University of California, San Francisco

Clinical Research Protocol: "PARADIGM"

Pancreatic Islets and Parathyroid Gland Co-transplantation for Treatment of Diabetes in the Intra-Muscular Site

VERSION 4.0

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1. PROTOCOL SUMMARY

Full Title: Pancreatic Islets and Parathyroid Gland Co-transplantation for Treatment of Diabetes in the Intra-Muscular Site: PARADIGM

Clinical Phase: Phase I/IIa

Sample Size: N=8 recipients with Type 1 (c-peptide negative) diabetes

Accrual Period: 2.5 years

Study Population: People with Type 1 (c-peptide negative) diabetes with stable kidney or liver allografts on chronic immunosuppression.

Participating Sites: UCSF

Study Design: Single-center, open label, non-randomized safety and efficacy trial to evaluate co-transplantation of allogeneic parathyroid glands (PTG) with adult pancreatic islets (both PTG and pancreatic islets obtained from same deceased donor) in people with Type 1 diabetes in the intramuscular (IM) site with stable function of liver or kidney allografts on chronic immunosuppression.

A total of 8 patients will be enrolled in the study and followed for a minimum of 1 year up to 2 years after the last islet transplant, depending on enrollment date. The islet manufacturing protocol and patient treatment and monitoring procedures are based on those used in the NIH CIT 06 trial.

Study Duration: 4 years

Primary Objective: The primary objective is to test the hypothesis that co-transplantation of allogeneic PTG with adult pancreatic islets (derived from same deceased donor) in the IM site in people with Type 1 diabetes with functioning kidney and/or liver transplants is safe, allows islet engraftment, and leads to insulin independence.

Secondary Objectives: The secondary objectives include defining the metabolic consequences of transplantation of islets and the safety of additional allogeneic PTG transplantation in normocalcemic patients.

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2. BACKGROUND AND RATIONALE

Over the last decade, pancreatic islet transplantation has become an attractive minimally invasive approach to restore normoglycemia in patients with brittle Type 1 diabetes (T1D) while avoiding the hypoglycemic complications observed with intensive insulin therapy and the surgical complications associated with pancreas transplantation. The consistent demonstration of insulin independence and excellent metabolic control following islet transplantation into patients with Type 1 diabetes by the Edmonton group marked a turning point in the history of islet transplantation [1]. Despite this progress however, widespread application of islet transplantation as a treatment for diabetes mellitus remains limited due to a few significant barriers. *First,* there are a finite number of donor organs available for transplant. *Second,* a significant number of islets infused into the portal vein die shortly after transplant due to instant blood mediated inflammatory reaction (IBMIR) and ischemia [2-5]. *Third*, despite multiple infusions of islets from up to three donors, insulin independence still cannot be reliably achieved after just a few years post-transplant [6-9] The cause of this progressive loss of function is multifactorial, but mounting evidence suggests that much of the islet loss after transplant is directly related to the intraportal transplant site that is used currently in clinical islet transplantation. [10-13]

In contrast, the intramuscular (IM) site has several physiologic attributes that may improve engraftment and durability of islet transplants compared with the conventional intraportal site. The IM site has already been shown to effectively support islet allograft function in pre-clinical animal models [14-18] and has been safely used in humans with minimal to no side effects and promising c-peptide values months to years after transplant [19,20]. Though these IM trials have shown promise, peri-transplant islet loss and lack of sustained long-term reversal of diabetes remain critical hurdles for wide-spread acceptance. Recently, our group at UCSF made an important breakthrough by showing that the parathyroid gland (PTG), a richly vascularized organ that is transplanted routinely in the IM, has the unique ability to support islets conferring complete protection when co-transplanted in the IM site in mice (unpublished data, see Study Reports SR-i-001, SR-i-002). Our work highlights the ability of PTG, specifically the CD34+ vascular endothelial progenitor cells that reside within PTG, to induce neoangiogenesis [20-22] and promote islet engraftment and survival when co-transplanted. The overall objective of this single center trial is to evaluate the safety and applicability of this novel co-transplantation of PTG and islets in the IM site for clinical islet transplantation.

The importance of developing extra-hepatic sites for clinical islet transplantation cannot be overstated, in that it will provide the foundation for the transplantation of stem cell derived beta cells and xenogenic islets. Several groups have developed beta cell like clusters from stem cells [23-25] , and as these cells approach clinical trials, successful clinical translation will depend on a suitable site for transplantation. Along these lines, the first clinical trials with stem cell derived beta cells will require an easily accessible site that permits graft retrieval in case a teratoma or other neoplasms forms from the pluripotent cells. Of equal significance, the low risks of a procedure which can be done under local anesthesia will open the procedure to the multitude of people with diabetes who carry high cardiovascular peri-operative risks [26]. The combination of anrenewable source of beta cells placed in a safe and easily retrievable site can expand the procedure to the millions of patients with Type 1 diabetes.

However, until a clinical grade stem cell derived beta cell is available, establishing the intramuscular transplant site with mature adult islets is feasible now and utilizing the PTG to support both adult islet engraftment and survival will be an essential stepping stone to ultimate expansion to stem cell derived beta cells in the future.

