue preparations were identified in 11 of 29 patients (38%) receiving autologous islets. The occurrence of serious infection morbidity (as defined as positive blood cultures, abscesses, or intra-abdominal infections) did not differ significantly between the positive and negative culture groups (p=0.99). In the allogeneic islet transplant group, 7 of 33 patients (21%) received tissue that retrospectively was determined to be contaminated. None of these patients developed serious infectious complications (despite broad-spectrum immunosuppression). Despite the occurrence of contaminated grafts, there was no serious increase in

infectious morbidity. Presumably the inocula were kept low by the multiple washing steps allowing the recipients to clear the organisms without serious sequelae.

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

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

5.4.4 Sensitization of the Recipient to Donor Antigens

As with any allogeneic transplant, islet transplant recipients may become sensitized to islet-donor histocompatibility antigens (HLA), leading to development of panel reactive alloantibodies (PRA). These alloantibodies may develop while the recipients demonstrate full or partial islet function on maintenance immunosuppression. Furthermore, donor specific alloantibodies may develop after loss of the islet transplant function and discontinuation of the immunosuppressant drug. Data on the development of cytotoxic antibodies against donor HLA in islet allotransplant recipients with failing grafts have been reported from several islet transplant centers (66-69). In the ITN-sponsored trial of islet transplantation using the Edmonton protocol of steroid-free immunosuppression, 5 of 36 subjects had evidence of elevated PRA post-transplant when measured by flow cytometry. Two of these 5 subjects experienced primary islet non-function. Moreover, data from five participating centers in the current CIT consortium indicate that approximately 25% of the islet alone transplant recipients developed a PRA >20% while on maintenance immunosuppression. These results are comparable to those reported for recipients of kidney transplant with stable serum creatinine and on maintenance immunosuppression (70-72). Importantly, the incidence of elevated PRA (>20%) in recipients who had lost their islet transplant function and discontinued their immunosuppression rose to approximately 84%.

The available information suggests that there is a strong correlation between islet allograft failure and a rise in anti-donor HLA sensitization as detected by PRA testing. A potential consequence of high PRA levels in type 1 diabetic recipients with failed islet transplants is that if these individuals develop diabetic nephropathy in the future, it may increase their time waiting on a transplant list to qualify for a suitable kidney (73).

5.4.5 Acceleration of Retinopathy with acute correction in glycemic control

In the DCCT study28, about 10% of patients with pre-existing retinopathy receiving intensive treatment experienced a transient worsening of their retinopathy during the first year, but nonetheless had a lower cumulative incidence of sustained progression when compared to the conventional group after the third year. A transient worsening of retinopathy has not been formally documented in islet transplantation trials, but it is assumed that a similar process might occur. Exclusion of patients with

unstable retinopathy and careful post-transplant follow-up will help to minimize the incidence of such occurrences and their morbidity should they occur.
When type 1 diabetic recipients of successful and unsuccessful pancreas transplants were compared for the end point of an increase of two or more grades in the retinopathy score, they did not differ significantly in the rate of progression whether retinopathy was mild (Grade P0 to P5) or advanced (Grade P6 to P14) at baseline (74). Long-term follow-up of both groups suggested that successful pancreas transplantation may have a late beneficial effect that becomes evident only after 36 months.

5.4.6 Psychological Impact of Successful or Failed Islet Transplantation

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

5.4.7 Risk of Induction and Maintenance Immunosuppressive Therapies

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

- <u>BASILIXIMAB (SIMULECT®)</u>

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

- <u>Rabbit ANTITHYMOCYTE GLOBULIN (THYMOGLOBULIN®)</u>

Rabbit Thymoglobulin® was approved by the FDA in 1999 for the treatment for acute renal graft rejection in conjunction with concomitant immunosuppression (see product monograph for details). It is a polyclonal IgG antibody obtained by immunization of rabbit with human thymocytes and contains cytotoxic antibodies directed against antigens expressed on human T lymphocytes. Thymoglobulin® has shown a consistent safety profile with most AEs being manageable and reversible; the most common events are fever, chills and leukopenia. While rare, the most severe events include allergic or anaphylactoid reactions and serum sickness. As with all immunosuppression, administration of Thymoglobulin® may be associated with an increased risk of infection and development of malignancy (especially of the skin and lymphoid system).
In 82 kidney transplant recipients receiving 1.5 mg/kg/day for 7 – 14 days, the principal AEs were fever (52%) and chills (47%) associated with the infusions, leucopenia (47%), and thrombocytopenia (30%). CMV infection (13%) and PTLD (2%). Neutropenia has been described; anaphylaxis has been reported rarely.
Published results of the use of Thymoglobulin® in clinical and experimental islet transplantation are limited to relative small cohorts. Hirshberg *et al.* described the successful role of rabbit ATG and sirolimus in reducing rejection of islet allografts in primates, with no evidence of direct islet toxicity from Thymoglobulin®. Hering *et al.* described a beneficial role of Thymoglobulin® induction (6mg/kg) in 8 patients with T1D receiving single donor islet grafts, all of whom achieved insulin independence and were protected against recurrence of hypoglycemia (75). Acute islet rejection was described in patients receiving calcineurin-free immunosuppression when sirolimus levels fell below 9ng/mL. The use of higher doses of sirolimus exacerbated the neutropenic side effects of Thymoglobulin®, but these could be managed safely without risk of opportunistic infections when appropriate dose reduction and/or administration of Granulocyte Colony Stimulating Factor (G-CSF; Neupogen®) if required.

- <u>Alemtuzumab (Lemtrada®)</u>

Alemtuzumab is directed against the CD52 antigen expressed on virtually all lymphocytes and monocytes. The antibody is a powerful depleting agent that causes lysis of all cells expressing this antigen and resulting in a dramatic reduction in the levels of lymphocytes and monocytes in the peripheral blood and in the bone marrow. Alemtuzumab has shown to be effective in promoting the engraftment of CD34+ enriched stem cell infusions given for the treatment of a variety of hematological disorders (106). In clinical trials it has also been used to treat kidney transplant rejection, to prevent graft versus host disease and to treat lymphoid malignancies (107-110). Alemtuzumab has also been used effectively in the treatment of various autoimmune diseases (autoimmune thrombocytopenic purpura (111), multiple sclerosis. The effect of Alemtuzumab on multiple inflammatory cell types may also provide a benefit by preventing the production of proinflammatory mediators by intrahepatic macrophages and endothelial cells, thus preventing early islet loss due to the deleterious effects of cytokines on islets (112, 113).