2.1. Consequences of intraportal islet transplantation.

Currently, the intraportal site is overwhelmingly the most common site that has been used for human islet transplantation, mainly because this site consistently resulted in insulin independence following allotransplantation in recipients with Type I diabetes [9,27]. During the procedure, the portal venous system is accessed by percutaneous transhepatic or mini-laparotomy assisted puncture of a portal vein tributary and the islets are then infused directly into the portal system where they lodge in the small portal venules within the hepatic parenchyma and eventually establish a microvascular blood supply [28]. Despite its widespread use, this site is not optimal for islet transplantation for a number of reasons. First, oxygen tension of portal blood is very low (15-20 mm Hg), and this relative hypoxia can compromise the viability and survival of the infused islets [29,30]. This state is further exacerbated by local stagnation of portal blood that develops in the small portal venous tributaries after islet transplantation and can persist for several days [31,32]. Second, intravenous infusion of the islets exposes them to a severe non-specific inflammatory response called the instant blood-mediated inflammatory reaction (IBMIR) which has been shown to destroy a significant percentage of the islet inoculum[33,34]. Third, the location of the islets directly within the portal system exposes them to high levels of immunosuppressive medications being absorbed from the gut, and many of these, such as tacrolimus and sirolimus have direct beta cell toxicity [35]. Together, these factors are thought to be responsible for a loss of 50-75% of the islet inoculum in the first several days after transplantation and are an important reason why most patients require islets from multiple donors to achieve independence [5,27]. Several approaches have been tried to minimize early destruction of intra-portally placed islets, including strategies to promote anticoagulation such as systemic therapy with heparin, and coating of the islet surface with heparin or urokinase [36-38]. In addition, several agents have been used to inhibit the inflammatory response - most promising are fusion proteins that target the inflammatory cytokines IL-1β and TNF-α [39-41]. Although these approaches have improved engraftment and early islet survival, the overall clinical outcomes are only modestly better, with most patients requiring 2 or more islet infusions to achieve independence and functional graft loss occurring in 50-60% of patients within 2-3 years after transplantation.[42]
In addition to these physiologic drawbacks, there are significant risks associated with intraportal transplantation such as hemorrhage, hypotension during the islet infusion, portal vein thrombosis, and periportal hepatic steatosis [43]. In one series, the risk of significant bleeding (hemoglobin drop > 2.5mg/dL or need for transfusion or surgery) after percutaneous islet transplantation was 9% [44]. Nonetheless, the need for systemic anticoagulation after the transplant places these patients at higher risk of bleeding and they must be monitored accordingly. Hypotension unrelated to bleeding can occur during the islet infusion and is thought to result from the inflammatory response caused by the intravascular inoculum. It is generally self-limited and responds to fluid resuscitation and transient vasopressor administration[45]. Portal vein thrombosis is estimated to occur in about 3% of patients[46]. The thrombosis is usually partial and resolves with long-term anticoagulation, but there is a report of complete portal vein thrombosis in a combined islet-liver transplant recipient which required emergent retransplantation of the liver [47]. Hepatic steatosis is seen in as many as 20% of intraportal islet recipients and is thought to result from increased insulin levels in the peri-islet parenchyma. The clinical relevance of this finding is not known at present [48,49]. All of these factors combined with the inability to monitor the graft for rejection or viability make the intra-portal site less than ideal for islet transplantation moving forward.

2.2. Rationale for Intramuscular site.

The search for extra-hepatic alternative sites for islet engraftment and survival is a current focal point in the field of islet transplantation. Most of the data available on alternative sites were achieved in animal models of islet transplantation and includes the subcutaneous tissue, muscle, omentum (epididymal fat pad), bone marrow and gastric submucosa 50. Furthermore, as stem cell-derived islets and xenogeneic islets begin to enter early phase clinical trials, there is an urgent need to define optimal extrahepatic sites for islet transplant grafts that permit graft monitoring and retrieval.
There are several distinct reasons that make the IM site an attractive transplant site. First, since the islets are not directly exposed to blood, IBMIR is minimized or eliminated. Second, the IM site is well vascularized and is perfused by arterial blood, minimizing hypoxia to the islets. Third, because the islets are not directly bathed by portal blood, the likelihood of exposure to high levels of immunosuppressive medications is reduced. Fourth, IM transplantation is a technically easy procedure to perform with very minimal risk to the patient. The islets are injected into a discrete area that can be imaged using non-invasive ultrasound, making it possible to monitor their function/status with biopsy or imaging techniques previously impossible after intraportal infusion.

Additionally, animal models have indicated that the vasculature within islets engrafted in striated muscle is functional and restored within 2 weeks after transplantation and vascular supply was similar to that in native islets 16. In an animal model for both human and murine islets, equivalent or superior graft function was observed for transplantation into skeletal muscle compared with the portal vein 17. In addition to these physiologic characteristics, the IM site already has an extensive body of literature describing its safety and ease with PTG auto and allotransplantation [51,52].

In summary, the IM site represents an attractive new site for islet transplantation that can be accessed safely and has the potential to allow better engraftment and function of the transplanted cells. This in turn may permit achievement of insulin independence with islets from single donors and improve the duration of insulin independence. The ability to retrieve islet allografts will also be important during the early clinical translation trials of stem cell derived beta cells.

2.3. First case report of intramuscular islet autotransplantation in man.

The first clinical islet autotransplantation into the brachioradialis muscle of the right forearm after total pancreatectomy in a 7-year-old female showed graft function up to 2 years later, and the islets were easily visualized through high-resolution magnetic resonance imaging and through GLP-1 receptor scanning[19] . C-peptide increased up to 1.37 ng/mL and a decreased insulin requirement and a normal HbA1c were reported during the 2-year follow-up.

2.4. First case series of intramuscular islet allotransplantation in human

Most recently, Bertuzzi et al in March 2017 reported the first clinical islet allotransplants outcomes in four patients[20]. These patients all had a functioning solid organ transplant and were on immunosuppression at the time of islet transplantation. Their case series showed islet graft function

was observed after transplantation in all the patients, from 3 to 24 months after the transplant but only to a small degree. Indeed, the metabolic impact of graft function remained limited and in 2 out 4 patients rapidly decreased. The authors reported a very favorable safety profile of the procedure with only a mild self-limiting local reaction in the muscle of one patient that spontaneously resolved after a few weeks. No other adverse reactions were noted by the authors. The authors concluded that the IM is a technically feasible and safe location, but better strategies were necessary to enhance peri-operative engraftment.

In light of the above-mentioned need for strategies to improve peri-operative engraftment of pancreatic islets in the muscle, we believe our preliminary data (outlined below in Section 2.5) provides evidence that the co-transplantation of PTG with pancreatic islets is safe and significantly improves the peri-operative engraftment of pancreatic islets in the muscle.

2.5. Preliminary data

2.5.1. Pre-clinical animal model data demonstrating engraftment protection with PTG co-transplantation.

We recently reported that nutrient deprivation acts synergistically with hypoxia to kill stem cell derived beta cell like clusters (BLCs) *in vitro,* particularly those with more mature phenotype[53]. Reducing oxygen tension (pO_2) during *in vitro* β cell differentiation and provision of amino acids locally at the time of islet transplant led to a significant improvement in preserving β cell mass after transplant. More recently, we have made significant advances in optimizing β cell engraftment in the intramuscular site. The novel approach to prevent peri-transplant β cell death stems from the long- standing observation that a different highly vascularized endocrine tissue, the parathyroid gland (PTG), successfully engrafts at a very high success rate (>90%) in thousands of patients every year[52]. The success in PTG transplantation has been attributed to an unusually high percentage (3-5%) of resident CD34+ vascular endothelial progenitor cells. These cells promote neovascularization by producing factors to induce angiogenesis from the host and by becoming mature endothelial cells themselves within days [21].

We thus tested if PTG can support β cell engraftment when co-transplanted. Using insulin-producing cells generated from a genetically modified human embryonic stem cell line (eBC.luc) and a B6 albino strain with a luciferase transgene on the insulin promoter (B6.Mip-luc) that both express luciferase, we monitored viable β cell mass when co-transplanted with PTG in mouse recipients over time using bioluminescence imaging (See Study Reports: SR-i-001, SR-i-005 for further experimental details). We observed unprecedented high level of engraftment with mature mouse and human embryonic stem cell line derived islet β cells in IM and SQ site. These sites are more challenging in achieving high engraftment due to limited vasculature when compared to kidney capsule but are more desirable than extrahepatic sites for clinical trials for the ease of graft monitoring and retrieval if needed. Previously, luciferase signals from transplanted β cells always decrease after transplant. With PTG co-transplantation, we have seen as high as a two-fold increase in the luciferase signal 2 weeks after transplant (compared to signal at day 0, the day of transplant).

Considering the low proliferative rate of the transplanted cells, the increase in luciferase signal is consistent with robust survival and neovascularization that allowed the luciferase substrate to penetrate the graft. Moreover, PTG co-transplantation enabled diabetes reversal using suboptimal doses of human islets [See Study Report SR-i-002, SR-i-003].

Together, these results demonstrate for the first time that β cell death after transplant can be largely prevented by co-transplanting PTG derived tissue. This discovery further suggests, that with improved engraftment, the therapeutic dose of β cells to achieve insulin independence may be greatly reduced from 10,000 IEQ/Kg based on previous clinical experiences[42].

2.6. Expertise of the UCSF clinical islet transplant program

The clinical islet transplant program at UCSF was established in November 2003 with the opening of the IND application for pancreatic islets and certification of the UCSF cGMP clinical islet isolation facility. Since that time, the program has participated in several JDRF and federally funded clinical trials. UCSF was a site for NIH-funded multicenter clinical trials in islet transplantation (CIT) in nonuremic patients with Type 1 diabetes as well as patients with Type 1 diabetes and prior kidney transplants[6].

During the past 7 years we have performed 45 islet isolations of which 37 met the release criteria for the NIH CIT trial, and were suitable for transplantation. This represents an 82% success rate in the isolation procedure, which is considerably better than the 40-50% historical processing/transplantation success rates described by clinically active centers [54]. To date, 29 patients have received allogeneic islet transplants at UCSF. All had evidence of graft function as demonstrated by reduced insulin requirements and detectable c-peptide levels, and 26 achieved insulin independence for at least 1 month. Currently, 8 of 21 patients transplanted since 2007 remain insulin independent 1-7 years after their final islet transplant and 3 others regained independence after receiving pancreas transplants. These outcomes are among the best in the world and have resulted in numerous presentations and peer-reviewed publications [55-58].