Alemtuzumab, the initial dose of 20mg IV over 3 hours will be given on Day –1 and a

second dose of 20mg IV will be administered on the day of transplant. Patients will pre-medicated 30 minutes before the first dose of alemtuzumab with Benadryl (50mg IV), acetaminophen (650mg PO), ibuprofen (400mg PO) and (Solumedrol 125mg iv). Pre-medication, WITHOUT Solu-Medrol, will also be given before the second dose of alemtuzumab. The most common side-effects of alemtuzumab include infusion-related reactions characterized by fever, chills, rigors, nausea, vomiting and hypotension. These usually occur with the initial dose of alemtuzumab. These events are prevented or improved with the premedication treatment administered prior to the infusion of alemtuzumab. An increased incidence in infectious complications (when compared to other immunosuppressive regimens) was not apparent in 31 renal transplant patients who received alemtuzumab as induction therapy (113). These patients also received prophylactic treatment against PCP and viral infections such as CMV as detailed below. Hematologic Toxicity: Serious and, in rare instances fatal, pancytopenia/marrow hypoplasia, autoimmune idiopathic thrombocytopenia, and autoimmune hemolytic anemia have occurred in patients receiving alemtuzumab therapy. Single doses of alemtuzumab greater than 30 mg or cumulative doses greater than 90 mg per week should not be administered because these doses are associated with a higher incidence of pancytopenia. A recent trial using alemtuzumabfor treatment of patients with multiple sclerosis revealed a high incidence of Grave's disease. One third of patients developed Graves' disease between 6 and 18 months after the alemtuzumab treatment (112). This complication has not been described in patients who received alemtuzumab for other indications than multiple sclerosis (lymphoid malignancies, renal transplant recipients). Thyroid function will be closely monitored in all patients. Another recent trial has demonstrated a small but significant incidence of immune thrombocytopenic purpura (ITP) 3 cases in 334 subjects, one resulting in death (this subject did not seek medical attention). alemtuzumabIn both trials patients were treated with very high doses of alemtuzumab (100mg given over 5 days).

- SIROLIMUS (RAPAMUNE®)

The FDA approved sirolimus (rapamycin, Rapamune®) as an immunosuppressive agent in 1999 (see product monograph for details). In 208 kidney transplant recipients receiving 5 mg of sirolimus daily compared to 124 receiving placebo, there was an increased incidence of hypercholesterolemia (46 vs. 23%), hyperlipemia (57 vs. 23%), rash (20 vs. 6%), arthralgia (31 vs. 18%), diarrhea (35 vs. 27%), anemia (33 vs. 21%), leucopenia (13 vs. 8%), thrombocytopenia (30 vs. 9%), and hypokalemia (17 vs. 9%). Side effects are related to drug concentration and are improved with maintenance of the sirolimus 24-hour trough level between 10–20 ng/mL. Of infections, only mucosal herpes simplex virus (HSV) occurred at a greater rate with sirolimus. There was no increase in rate of malignancy (3.4 vs. 3.1%). While sirolimus was originally proposed as a non-nephrotoxic agent, it is becoming apparent that sirolimus-associated nephrotoxicity does occur in clinical practice. Crew *et al.* described two patients with thrombotic microangiopathy secondary to sirolimus exposure121. Sirolimus alters the pharmacokinetic profiles of other CNIs (*e.g.*, tacrolimus) and may thereby potentiate nephrotoxicity (76). Fervenza *et al.* described nephrotoxicity from sirolimus in patients with chronic glomerulopathies that was non-reversible on cessation of therapy (77).

Nephrotoxicity from combined sirolimus and tacrolimus has been described in patients with T1D undergoing islet transplantation, particularly where there is underlying pre-existing renal damage from diabetes (78, 79).

The majority of islet transplant recipients receiving sirolimus in conjunction with tacrolimus have experienced transient mouth ulceration, lower extremity edema; perinephric edema and a high incidence of benign ovarian cysts have also been described in islet recipients in association with sirolimus. Pneumonitis and colitis have also occurred (80-82). The most common (> 30%) adverse reactions are: peripheral edema, hypertriglyceridemia, hypertension, hypercholesterolemia, creatinine increased, abdominal pain, diarrhea, headache, fever, urinary tract infection, anemia, nausea, arthralgia, pain, and thrombocytopenia.

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

- TACROLIMUS (PROGRAF®)

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

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

CellCept® and Myfortic® are associated with: diarrhea, leucopenia, vomiting, and evidence of higher frequency of certain types of infections. CellCept® and Myfortic®

may increase the risk of developing lymphoproliferative disease, lymphomas, and other malignancies, particularly of the skin, and have been known to cause fetal harm when administered to a pregnant woman. Cases of progressive multifocal leukoencephalopathy, sometimes fatal, and pure red cell aplasia have been reported in patients treated with CellCept® or Myfortic®.

5.4.8 Risks of Immunosuppressive / Anti-inflammatory Therapy: Adalimumab (Humira®)

Adalimumab is a recombinant human IgG1 monoclonal antibody specific for human tumor necrosis factor (TNF). It is FDA-approved for use in rheumatoid arthritis, juvenile arthritis, ankylosing spondylitis, psoriatic arthritis, Crohn's disease, ulcerative colitis and uveitis. Inhibitors of tumor necrosis factor (TNF)-alpha represent important treatment advances in a number of inflammatory conditions, including rheumatoid arthritis (RA), the seronegative spondyloarthropathies, and inflammatory bowel disease (IBD). TNF-alpha inhibitors offer a targeted strategy that contrasts with the nonspecific immunosuppressive agents traditionally used to treat most inflammatory diseases. However, multiple adverse effects of TNF-alpha inhibition have been identified through both clinical trials and post-marketing surveillance. These include: Injection site reactions, infusion reactions, neutropenia, infections, demyelinating disease, heart failure, cutaneous reactions, malignancy and induction of autoimmunity.

5.4.9 Risks from Adjuvant Interventions

a. Exenatide Extended-Release (Bydureon®).

Nausea, vomiting, dyspepsia, constipation, diarrhea, headache and injection-site pruritus were the most common adverse effects reported with ER exenatide in clinical trials. Gastrointestinal effects occurred in about 35% of patients, but decreased over time. Acute renal failure and acute pancreatitis have been reported in patients with diabetes taking exenatide; whether they were caused by the drug or the disease is not clear. Injection-site nodules have been reported in about 77% of patients and injection-site reactions in about 17% of patients. Weight loss is reported with exenatide. In subjects with T2D who took exenatide for 4 weeks, the average weight loss was 1.3±0.3 kg. Weight loss was also seen in the DURATION-5 trial of Bydureon.. Impaired recovery from hypoglycemia is a potential adverse effect of GLP-1 receptor agonists. However, the risk of hypoglycemia in patients with T2D treated with combination of exenatide (Byetta) and exogenous insulin has been low. In the DURATION 4 study, a non-inferiority study comparing Bydureon to Metformin, Pioglitazone, and Sitagliptin, no major hypoglycemia was seen.

Exenatide may affect absorption of other drugs such as oral contraceptives, antibiotics, digoxin, lovastatin and warfarin. Participants will be informed to take their routine medications at least 1 hour prior to taking exenatide.

Thyroid C-cell hyperplasia has been reported with use of exenatide in rats, and the FDA has required a boxed warning about the risk of thyroid C-cell tumors in the package insert.

Contraindications for the use of Exenatide include:

- Medullary Thyroid Carcinoma: Exenatide is contraindicated in patients with a personal or family history of medullary thyroid carcinoma (MTC) or in patients with Multiple Endocrine Neoplasia syndrome type 2 (MEN 2).
- Hypersensitivity: Exenatide is contraindicated in patients with a prior serious hypersensitivity reaction to exenatide or to any of the product components.

Warning and precautions:

- Risk of Thyroid C-cell Tumors: In both genders of rats, exenatide extended-release caused a dose-related and treatment-duration dependent increase in the incidence of thyroid C-cell tumors (adenomas and/or carcinomas) at clinically relevant exposures compared to controls. A statistically significant increase in malignant thyroid C-cell carcinomas was observed in female rats receiving exenatide extended-release at 25-times clinical exposure compared to controls and higher incidences were noted in males above controls in all treated groups at ≥2-times clinical exposure. The potential of exenatide extended-release to induce C-cell tumors in mice has not been evaluated. Other GLP-1 receptor agonists have also induced thyroid C-cell adenomas and carcinomas in male and female mice and rats at clinically relevant exposures. It is unknown whether Bydureon will cause thyroid C-cell tumors, including medullary thyroid carcinoma (MTC), in humans, as the human relevance of exenatide extended-release-induced rodent thyroid C-cell tumors could not be determined by clinical or nonclinical studies. Serum calcitonin was not assessed in the clinical trials supporting the approval of Bydureon.
Serum calcitonin is a biological marker of MTC. Patients with MTC usually have calcitonin values >50 ng/L. Patients with thyroid nodules noted on physical examination or neck imaging should be referred to an endocrinologist for further evaluation. Routine monitoring of serum calcitonin or using thyroid ultrasound is of uncertain value for early detection of MTC in patients treated with Bydureon. Such monitoring may increase the risk of unnecessary procedures, due to the low specificity of serum calcitonin testing for MTC and a high background incidence of thyroid disease.

- Acute Pancreatitis: Based on post marketing data, exenatide has been associated with acute pancreatitis, including fatal and non-fatal hemorrhagic or necrotizing pancreatitis.

- Hypoglycemia: The risk of hypoglycemia is increased when exenatide is used in combination with a sulfonylurea. Therefore, patients receiving Bydureon and a sulfonylurea may require a lower dose of the sulfonylurea to minimize the risk of hypoglycemia. It is also possible that the use of Bydureon with other glucose-independent insulin secretagogues (e.g. Meglitinides) could increase the risk of hypoglycemia.

- Renal Impairment: Bydureon should not be used in patients with severe renal impairment (creatinine clearance < 30 mL/min) or end-stage renal disease and should be used with caution in patients with renal transplantation. In patients with end-stage renal disease receiving dialysis, single doses of Byetta 5 mcg were not well tolerated due to gastrointestinal side effects. Because Bydureon may induce nausea and vomiting with transient hypovolemia, treatment may worsen renal function. Use Bydureon with caution in patients with moderate renal impairment (creatinine clearance 30 to 50 mL/min). Bydureon has not been studied in patients with end-stage renal disease or severe renal impairment.

  There have been post marketing reports of altered renal function with exenatide, including increased serum creatinine, renal impairment, worsened chronic renal failure and acute renal failure, sometimes requiring hemodialysis or kidney transplantation. Some of these events occurred in patients receiving one or more pharmacologic agents known to affect renal function or hydration status such as angiotensin converting enzyme inhibitors, nonsteroidal anti-inflammatory drugs, or diuretics. Some events occurred in patients who had been experiencing nausea, vomiting, or diarrhea, with or without dehydration. Reversibility of altered renal function has been observed in many cases with supportive treatment and discontinuation of potentially causative agents, including exenatide. Exenatide has not been found to be directly nephrotoxic in preclinical or clinical studies.

- Gastrointestinal Disease: exenatide is commonly associated with gastrointestinal adverse reactions, including nausea, vomiting, and diarrhea.

- Immunogenicity: Patients may develop antibodies to exenatide following treatment with Bydureon. Antiexenatide antibodies were measured in all Bydureon -treated patients in the five comparator-controlled 24-30 week studies of Bydureon. In 6% of Bydureon -treated patients, antibody formation was associated with an attenuated glycemic response.

- Hypersensitivity: There have been post marketing reports of serious hypersensitivity reactions (e.g. anaphylaxis and angioedema) in patients treated with exenatide.

- Macrovascular Outcomes: There have been no clinical studies establishing conclusive evidence of macrovascular risk reduction with Bydureon or any other antidiabetic drug.

- Pancreatic Cancer: A recent meta-analysis suggested an increase rate of pancreatic cancer in patients with T2D who used GLP-1 receptor agonists or DPPIV inhibitors. More recently, an analysis of histologic sections from patients with T2D suggested expansion of exocrine and endocrine pancreas with incretin therapy and increased exocrine pancreas dysplasia and the potential for glucagon-producing neuroendocrine tumors . As a result of these reports, the FDA is initiating an investigation of the safety of DPPIV inhibitors and GLP-1 receptor agonists. In the

event that this and other studies show efficacy of this and other agents in T1D, further assessment of the risk/benefit ratio will be necessary in the context of the forthcoming investigations. The potential risks for pancreatic cancer or medullary carcinoma of the thyroid are believed to be minimal for participants in this study because of the very short exposure to study drug.

b. Pegylated G-CSF (Neulasta®).

There are some potential risks associated with pegylated G-CSF therapy. Although the risk is entirely theoretical at this time, there is a possibility that the G-CSF injections could worsen and not improve the autoimmunity associated with T1D. The side effect profile of pegylated G-CSF injection will be monitored by close clinical observation of the subjects for 1 hour after the first G-CSF injection as well as with daily phone calls to the subjects during the first 5 days of G-CSF therapy and weekly thereafter. Pegylated G-CSF carries a risk (10-20% of patients) of bone pain and a risk (less than 10%) of fever, pain or redness at the site of injection, headache, and dizziness. There is also a risk that the subjects' WBC could become elevated while taking G-CSF. There are also now a small number of reports in which recipients of GCSF have developed splenomegaly and/or splenic rupture. This risk will be explicitly discussed in the consent form and subjects will have close physical examination and phone follow-up to screen for any signs or symptoms. In addition, weekly phone calls for the month following therapy will be used to document any adverse reactions. The CBC and complete metabolic profile at each blood draw will be carefully reviewed for any clinically relevant abnormalities. There are no other known long-term risks from a 12 week course of G-CSF.

c. Ultra-refined omega-3 EPA/DHA concentrate (ZoneLabs® OmegaRx®2)

Omega-3 Fatty Acids may cause gastrointestinal side- effects including stomach pain or discomfort, burping, heartburn, vomiting, constipation, diarrhea, nausea, change in the sense of taste and prolongation of bleeding time. Joint pain may be a side effect as well. As with any drug, it is possible that subjects could experience an allergic reaction to OMEGA-3. Such allergic reactions include: itching, skin rash, sudden drop in blood pressure, loss of consciousness and/or associated with seizures, including the possibility of death.

d. Supplemental oxygen.

Low flow supplemental oxygen therapy via nasal cannula can lead to dryness of nares and bleeding after prolonged use. Oxygen toxicity has been associated with hyperoxia from high FiO2. However, as per protocol, subjects will receive supplemental oxygen at FiO2 36% and data in the literature suggest that by keeping FiO2 below 40 to 50%, oxygen toxicity can be minimized.

5.4.10 Risk of Study Procedures

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

- BLOOD DRAW TESTING

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

- METABOLIC STIMULATION TESTING

The risks associated with metabolic testing are generally regarded as minor. Placement of IV cannulae may be associated with pain and discomfort at the puncture site, bruising, bleeding, interstitial infusion of fluids, local vein thrombosis, infection or thrombophlebitis.
The administration of bolus glucose or insulin by mouth or intravenously may lead to acute hypoglycemia or hyperglycemia, or rarely may induce ketoacidosis.