Recently Completed Trials: Long-term (10 year) data are available from the JDRF-funded trial that examined the outcomes of islet transplantation in patients treated with antithymocyte globulin (thymoglobulin) induction followed by maintenance immunosuppression with the anti-leukocyte functional antigen-1 (anti-LFA-1) antibody efalizumab (EFA, Raptiva®), or the co-stimulation blocker belatacept (BELA, LEA29Y). [56,57] These biologic agents were selected for their potent immunosuppressive properties, absence of beta cell and renal toxicity, and lack of many of the side effects observed with other currently employed drugs. The immunosuppressive protocol also included sirolimus and/or mycophenolate maintenance immunosuppression and avoided corticosteroids and calcineurin inhibitors (CNIs). All ten patients (5 treated with EFA and 5 with BELA) in this trial became insulin independent after their last islet transplant and had complete resolution of their hypoglycemic episodes. EFA was discontinued in all 5 pts 12-28 months after initial transplant. 3/5 remain insulin independent on MMF and/or sirolimus (81,84,86 months after last transplant). Two resumed partial insulin use and underwent successful pancreas transplant. 3/5 BELA-treated patients remain insulin-independent (56,58,59 months after last transplant), one has resumed low dose insulin use, and one developed insulin resistance, received a pancreas transplant, and is insulin independent. All patients maintain stable renal function (mean pre-transplant GFR = 81.4; mean most recent GFR=70.2). One patient in the EFA group developed post-transplant lymphoproliferative disorder (PTLD) approximately 5 years after transplant which resolved with therapy and discontinuation of immunosuppression. She remains insulin independent off all immunosuppression.

We continue to follow islet recipients who were transplanted as part of the recently completed NIH CIT consortium trials [6]. These trials were designed to optimize the islet isolation protocol, evaluate novel immunosuppressive regimens, and obtain the data necessary for FDA approval of islet transplantation as a viable cellular therapy for Type 1 diabetes mellitus.

Current Trials: We are currently enrolling patients in a NIH U01 trial (Andrew Posselt, PI) which is examining the safety and efficacy of islet transplantation in the gastric submucosal site in Type 1 diabetic patients with functioning renal transplant (See Dr. Andrew Posselt's Cross Reference Letter attached).

2.7. Metabolic core

Our clinical research center has extensive expertise with a wide variety of metabolic tests to assess islet function and insulin sensitivity. Glycemic control in our current islet transplant patients is routinely evaluated with periodic hemoglobin A1c (HbA1c) measurements, insulin requirements, fasting C-peptide levels, mixed meal tolerance test (MMTT), BETA-2 score, and mean amplitude of glucose excursions (MAGE) estimations. In addition, the metabolic core in the UCSF clinical research center has established protocols for oral glucose tolerance tests (OGTT), glucagon stimulated C-peptide release, arginine stimulated (fasting or glucose potentiated) C-peptide release, HOMA, stepped hypoglycemic clamp, euglycemic hyperinsulinemic clamp, and stable isotope studies. All of these protocols have been used extensively in several studies, and our staff is familiar with the procedural details of each test. [59-61]

3. KNOWN AND POTENTIAL RISKS AND BENEFITS TO HUMAN PARTICIPANTS

3.1. Human Subjects Involvement:

Human subjects will be enrolled in this study using informed consent that have been reviewed and approved by the institutional review board at UCSF. All study protocols will also be reviewed and approved by the institutional review board at UCSF prior to implementation. No human studies will be undertaken until appropriate approval has been obtained.

This study examines the outcomes of human islet transplantation, and thus there are no suitable alternatives to using human subjects, blood, and tissue. The UCSF clinical islet transplant program is highly experienced in performing human islet isolation and transplantation and has a proven track record at performing these procedures safely.

Subjects with Type 1 diabetes who have received a kidney or liver transplant will participate in this study. The inclusion and exclusion criteria listed in the study design section must be met by all subjects prior to enrollment.

3.2. Research Materials:

The following research materials will be used:

- Demographic information
- Medical records and physical exam results
- Results of diagnostic procedures
- Blood samples
- Tissue and pathology specimens

Patient privacy and confidentiality will be protected by assigning unique study identification numbers and using these numbers rather than names to collect, store, and report subject information and samples. These numbers will be kept secure at all times and only the PI and co-investigators will have access to the information. Shared biologic specimens or health information will not contain identifiers but will be coded with the unique study number.

3.3. Potential Risks:

The risks to participating in this trial can be grouped into four general categories: 1) risks associated with allogeneic islet transplantation, 2) risks of allogeneic PTG transplantation 3) risks of medications used in this study, and 4) risks associated with the study procedures.

3.3.1. Risks associated with allogeneic islet transplantation

a) *Transmission of disease from donor to recipient*
Although all donor blood samples are screened for conditions including (but not limited to) Human Immunodeficiency Virus (HIV)1, HIV2, hepatitis B, hepatitis C, Cytomegalovirus (CMV), Epstein Barr Virus (EBV) and syphilis, there is a low risk of transmission of these diseases from donor to recipient.

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 and valgancyclovir prophylaxis. For instance, there have been no episodes of CMV disease in 77 consecutive islet recipients transplanted at the University of Alberta (personal communication). EBV polymerase chain reaction (PCR) monitoring will be carried out routinely after transplantation at defined intervals throughout the trial.

b) *Risk of microbial contamination of islet preparations*
There is a low risk of microbial contamination of the islet product. At UCSF, 29 patients have received 37 allogeneic islet infusions, and there has been only one contaminated islet prep. Routine peri-transplant prophylactic antibiotics covered the bacterial contaminant in this case and no clinical complication of bacterial transmission or infection was noted in the islet recipient.

Historically at active islet centers in the US and Canada show low risk of bacterial or fungal contamination of the islet product. 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 (unpublished data). Similar findings were reported in 74 islet preparations transplanted consecutively at the University of Miami since 1999 (unpublished data). The University of Minnesota investigators have previously reported on the incidence and significance of contaminated islet preparations in clinical islet auto- and allotransplantation. 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 by positive blood cultures, abscesses, or intra-abdominal infections) did not differ significantly between the positive and negative culture groups (p=0.99) (unpublished data). In the allogeneic islet transplant group, 7 of 33 patients (21%) received tissues that retrospectively were determined to be contaminated. None of these patients developed serious infectious complications

(despite broad- spectrum immunosuppression). Despite the occurrence of contaminated grafts, there was no serious increase in infectious morbidity (unpublished data). Overall, the risk of islet transplantation-related septicemia is considered very low with the current islet isolation protocols. Of equal significance, currently utilized antibiotic prophylactic regimens have proven to be effective in preventing infections at the surgical site of implantation in previous trials.

c) *Sensitization of the recipient to donor antigens*
As with any allogeneic transplant, the recipient of an organ transplant may become sensitized against donor antigens, and this may limit the number of compatible donor kidneys/livers or other organs should a transplant be needed in the future. Islet transplant recipients who lose their islet graft due to allorejection may develop alloantibodies directed at donor alloantigens. Once an islet graft fails in islet alone patients, immunosuppressive medicines are usually stopped which can lead to the development of antibodies against the transplanted tissues. Islet after kidney (IAK) or islet after liver (IAL) patients are also at risk for developing antibodies against the transplanted islet tissue; however, it is thought to be less than in patients who receive islet alone transplants, since patients with kidney or liver transplants will continue taking maintenance immunosuppressive medications even if the islet graft fails. This is also supported by data from whole organ pancreas transplantation. It is estimated that the overall risk of significant levels of additional sensitization from the islet graft is approximately 5-10% in IAK/IAL patients who continue on maintenance immunosuppressive medications[62,63].

d) *Acceleration of retinopathy with acute correction in glycemic control*
In the DCCT study, 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.

e) *Risk of triggering renal or liver allograft rejection*
A risk unique to IAK/IAL subjects is the possibility that the immune response to the islet transplant could trigger renal/hepatic graft rejection. While the magnitude of this risk is unknown, data from IAK/IALs performed to date, simultaneous kidney- pancreas transplants, and PAK transplants suggests that the risk should be small (see summary above)[63]. Naturally, an important component of follow-up in these subjects will include monitoring the function of the renal and liver allograft and prompt treatment of rejection if it ensues.

f) *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 enrolled in the study or transplanted.

3.3.2. Risks associated with PTG allogeneic transplantation

a) *Risk of clinical hyperparathyroidism from addition of allogeneic parathyroid tissue*

Animal Model of Allogeneic Supernumerary Parathyroid Transplantation **[See Study Report SR-i-004]**.
An unknown potential risk with co-transplantation of allogeneic parathyroid glands with allogeneic human islets is the risk of allogeneic derived hyperparathyroidism. Auto and allogeneic whole parathyroid gland transplants have been safely and effectively performed in multitudes of patients with hypoparathyroidism but in the current literature, there no clinical experience with transplantation of additional parathyroid glands in euparathyroiditic patients.
Of note, there is a minor body of literature that has documented supernumerary parathyroid gland locations as natural occurrence. In random surgical autopsies, 13% of patients were found to have supernumerary (>4) parathyroid glands and this elicited no additional risk of hyperparathyroidism in this small retrospective case series[64].

As outlined in SR-i-004, to address the potential toxicity of extra parathyroid transplant, we performed syngeneic and allogeneic mouse parathyroid gland (mPTG) transplants. Mice normally have 2 parathyroid glands. We transplanted 2 to 5 additional syngeneic or allogeneic parathyroid glands to assess effects of supernumerary parathyroid gland transplantation.

No significant difference was observed in serum calcium and phosphate levels in syngeneic versus allogeneic treatment transplanted mice. Additionally, no significant difference was observed in mice transplanted with 2-5 additional mPTG's versus controls. Microcomputed tomography scans of femur and tibia showed no significant difference in bone mineralization or bone density.

Together with historical animal study data, this study provides further evidence to effective autoregulatory feedback between PTH and calcium in both the host and transplanted normal parathyroid glands[65,66].