- HYPOGLYCEMIA

Severe hypoglycemia is a risk associated with the infusion of islets. Iatrogenic hypoglycemia in the immediate post-transplant period is a rare event. Frequent blood glucose monitoring immediately following islet transplantation is recommended to avoid severe unrecognized hypoglycemia in the early post-transplant period. In longer-term follow-up, life-threatening hypoglycemia (Grade 4) occurred in six of the 236 SAEs reported to CITR (93) For these six occurrences, the events occurred at the following time intervals; 59 days post the third infusion, 230 days post the second infusion, 296 days post the second infusion, 360 days post the third infusion, 673 days post the third infusion, and 318 days post the second infusion. The local CITR investigators did not attribute any of the six events to the infusion procedure or to the immunosuppression medication.

- CHEST X-RAY

A chest x-ray exposes subject to a dose of radiation equal to approximately the amount one receives in 10 days of normal day-to-day activity. This amount of radiation should not create additional risk to the subject, but this is not known for sure.

- MAGNETIC RESONANCE IMAGING (MRI)

The risk of MRI performed with intravenous gadolinium contrast is that of an allergic reaction which can be life threatening (anaphylaxis). Some patients with impaired kidney function who have received gadolinium have experienced a worsening of their kidney function that in some cases has been severe and irreversible and has required dialysis. Some patients may also experience claustrophobia and if this should happen the test will be stopped. The MRI center has a physician available in the building while patients are being injected with contrast. A crash cart with medications, a defibrillator, an Automated External Defibrillator (AED), oxygen, intubation

equipment, and automatic blood pressure monitor are in place. The technologists are all Basic Cardiac Life Support (BCLS) certified. In the case of an emergency, 911 is called. For non-emergent problems that require admission, ambulance transportation can be arranged.

- FLUORODEOXYGLUCOSE POSITRON EMISSION TOMOGRAPHY INTEGRATED WITH COMPUTED TOMOGRAPHY (^{18}F-FDG PET/CT)

The risk associated with this procedure is the exposure to radiation. To better understand the amount of radiation that people receive from various medical X-ray exams it is best to compare it to the radiation people are exposed to from natural sources in the environment. The unit of measurement for an effective radiation dose is called the millisievert (mSv). The average person in the United States receives a dose of about 3 mSv per year from naturally occurring radiation. As a comparison, the radiation exposure from one chest X-ray is the same as the amount of radiation a person is exposed to from their natural surroundings in 10 days (94).

The radiation exposure from a PET-CT can range from 12 to 30 mSv, which is comparable to what a person would receive from natural background radiation over a period of 3 to 9 years (94-95). This degree of radiation exposure has been associated with a less than 1% increase in lifetime risk of cancer (95, 96, 97).

- RISKS FROM ISLET TRANSPLANT PROCEDURE IN RADIOLOGY:

Islets will be transplanted under fluoroscopic guidance (X-RAY imaging). The procedure will expose subject to a dose of radiation equal to approximately the amount one receives in 7 years of normal day-to-day activity. This amount of radiation should not create an additional risk for the subject, but this is not known for sure.

- CENTRAL LINE

A Central line will be needed for induction. The risks related to a PICC line are bleeding at the site of the vein puncture, infection, and clotting of the veins. In case a PICC line is not possible, a central line in the neck might be placed. The risks related to central line in the neck are: bleeding (internal or external), lung collapse, vein thrombosis, damage of adjacent structures, irregular heart rhythm, infection, shortness of breath, and hypotension.

- CONTINUOUS GLUCOSE MONITORING

It may cause skin irritation at the site of implantation. It may be related to: bruising, discomfort, pain, bleeding, redness, raised bumps, local infection, and appearance of a small “freckle-like” dot where the needle was inserted. It may lead to an infection where the sensor was inserted. If there is pain, redness, irritation, or rash at insertion site, the sensor will be removed and re-inserted in a different site.

5.4.11 Benefits

Successful islet transplantation alleviates T1D patients from life-threatening hypoglycemia and psychosocially debilitating glycemic lability. While the long-term durability of these responses is at present uncertain, they persist for as long as some graft function is maintained, despite the eventual return to insulin therapy in the majority of recipients. This partial function, as indicated by continued c-peptide production, may be present in as many as 80% of recipients after 5 years (101). Furthermore, as long as graft function is maintained, fear of hypoglycemia and anxiety are significantly lower after islet transplantation (102). Indeed, T1D subjects in the DCCT who had persistent c-peptide production had a significantly reduced risk of severe hypoglycemia despite intensive insulin therapy (103). Additionally, while most transplant recipients experience only a temporary reprieve from exogenous insulin therapy, a few have maintained insulin-independent graft function for more than 3 years. Novel strategies aimed at promoting the engraftment or survival of transplanted islets may lead to improved long-term graft function and further the duration of insulin-independence after transplantation, and hopefully lead to reductions in the secondary complications of T1D.

5.5 Assessment of Compliance with Study Treatment

Assessment of subject compliance will be determined by the completion of scheduled study visits and required documentation that the specific subject is responsible for (*e.g.*, Blood Glucose Logs, AE and Insulin Use recording) as well as their willingness to comply with the recommendations of the study investigators. Any aberration of trough levels of immunosuppressive agents that could indicate no adherence, lack of compliance that poses a significant clinical risk and or derangement of protocol data collection will be documented.

5.6 Modification or Discontinuation of Study Treatment

5.6.1 Islets are Unsuitable

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

5.6.2 Graft Failure

Immunosuppression will be stopped in subjects who experience graft failure and the subject will continue follow up as per SOE on appendix A. Post graft failure follow up will exclude graft function measurements: 90min MMTT, BSR, CGM, LI, HYPO, Beta Score, C-peptide ratio. Physical exam after graft failure will only be done at study visit 19 (M12) and Visit 23 (M24) or as medically indicated.

5.6.3 Allergic Reaction to ATG

If a subject demonstrates an allergic reaction to thymoglobulin that results in cancellation of the initial transplant and the investigators feel that future use of the drug in that subject is contraindicated, the steps outlined in section 5.6.10 should be followed. Once another organ becomes available, the subject will receive an alternate induction immunosuppressive regimen with basiliximab (IL-2 receptor blocker).

5.6.4 Intolerance of Protocol Medications

In the event that the immunosuppression regimen is not tolerated, the principal investigator (PI) may elect to prescribe an alternative immunosuppression regimen. The intent would be for the alternative regimen to be temporary in nature where possible. Any non-protocol directed study treatment modification that the site PI determines is necessary should be reported as a protocol deviation.

5.6.5 RABBIT ANTI-THYMOCYTE GLOBULIN-Induced Anaphylaxis

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

5.6.6 RABBIT ANTI-THYMOCYTE GLOBULIN-Induced Cytokine Release

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

5.6.7 Neutropenia

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

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

• Reduce rabbit ATG by 50%.

• Reduce the prophylactic use of valganciclovir from 900 mg per day to 450 mg per day or hold valganciclovir.

• Reduce trimethoprim/sulfamethoxazole to 80/400 mg on Monday, Wednesday, and Friday or hold trimethoprim/sulfamethoxazole.

• If subject is using mycophenolate mofetil or mycophenolate sodium, consider dose reduction.

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

If a subject's absolute neutrophil count is less than 1000 cells/μL and the subject is febrile, then the following will be done:
• Obtain Infectious Disease Consult.
• Hold rabbit ATG.
• Hold valganciclovir and trimethoprim/sulfamethoxazole.
• If subject is using mycophenolate mofetil or mycophenolate sodium, consider dose reduction.
• Administer G-CSF.
• Monitor temperature BID.
• Follow up within 48-72 hours to obtain: repeat CBC with differential, subject symptoms, and measured temperatures.

If a subject's absolute neutrophil count is measured as less than 500 cells/μL and the subject is afebrile, then the following will be done:
• Hold rabbit ATG.
• Hold administration of trimethoprim/sulfamethoxazole and/or valganciclovir.
• If subject is using mycophenolate mofetil or mycophenolate sodium, consider holding dose.
• Obtain CMV antigenemia or PCR for CMV.
• Consider fluoroquinolones in afebrile subjects.
• Consider clotrimazole.
• Administer G-CSF.
• Monitor temperature BID.
• Follow up within 24 hours to obtain repeat CBC, subject symptoms, and measured temperatures.

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

• The subject will be hospitalized under neutropenic precautions and Infectious Disease/Hematology consult will be obtained.
• Hold rabbit ATG.
• Hold administration of trimethoprim/sulfamethoxazole and/or valganciclovir.
• If subject is using mycophenolate mofetil or mycophenolate sodium, consider holding dose.
• Obtain CMV antigenemia or PCR for CMV.
• Administer G-CSF.

5.6.8 Thrombocytopenia

If the subject is found to have a platelet count (PLT) of $<50 \times 10^9$/L, ATG will be withheld until PLT $>50 \times 10^9$/L, then resume at a 50% reduced dose. If the PLT is <50

x 10^9/L, sirolimus will be withheld for 24 hours, then resumed at a 50% reduced dose. If PLT fails to return to >50 x 10^9/L within one week, sirolimus is to be withheld until PLT >50 x 10^9/L, after which sirolimus is resumed at 50% of the dose that preceded the drop in PLT to <50 x 10^9/L. If the PLT is between 50 and 75 x 10^9/L, reduce anti-thymoglobulin dose by 50% until PLT is >75 x 10^9/L.

5.6.9 Nephrotoxicity

A sustained increase in serum creatinine ≥ 2 times pre-transplant baseline or decrease in their GFR of ≥ 33% compared to baseline warrants a prompt referral to a nephrologist for evaluation. If it is determined that the decrease in renal function is attributable to CNI immunosuppressive therapy, the treating physician should choose a therapeutic alternative. A repeat assessment of creatinine should be performed 3 months after the change in immunosuppression.
Anti-hypertensives, anti-hyperlipidemics and other approved therapies for pre-existing and new medical conditions will be provided per standard of care.

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

Study treatment may be prematurely discontinued for any subject for any of the following reasons:
1. The subject is unwilling or unable to comply with the protocol.
2. The investigator believes that the study treatment is no longer in the best interest of the subject.
3. Graft Failure.
4. An unexpected related SAE. The agent(s) to which the event is attributed will be discontinued.
Subjects who prematurely discontinue study treatment will remain in the study until normal termination, for the purpose of monitoring safety and efficacy parameters and will enter the post graft failure follow-up (section 5.6.2). Data from these subjects will be used in the intent-to-treat analysis. These subjects are permitted to simultaneously enroll in a specific graft failure follow-up protocol, if available.

5.7 Risks of surgery and anesthesia

Islet cells will be placed in the omentum by an open surgical approach under general anesthesia. The general risks of surgery include bleeding, wound infection, wound hernia, bowel obstruction (adhesions), deep vein thrombosis and pulmonary embolism. Risks associated with anesthesia include difficulties with airway management, cardiac arrhythmias and drug-related anaphylactic reactions. Pain and discomfort at the surgical site is expected in the early period following surgery, and may be reduced by administration of opiate, opioid or non-steroidal analgesic medications. If an ileus develops, a prolonged hospital stay may be anticipated. The risks related to the intrahepatic islet transplantation are listed below:

- moderate pain or discomfort;

- bleeding from the liver puncture, which may require a blood transfusion;
- damage to liver or blood vessels, which may require surgery;
- damage to gallbladder, which may lead to the removal of gallbladder;
- bleeding as a result of the blood thinners used to prepare the islets;
- portal vein thrombosis, which may heal itself or may lead to the need for a liver transplant;
- shortness of breath or apnea as a result of the sedation used for the procedure;
- pulmonary edema or/and pleura effusion;
- death from complications (this risk is rare but possible);
- liver or kidney injury that may or may not resolve; and severe hypoglycemia during the procedure, or in the hours afterwards.

5.8 Study Stopping Rules

Study enrollment will be suspended if any one of the following occurs:

1. Any unexpected fatal or life-threatening AE possibly related to the use of the test therapy.
2. Primary non-function (PNF) occurs in 2 consecutive subjects. PNF is defined as no c-peptide detection up to 75 days post-transplant.
3 C-peptide less than 0.3 ng/mL (on random testing, at baseline and 1-3 hours post MMTT) at 75-180 days post-transplant in 2 consecutive study subjects.
4. Torsion of omentum, GI obstruction, or abscess in 2 consecutive subjects.

6 STUDY PROCEDURES

6.1 Enrollment and Screening

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

Once informed consent has been obtained, the subject will be enrolled and assigned a unique subject identification number. Subject eligibility will be confirmed through the performance of the screening visit procedures detailed in the Schedule of Events (SOE). More than one visit may be necessary to complete all of the screening procedures.

6.2 Waiting List/Baseline

After completion of the screening assessments required to confirm eligibility for the study, he/she will be listed for an islet transplant. Waitlist assessments will be repeated at pre-defined intervals as detailed in SOE. Results from assessments done closest to the start of immunosuppression will be used as the subject's baseline values. All one-time waitlist/baseline assessments should be completed on Day -2, whenever possible, but always prior to the start of immunosuppression. As in any other transplant situation, medical conditions that arise (*e.g.*, new serious infection, malignancy, compliance issues,

etc.) will automatically trigger a re-evaluation to determine if the subject remains qualified for the protocol. Only qualified subjects may proceed to donor organ matching and transplant.

6.3 Islet Transplant, and Study Treatment Visits

Once compatible islet prep becomes available, subject eligibility will be re-confirmed. Subjects will receive the initial islet transplant on Day 0 and will continue the immunosuppression regimen as per protocol.

6.4 Follow-up Visits

Subject will undergo a 24-month follow-up period following their islet transplant. Please refer to the Schedule of Events, for the clinical time points of specific follow-up study procedures.

Subjects will be followed for adverse events only until 24 months after their islet transplant.

6.5 Imaging modalities to localize the transplanted islet allograft.

A major challenge in islet transplantation has been the identification of imaging studies that may allow for *in-vivo* visualization of the transplanted islet allograft. Ideally, such imaging techniques should allow to adequately correlate the visualized islet mass with islet graft metabolic function and also permit quantification of the islet mass.

The use of MRI in the assessment of the peritoneum, particularly for peritoneal malignancies, is well established in clinical practice. In addition, MRI is the imaging modality of choice for primary pelvic/gynecologic malignancies due to its superior contrast resolution. Considering that in this protocol islets are transplanted onto the omentum and that it is possible that some of the islets may have not adhered onto the omentum but travelled down and engrafted in the pelvis, we have opted for the utilization of MRI with intravenous contrast as the imaging modality for localization of the islet graft in the abdomen and pelvis.