Current practice guidelines for parathyroid gland autotransplantation with frozen hyperplastic tissue.
Currently, patients who are diagnosed with hyperparathyroidism due to 4-gland hyperplasia undergo 4 gland exploration and either 1) total parathyroidectomy and autotranplantation of part of a parathyroid gland or 2) 3 and ½ gland removal and have their glands frozen in the case of post-operative persistent hypoparathyroidism. If it is deemed clinically necessary, subsequent autotransplantation of the hyperplastic tissue is performed in a retrievable site in the brachioradialis muscle of the forearm. In that case, the gland can be retrieved easily if hyperparathyroidism re-develops after autotransplantation. We highlight this current clinical practice to illustrate that we are following current endocrine surgical practices to prevent and ultimately treat possible allogeneic derived hyperparathyroidism and ultimately, believe this to be a low risk to the recipient.

Donor Exclusion Criteria. Regarding donor exclusion criteria specific to PTG (see exclusion criteria in subsequent section in more detail), we will be 1) performing a careful screening of donor for history or laboratory evidence of hyperparathyroidism (Ca, Phos, PTH) and 2) including strict exclusion

criteria of MEN 1 or MEN 2a family history in addition to in-place criteria excluding extra-cranial malignancies.

b) Transmission of disease from donor to recipient
Although all 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, there is a low risk of transmission of these diseases from donor to recipient. The risks would be similar to results summarized above for islets due to similar digestion and processing of tissue prior to transplantation. Deceased donors deemed to be at a higher risk for infection by UNOS will not be used in this trial.

c) Risk of microbial contamination of parathyroid preparations
There is a low risk of bacterial contamination of the PTG product as it will be removed in sterile fashion from donor and is not in contact with airway or gastrointestinal tract. The risks for microbial contamination should be equal to or below risks stated for islets, since PTG tissue is not in continuity with the bowel, and therefore the risk of bacterial contamination should be less. We will perform microbial cultures of the UW effluent post-transplant for final product quality testing; performed standardly for islet product.

d) Risk of *triggering renal, liver or islet allograft rejection* or *sensitization of the recipient to donor antigens*

There is limited clinical experience with multi-organ transplantation involving parathyroid in the literature. Here, we detail the parathyroid allotransplant experience from the available literature and further focus on the parathyroid transplant experience in recipients who have previously obtained a solid organ allograft on stable immunosuppression. This population affords the best clinical data as to the possible risks of sensitization of current solid organ allograft.

As stated previously, the prior parathyroid allotransplant clinical experience is strictly for treatment of persistent hypoparathyroidism (HP). Parathyroid allotransplantation has not gained wide acceptance as the main treatment modality of HP due to the need for chronic immunosuppression. Summary of the available literature on parathyroid allotransplantation in humans is outlined in Table 1.

Table 1: **Summary of normal parathyroid gland allotransplantation in humans on immunosuppression with stable solid organ graft**

Year	Authors	Study	N	Match	Immunosuppression	Graft Survival	Notes
2009	Chapelle et al[67]	Case report	1	ABO, partial HLA	Yes, triple IS for renal tx	1 year	Normal gland in prior renal transplant recipient
2014	Giulanotti et al[68]	Case report	1	ABO, HLA identical	Yes, triple IS for renal tx	2 years	Kidney and PTG from HLA identical sister
2016	Agha et al[69]	Case report	1	ABO	Simulect®, steroids, tacrolimus	3 years	Normal gland, living donor
2016	Garcia-Roca et al[70]	Case report	1	ABO, HLA haplo	Yes, triple IS for renal tx	9 mo	Kidney and PTG from sister

None of the published reports cited in the literature describe loss of solid organ allograft due to the addition of parathyroid allotransplantation[67-70]. Rahusen et al in 1997 also reported the first kidney-pancreas and parathyroid transplant for autoimmune polyglandular syndrome and medullary cystic disease. The patient underwent quadruple induction therapy and all three grafts functioned for greater than 2 years, this is the only concurrent pancreas and parathyroid transplant trial to date[71].

The only available literature regarding HLA expression in normal parathyroid glands comes from a study by Bjerneroth in 1993 that showed very low to no HLA I and II expression in normal glands, even with interferon gamma stimulation [72]. In summary, we believe the addition of PTG from the same donor as the islets confers very low risk of additional sensitization or risk of triggering rejection to a recipient on stable immunosuppression.

3.3.3. Risks of medications used in this study:

All of the medications that may be used in this study and their side effects are listed in **Table 2** below. Since recipients will be maintained on their current immunosuppressive protocols, all potential agents are listed in **Table 2**. Immunosuppressive drugs can increase the chance of infections. These drugs have also been associated with a higher risk of malignancy, particularly skin cancers and lymphomas, and decreases in blood cell counts. The immunosuppressive regimen for subjects with a pre-existing renal and liver transplant will consist of thymoglobulin induction (a total of 3mg/kg) along with two doses of methylprednisolone (2 mg/kg prior to the first dose of thymoglobulin, 1 mg/kg prior to the second dose of thymoglobulin; maintenance immunosuppression will consist of medications that the patient was taking for their renal or liver transplant respectively. Severe and potentially life-threatening Cytokine Release Syndrome (CRS) has been reported with the use of thymoglobulin. Post- marketing reports of severe CRS have included cardiorespiratory dysfunction (including hypotension, acute respiratory distress syndrome, pulmonary edema, myocardial infarction, tachycardia, and/or death). If subjects require retransplantation of islets in the future, basiliximab will be used in place of thymoglobulin and steroids.

Table 2. Duration and Principal Side Effects of Medications that may be administered in this Study

DRUG (Purpose)	Typical Duration of Therapy	PRINCIPAL SIDE EFFECTS
Immunosuppressants/ Anti-Inflammatory	**See below**	**In general, the more immunosuppressed a person is, the higher the risk of developing serious infections and cancer, particularly of the skin. Side effects will be treated appropriately. Serious adverse effects may necessitate lowering the dose or discontinuing the drug, which may in turn lead to increased risk of rejection of the transplanted islets or other transplanted organ. If appropriate, another immunosuppressant drug from this list will be substituted or added to the regimen in an effort to reduce the risk of rejection.**

Cyclosporine (Neoral®, Gengraf®) Immunosuppression	Maintenance therapy for kidney/liver transplant	Frequent side effects include headache, diarrhea, nausea, vomiting, **overgrowth of the gums**, hair growth, shaking and numbness, decline in kidney function, elevated blood sugar (diabetes), and high blood pressure. Cyclosporine is known to cause side effects in the unborn child and should not be taken by pregnant or breast-feeding women.
Tacrolimus (FK-506, Prograf®,) Immunosuppression	Maintenance therapy for kidney/liver transplant	Frequent side effects include headache, diarrhea, nausea, and vomiting, shaking, and numbness. **Less frequent** side effects may include diabetes. Rare, but more serious side effects may include seizures, diabetes, decline in kidney function, and high blood pressure.
Sirolimus (rapamycin, Rapamune®) Immunosuppression	Maintenance therapy for kidney/liver transplant	Frequent side effects include increased cholesterol and triglycerides (fats in the blood), liver enzymes (usually temporary and not harmful; very rarely, these increases can indicate irreversible liver damage). Occasional side effects include a decrease in platelets (blood cells involved in clotting), a decrease in white blood cells (cells that fight infection), headache, indigestion, nose bleed, infection, irritation of the mouth, mouth ulcers, rash, and anemia (low red blood cell count).
Prednisone (Used as premedication for thymoglobulin) Immunosuppression	Given prior to thymoglobulin dose to reduce side effects of the thymoglobulin. Low doses (<10mg/day) may be used as part of maintenance therapy for kidney/liver transplant	Side effects are minor and self-limited during a short treatment course but include hyperglycemia, hypertension, depression, euphoria, visual disturbances, impaired healing, and irritation of the stomach.
Mycophenolate mofetil (Cellcept®, MMF) Immunosuppression	Maintenance therapy for kidney/liver transplant	Occasional side effects of MMF are diarrhea, nausea, vomiting, abdominal pain, constipation, loss of appetite, and indigestion. Anemia (a decrease in red blood cells),low platelet counts (blood cells involved in clotting), and a decrease in white blood cells (cells that fight infection)
Mycophenolic Acid (Myfortic®) Immunosuppression	Maintenance therapy for kidney/liver transplant	A version of mycophenolate mofetil coated to protect the stomach, usually resulting in fewer gastrointestinal side effects
Basiliximab (Simulect ®) Immunosuppression	On the day of transplant and the 4th day after transplant (second transplants only)	Occasional side effects of basiliximab include nausea, vomiting, abdominal pain, itching, rash, fever, rapid heart rate, shortness of breath. If any of these occur, appropriate treatment will be initiated
Belatacept (Nulojix®) Immunosuppression	Maintenance therapy for kidney/liver transplant	Side effects are generally minor but include low red blood cell count which may make you feel fatigued, weak, short of breath, and dizzy or lightheaded when you change positions quickly, and low white

		blood cell count which increases the risk of infection; gastrointestinal disorders such as nausea, vomiting, diarrhea, and constipation; swelling, fevers, infections (such as viral, bacterial), low phosphorus levels in the blood which causes a decrease in blood-calcium levels and can lead to bone loss, headaches, cough, joint and back pain, tremors, trouble sleeping, high blood pressure, high cholesterol, incision-site complications, post-procedural pain and mild skin reactions at injection sites. If any of these occur, appropriate treatment will be initiated. One kidney transplant recipient receiving Belatacept in addition to other immunosuppressive drugs developed a progressive and potentially fatal neurologic disease c alled progressive multifocal leukoencephalopathy (PML).
Thymoglobulin Immunosuppression	2 days at time of 1st islet transplant	Frequent side effects include nausea, chills, headache, joint and muscle aches, fever, decrease in white blood cells (neutropenia), and decrease in platelets. Occasional side effects include shortness of breath. Medications to decrease the likelihood of these symptoms occurring will be given before each dose. If any of these occur, appropriate treatment will be initiated. Rare side effects may include heart and lung problems (including low blood pressure, severe difficulty breathing, heart failure, heart attack, and/or death).
Etanercept Anti-inflammatory	4 times, pre-transplant(Day 0), and then days 3,7,10 post transplant	Common side effects include: local skin irritation from injection and upper respiratory infections. Serious side effects can include multiple sclerosis, seizures, or inflammation of the nerves of the eyes; new or worsening heart failure; new or worsening psoriasis; autoimmune reactions, including a lupus-like syndrome and autoimmune hepatitis.
Antibiotics, Antivirals	See below	**There is a risk of an allergic, or more serious, anaphylactic reaction to any of the antibiotics that that may need to be administered. Anaphylaxis is a life-threatening emergency that can be reversed with appropriate medical care but can also lead to death in some instances. Antibiotics will be discontinued if serious reactions occur and appropriate treatment will be given.**
Trimethoprim/ sulfamethoxazole (Septra®, Bactrim®, Cotrim®) (antibiotics)	Every day for **6- 12** months beginning at time of transplant	Low white blood cell and platelet counts, anemia, fever, chills, vertigo, seizures, meningitis, depression, reduced appetite, nausea, vomiting, liver damage, large bowel inflammation, rash, urticaria (hives).
Pentamidine (antibiotic)	Inhaled once a month for 6-12 months. Used only if Trimethoprim/ sulfamethoxazole cannot be given	Low white blood cell and platelet counts, low blood pressure, chest pain, and rash.
Atovaquone (antibiotic)	Once daily for 6- 12 months if trimetho-prim/sulfamethox a-	