Positron Emission Tomography integrated with Computed Tomography (PET/CT) has been used as a functional imaging modality for identification and visualization of islet allografts. Several tracers have been evaluated including ^{18}F-FDG, GLP-1, Exendin-4 and most recently [^{11}C]5-HTP (98-102). We will perform an ^{18}F-FDG PET/CT study for localization of the islets. PET/CT is a functional imaging technique mostly used in the field of oncology. It comprises the use of a radiopharmaceutical which is administered intravenously to the patient. The uptake of the tracer by tissues is then evaluated by a PET scanner and CT providing two and three-dimensional images of the distribution of the tracer in the body. The most common tracer used is 2-deoxy-2-[fluorine-18]fluoro-D-glucose (^{18}F-FDG), also known as fluorodeoxyglucose, an analogue of glucose containing the positron-emitting radionucleide fluorine-18. ^{18}F-FDG is taken up by cells that rely on glucose as an energy source and primarily by cells with high-metabolic activity such as cancer cells thus providing significant information regarding tumor identification and

response to treatment in the field of oncology. We expect that the uptake of ^{18}F-FDG, a glucose analog, by the islets will be superior to the expected physiologic background uptake by the omentum thus allowing for functional visualization of the engrafted islet mass.

Both studies will be performed at the 1-year post-islet transplant visit in patients with persistent graft function and fasting C-peptide ≥0.5 ng/mL.

6.6 Mechanistic assays

Immunologic assays

We will obtain islet transplant donor splenocytes and recipient lymphocytes to perform T-cell assays. A maximum of 50 ml of blood will be obtained from recipients to perform these tests in order to evaluate for anti-donor reactivity. Frequency of T-cell assays will be determined by the principal investigator but will occur no less than every 6 months.

Cytokine/Chemokines

We will perform a cytokine/chemokine panel to explore the inflammatory milieu during the peri-transplant period. In addition, we will also assess cytokine/chemokine profiles at 6 and 12 months post-transplantation to evaluate the role of inflammation on islet allograft function.

Unmethylated preproinsulin DNA

It has been postulated that as β-cells die unmethylated preproinsulin DNA emanates from the β-cells and is detectable in the circulation. Thus, changes in circulating levels of unmethylated preproinsulin DNA during the peri-transplant period may correlate with beta cell loss.

Investigators from the Human Islet Research Network (HIRN) have developed assays to detect circulating unmethylated preproinsulin DNA. We will collaborate with the HIRN for validation of these assays by providing samples. Serum and plasma samples will be obtained pre-transplant, 1hr, 3hr and 3 days post-transplant. Urine samples will be obtained pre-transplant and 5hr post-transplant. Samples will be shipped to the Juvenile Diabetes Research Foundation (JDRF) Core for Assay Validation at the Benaroya Research Institute. Results from these assays will not be made available to our center.

7 ADVERSE EVENTS

This section defines the types of AEs and outlines the procedures for appropriately collecting, grading, recording, and reporting them. Information in this section complies with *International Conference on Harmonization (ICH) Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting and ICH E6: Guideline for Good Clinical Practice,* and the CIT-TCAE V5.0 Date: 03Aug2011.

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

7.1 Serious Adverse Events

An SAE is defined per 21CFR§312.32 as "an adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes." This includes but is not limited to any of the following events:

1. Results in death,
2. Is life threatening, (Note: the term "life-threatening" refers to an event/reaction in which the patient was at risk of death at the time of the event/reaction; it does not refer to an event/ reaction which hypothetically might have caused death if it were more severe),
3. Requires inpatient hospitalization or results in prolongation of existing hospitalization,
4. Results in persistent or significant disability/incapacity,
5. Is a congenital anomaly/birth defect, or
6. Is a medically important event or reaction. Medical and scientific judgment should be exercised in deciding whether other situations should be considered serious, such as important medical events that might not be immediately life-threatening or result in death or hospitalization, but might jeopardize the patient or might require intervention to prevent one of the other outcomes listed in the definition above.
7. Other conditions specified in the protocol.

In addition, events that occur at a higher than expected frequency, as determined by appropriate medical judgment, may be consider SAE's. Regardless of the relatedness of the AE to study drug, the event must be reported as an SAE if it meets any of the above definitions.

7.2 Unexpected Adverse Event

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

7.3 Collecting Procedures

AEs that are associated with a protocol-mandated procedure, which is not part of the normal standard of care for the participant and hypoglycemic events, will be collected beginning immediately after enrollment consent has been obtained. All other AEs will be collected beginning immediately after transplant. All AEs will continue to be collected until study completion, or for 30 days after the subject prematurely withdraws from the study.

AEs will be followed until the time the event is resolved, stabilized, or the subject completes or withdraws form the study, whichever comes first. AEs may be discovered through any of these methods:

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

7.4 Recording Procedures

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

7.5 Reporting Procedure

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

7.5.1 Standard reporting. (*i.e*., should be included in the investigational new drug [IND] annual report to the health-authorities). This requirement applies if the AE is classified as any of the following:
Serious, expected, and drug related.
Serious, expected, and *not* drug related.
Serious, *unexpected,* and not drug related.

7.5.2 Expedited reporting. This requirement applies if the AE is considered serious, unexpected, and drug related as defined in 21 CFR 312.32. This type of SAE must be reported by the sponsor to the appropriate health authorities within 15 days; fatal or life-threatening events must be reported within 7 days.

7.5.3 Notifying the Institutional Review Board (IRB) and Ethics Committee

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

- PREGNANCY

Sexually-active WOCBP must use an effective method of birth control during the course of the study, in a manner such that risk of failure is minimized. Prior to study

enrollment, WOCBP must be advised of the importance of avoiding pregnancy during trial participation and the potential risk factors for an unintentional pregnancy. The subject must sign an informed consent form documenting this discussion. In addition, all WOCBP should be instructed to contact the investigator immediately if they suspect that they might be pregnant (*e.g.*, missed or late menstrual period) at any time during study participation. Male subjects should also notify study team in case their female partners become pregnant during the study participation.

- REPORTING PREGNANCY AS A SERIOUS ADVERSE EVENT

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

7.6 Grading and Attribution

7.6.1 Grading Criteria

The study site will grade the severity of AEs experienced by study subjects according to the criteria set forth in the CIT-TCAE V5.0 Date: 03Aug2011. This provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all AEs.

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

Grade 1 = Mild AE.

Grade 2 = Moderate AE.

Grade 3 = Severe and undesirable AE.

Grade 4 = Life-threatening or disabling AE.

Grade 5 = Death.

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

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

7.6.2 Definition of Attribution

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

The relatedness, or attribution, of an AE to islet transplantation, which includes the transplant procedure and/or the islet product, the secondary investigational agent, or to the immunosuppression and/or infection prophylaxis will be determined by the investigator. The investigator will also record the determination of attribution on the

appropriate eCRF and/or SAE report form. The relationship of an AE (attribution of AE) to islet transplantation (islets or transplant procedure),

7.6.3 Attribution of adverse events

UNRELATED CATEGORY

1 The AE is clearly not related to allogeneic islets; the islet transplant procedure; immunosuppression or infection prophylaxis.

RELATED CATEGORIES

2 Unlikely: The AE is doubtfully related to allogeneic islets; the islet transplant procedure; immunosuppression or infection prophylaxis.
3 Possible: The AE may be related to allogeneic islets; the islet transplant procedure; immunosuppression or infection prophylaxis.
4 Probable: The AE is likely related to allogeneic islets; the islet transplant procedure; immunosuppression or infection prophylaxis.
5 Definite: The AE is clearly related to allogeneic islets; the islet transplant procedure; immunosuppression or infection prophylaxis.

8 REVIEW OF SAFETY INFORMATION

This trial will be conducted following Good Clinical Practice (GCP) and International Committee on Harmonization Guidelines (ICH). All research project personnel who work with subjects or subject data, or subject research samples will be required to complete ethics and protection of human research participants training as per guidelines issued by the US Department of Health and Human Services, Office for Human Research Protection. This protocol will undergo review and approval by the University of Miami (UM) Miller School of Medicine IRB. UM Office of Clinical Research Operations and Regulatory Support (CRORS) will monitor this study and provide the investigator with the overall monitoring plan, visit follow up letters that will include the information regarding of what was monitored and any action items noted during the visit. CRORS will monitor according an executed monitoring plan.

8.1 Data Safety Monitoring Board (DSMB)

Diabetes Research Institute (DRI) will appoint the DSMB to monitor the progress of the trial. DRI will provide DSMB with listings of all AE / SAEs on an ongoing basis, i.e. at least annually.

9 QUALITY CONTROL AND QUALITY ASSURANCE

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

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

9.1 Compliance, Access, Entry and Handling of Study Data

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

The results of all clinical and laboratory evaluations will be maintained in the subjects medical records and the data will be transferred from these source documents directly to the electronic study CRFs.

9.2 Ethical Considerations and Compliance with Good Clinical Practice

9.2.1 Statement of Compliance

This clinical study will be conducted using cGCP, as delineated in *Guidance for Industry: E6 Good Clinical Practice Consolidated Guidance* 163, and according to the criteria specified in this study protocol. Before study initiation, the protocol and the informed consent documents will be reviewed and approved by UM IRB. Any amendments to the protocol or to the consent materials must also be approved by UM IRB and submitted to the applicable Health Authorities before they are implemented.

9.2.2 Informed Consent and Assent

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

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

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

9.2.3 Privacy and Confidentiality

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

9. Archived Serum Samples

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

11. Financing section and Funding Agency:

The study medications will be provided by study team upon to 2 years after the last islet infusion. Thereafter subject, or subject's insurance will be responsible for the costs related to immunosuppression.

Study team will be responsible for medical costs of AE related to study treatment upon to 2 years after last islet infusion. Thereafter subject or/and subject insurance carrier will be responsible for future medical events.

Name: JDRF Grant: 17-2012-361
Address: 26 Broadway 14th floor NY, NY 10004

Name: Diabetes Research Institute Foundation (DRIF)
Address: 3440 Hollywood Blvd. Ste. 100 Hollywood, FL

12. Publication Policy:

University of Miami Publications policy will be used for results publication.

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APPENDIX A: SCHEDULE OF EVENTS (SOE)

Time points (specified in Days relative to transplant)	SCR	WL/BL[1]	0 [2]	3 [3]	7	14	21	28	56	75	120	150	180	210	240	270	300	330	365
Visit Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Visit Windows (specified in days)	N/A	N/A	N/A	N/A	±3	±3	±3	±3	±7	±7	±7	±7	±14	±14	±14	±14	±14	±14	±14
Equivalent Week/Month	N/A	N/A	N/A	N/A	**W1**	**W2**	**W3**	**W4**	**M2**	**M2.5**	**M4**	**M5**	**M6**	**M7**	**M8**	**M9**	**M10**	**M11**	**M12**
GENERAL ASSESSMENTS																			
Informed Consent	X	X	X[22]																
Med/Diabetes Hx & Demographics	X																		
Evaluation of Inclusion / Exclusion	X	X	X[22]																
Physical Exam[17]	X	X-yrly	X[22]		X			X		X			X						X
Telephone Consult						X	X		X		X	X				X			
QOL	X	X-Q6mo								X									X
Chest X-Ray	X	X-yrly																	X
Abdominal US (total)	X	X-yrly			X														X
MRI abdomen and pelvis with IV contrast[30]																			X
^{18}F-FDG PET/CT[30]																			X
ECG	X	X-yrly																	X
Psychological Evaluation	X																		
Cardiac Stress Test	X																		
PPD	X	X-yrly																	X
AE /Hypoglycemic Events/Toxicity Assess			X[22]		X	X	X	X	X	X	X	X	X			X			X
LOCAL LABORATORY ASSESSMENTS																			
CBC (WBC + Diff & Plat)	X	X-Q6mo	X[22]		X	X	X	X	X	X	X	X	X			X			X
Chemistry[3]	X	X-Q6mo	X[22]		X	X	X	X	X	X	X	X	X			X			X
Lipids	X	X-Q6mo								X			X			X			X

Thyroid Function (TSH)	X	X-Q6mo																	X
Pregnancy test (females)	X	X[4,22]																	
Serology[5]	X	X-yrly	X[22]																X

Time points (specified in Days relative to transplant)	**SCR**	**WL/BL[1]**	**0 [2]**	**3 [3]**	**7**	**14**	**21**	**28**	**56**	**75**	**120**	**150**	**180**	**210**	**240**	**270**	**300**	**330**	**365**
Visit Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Visit Windows (specified in days)	N/A	N/A	N/A	N/A	±3	±3	±3	±3	±7	±7	±7	±7	±14	±14	±14	±14	±14	±14	±14
Equivalent Week/Month	N/A	N/A	N/A	N/A	**W1**	**W2**	**W3**	**W4**	**M2**	**M2.5**	**M4**	**M5**	**M6**	**M7**	**M8**	**M9**	**M10**	**M11**	**M12**
LOCAL LABORATORY ASSESSMENTS																			
EBV IgG/IgM	X																		
CMV IgG, CMV IgM	X	X-yrly[6]	X[22]																X
Coagulation (PT, PTT, INR)	X	X-yrly	X[22]					X		X			X						X
Blood Type	X																		
HLA	X[31]																		
Crossmatch [7]		X[22]																	
Fasting & post-prandial glucose and C-peptide				X[8]	X[8]														
CMV by PCR			X[22]							X									X
EBV by PCR[10]			X[22]																
AA/EPA ratio	X	X-yrly	X[22,28]																
DRI / UM LAB																			
Urinalysis	X	X-yrly	X[22]																
Spot urine[11]	X	X-yrly	X[22]					X		X			X						X
Urine C-peptide/Creatinine ratio[25]	X									X			X			X			X
Calculated eGFR	X	X-yrly						X		X									X
HbA1c[12]	X	X-Q6mo								X			X						X
Fasting serum glucose and C-pep[12]	X							X	X	X	X	X	X	X	X	X	X	X	X

MMTT glucose C-peptide, and Acetaminophen [12,13]	X									X			X						X

Time points (specified in Days relative to transplant)	SCR[16]	WL/BL[1]	0 [2]	3	7	14	21	28	56	75	120	150	180	210	240	270	300	330	365
Visit Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Visit Windows (specified in days)	N/A	N/A	N/A	N/A	±3	±3	±3	±3	±7	±7	±7	±7	±14	±14	±14	±14	±14	±14	±14
Equivalent Week/Month	N/A	N/A	N/A	N/A	**W1**	**W2**	**W3**	**W4**	**M2**	**M2.5**	**M4**	**M5**	**M6**	**M7**	**M8**	**M9**	**M10**	**M11**	**M12**
IMMUNOSUPPRESSION LEVELS																			
Tacrolimus 12-hour trough levels[18]				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
MECHANISTIC ASSAYS																			
Alloantibody (PRA) [15]	X	X-Q6mo								X			X			X			X
Autoantibody (GAD, IA-2, IAA, Znt8)	X	X-Q6mo						X	X	X	X	X	X	X	X	X	X	X	X
Anti-rabbit antibodies[27]	X	X-yrly																	
Plasma D-dimer	X	X-yrly	X[21]	X[21]	X	X	X	X											
Pro-inflammatory cytokines/chemokines [19]	X	X-yrly	X[20]	X	X	X	X	X		X			X						X
Immunological assays[26]		X[22]											X						X
Lymphocyte Subsets		X[22]			X	X	X	X	X	X			X						X
Unmethylated preproinsulin DNA assays			X[29]																
LOCAL METABOLIC ASSESSMENTS																			

Glycemic Stability (CGM) [12]		X-yrly	X[23]							X									X
BSR [12,14]	X	X-Q6mo								X			X						X
CALCULATED METABOLIC ASSESSMENTS																			
LI	X	X-Q6mo								X			X						X
Clarke Score	X	X-Q6mo											X						X
HYPO	X	X-Q6mo								X			X						X
Beta Score	X									X			X						X
C-peptide/glucose creatinine ratio[24]	X							X	X	X	X	X	X						X
ARCHIVED SAMPLES																			
Serum		X	X[22]							X			X			X			X
IN CASE OF ALEMTUZUMAB INDUCTION																			
Urinalysis			X[22]					X	X	X	X	X	X	X	X	X	X	X	X
CBC w/Diff and Plat			X[22]					X	X	X	X	X	X	X	X	X	X	X	X
Serum Creatinine			X[22]					X	X	X	X	X	X	X	X	X	X	X	X
Thyroid function tests			X[22]							X			X			X			X

[1] WL = Waiting List. BL = Baseline. These samples can be obtained at any time before transplant. Repeat assessments as indicated while subject is on the waiting list every 6 months or annually from screening. All one-time WL/BL assessments should be completed on Day -2 whenever possible, but always prior to start of immunosuppression. For repeat WL/BL assessments, results from test done closest to the start of immunosuppression will be used as the baseline value.

[2] Day 0 = the day of transplant.

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

[4] Complete pregnancy test within 72 hours prior to the initiation of study medication.

[5] Serology includes: HBc Ab, HBs Ab, HBs Ag, HCV Ab, and HIV. Do not repeat Hepatitis B tests if HBs Ab was previously positive.

[6] Repeat only if previous test was negative.

[7] Sample used for crossmatch may be obtained up to 60 days prior to the start of immunosuppression, as long as there is no evidence of infections or transfusions since the time the sample was drawn.

[8] C-peptide should be done locally and drawn fasting, and twice between 1-3 hrs post-prandial on Day 3 and Day 7 post-transplant.

[9] Error.

[10] EBV by PCR should only be done post-transplant if reactivation is suspected.

[11] Spot urine testing includes: albumin, protein, and creatinine

[12] Do not collect for participants with graft failure. Results of tests performed at the time of graft failure will be used for day 75 endpoint calculations.

[13] MMTT with Acetaminophen (to measure gastric emptying) includes 0, 60 and 90 minute samples for glucose and islet hormones, and Acetaminophen. *Following transplantation we will perform an extended 5 hr MMTT collecting samples at -10, 0, 15, 30, 60, 90, 120, 180, 240, and 300 minutes.*

[14] Blood Sugar Record (BSR) is completed using information gathered from subject diary logs, glucometer download data, and insulin requirements.

[15] For transplant, complete alloantibody assessment every 6 months and again on Day -2, regardless of the most recent draw.

[16] Data from 20053135 protocol can be used for this visit. If data was collected more than 12 months prior to enrollment, screening labs from visit 2 should be repeated.

[17] All visits that include a physical exam are in person at Miami

[18] If Sirolimus is used, levels should be checked concomitant with Tacrolimus levels.

[19] Pro-inflammatory cytokines/chemokines

[20] Samples for pro-inflammatory cytokines/chemokines for day 0 will be collected at 12 and 24 hrs after transplant

[21] Daily while in the hospital. For D-dimer, a sample will be obtained prior to placement of peripherally inserted central catheter (PICC) for Thymoglobulin infusion.

[22] Day of admission

[23] Day of admission and will be continued as clinically indicated

[24] C-peptide/glucose creatinine ratio calculation is: [C-peptide (ng/mL) x100]/[glucose (mg/dL) x creatinine (mg/dL)]

[25] Urine C-peptide/Creatinine ratio is obtained from a spot urine. During protocol scheduled site visits coinciding with MMTT, spot urine for C-peptide/creatinine ratio will be obtained before and at completion of MMTT.

[26] A maximum of 50 ml of blood will be obtained to perform T-cell assays to evaluate for anti-donor reactivity. Frequency of T-cell assays will be determined by the principal investigator but will occur no less than every 6 months.

[27] IgG and IgE to rabbit serum proteins and epithelium will be obtained at screening. After transplantation, samples for these antibodies may be obtained as clinically indicated (e.g. in case of a patient developing an immunologic reaction against Thymoglobulin).

[28] AA/EPA ratio will be measured monthly starting at POD -2 until target levels are achieved and as clinically indicated thereafter.

[29] Samples for unmethylated preproinsulin DNA will be collected as follows:

- Serum and plasma samples will be obtained pre-transplant, 1hr, 3hr and 3 days post-transplant.
- Urine samples will be obtained pre-transplant and 5hr post-transplant.

[30] To be performed in patients with persistent graft function and fasting C-peptide ≥0.5 ng/mL.

[31] Test will be done prior to listing subject in UNOS waiting list.

Time points (specified in Days relative to transplant)	**450**	**540**	**630**	**730**
Time Point (months [M] relative to final islet transplant	**M15**	**M18**	**M21**	**M24**
Visit Number	20	21	22	23
Visit Windows (specified in days)	±30	±30	±30	±30
GENERAL ASSESSMENTS				
Physical Exam		X		X
Telephone Consult	X		X	
QOL				X

AE /Hypoglycemic Events/Toxicity Assessment	X	X	X	X
LOCAL LABORATORY ASSESSMENTS				
CBC (WBC + Diff & Plat)	X	X	X	X
Chemistry	X	X	X	X
Lipids		X		X
DRI LABORATORY/METABOLIC ASSESSMENTS				
Spot urine		X		X
Fasting glucose and C-peptide	X	X	X	X
Urine C-peptide/creatinine ratio	X	X	X	X
Calculated eGFR				X
HbA1c		X		X
MMTT glucose C-peptide, and Acetaminophen [12,13]		X		X
Glycemic Stability (CGM)				X
BSR eCRFs		X		X
Lymphocyte Subsets		X		X
Immunological assays[26]		X		X
CALCULATED METABOLIC ASSESSMENTS				
LI, Clarke Score, HYPO, Beta SCORE, C-peptide/glucose ratio		X		X
IMMUNOSUPPRESSION LEVELS				
Tacrolimus Levels	X	X	X	X
MECHANISTIC ASSAYS				
Autoantibodies	X	X	X	X
Alloantibodies		X		X