	zole or pentamidine cannot be used	Upset stomach, diarrhea, constipation, headache, sleeping difficulties, sweating, dizziness, drowsiness, altered sense of taste.
Clotrimazole (Mycelex ®, an antifungal)	2 months	Nausea, vomiting, stomach distress, and diarrhea.
Fluconazole (Diflucan®, an antifungal)	Once weekly for two months	Nausea, vomiting, diarrhea, loss of appetite, skin rash, fever and headache.
Vancomycin (antibiotic)	Single infusion into the vein on the day of transplant	Damage to hearing, nausea and vomiting, kidney damage, itching, rash, and pain in muscles and bones.
Imipenem/cilastin Primaxin® (antibiotic)	Single infusion into the vein on the day of transplant	Blood clots, low blood pressure, dizziness and seizures.
Levofloxacin (Levaquin®, an antibiotic)	Single infusion into the vein on the day of transplant	Nausea, vomiting, diarrhea, headache, restlessness, rash, seizures.
Valganciclovir **(**Valcyte® an antiviral)	orally, once a day for 6 months	Decreased production of white and red blood cells by your bone marrow, fever, blood clots, and decreased appetite.
Acyclovir (Zovirax®, an antiviral)	orally up to 4 times a day for 3- 6months	Abdominal pain, nausea, decreased appetite, fatigue, headache.
OTHER DRUGS		**If serious side effects occur, the drug will be discontinued or dose will be decreased and appropriate equivalent therapy will be given if available.**
pantoprazole (Protonix®, to minimize the risk of ulcers)	at time of transplant and continued indefinitely	Nausea, vomiting, gas, or bloating
Neupogen® (a White Blood Cell Growth Stimulant)	Injections as needed	Nausea, vomiting, skeletal pain.
Insulin	Given by continuous intravenous infusion around the time of transplant; otherwise the way the subject is used to giving it to him/herself.	Hypoglycemia (blood sugar too low), which can be mild to severe and life- threatening. Mild side effects include a rash (usually temporary).
Cinacalcet, Sensipar®, a calcimimetic, to lower blood Ca levels	Orally, if needed for hypercalcemia	Nausea, vomiting, diarrhea
Mepivacaine 2%	Injection locally for regional anesthesia block	Nausea, pruritis, urticaria, indefinite anesthesia

3.3.4. Risks associated with the study procedures:

The procedures that will be performed as part of the care of research subjects undergoing islet transplantation include risks pertaining to: 1) blood draw testing, 2) metabolic stimulation testing, 3) the procedural risks co-transplantation of islet and PTG into the IM space, and 4) other risks associated with islet transplantation.

a. *Blood draw testing*
The subject may experience some discomfort at the site of the needle entry, and there is risk of bruising and bleeding at the site. There is a remote risk of fainting or local infection.

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

c. *Procedural risks of transplantation into the IM space*
The risks during and immediately after an islet transplant into the IM space include: local irritation, post-op pain, infection, low blood glucose, decrease in blood pressure, and death.

c. *Procedural risks of regional anesthesia axillary brachial plexus block*
The risks during and immediately after axillary brachial plexus block include: local irritation, allergic reaction to anesthesia, infection, indefinite anesthesia, bleeding from axillary artery iatrogenic puncture, decrease in blood pressure and depressed myocardium from intravascular iatrogenic injection, and death.

Protection against risks:

A number of features of this proposal have been designed to maximize the safety of subjects in the trial. Important selection criteria include avoidance of any medical condition that might significantly increase the risk related to islet transplantation. Importantly, this includes selection of kidney recipients who have stable graft function and who are at a low risk for rejection of their graft. In addition, renal and liver graft function in potential trial subjects has to be sufficient (*Kidney*: GFR by CKD-EPI > 40 mL/min/1.73 m2, as well as absence of a rejection episode in the 6 months prior to islet transplant; *Liver*: Stable liver function tests as defined by: SGOT (AST), SGPT (ALT), alkaline phosphatase values < 1.5, or total bilirubin < 1 times normal upper limits at time of study entry, as well as absence of a rejection episode in the 6 months prior to islet transplant) so that it is unlikely that that they will experience imminent graft failure and face the need for ongoing immunosuppression for an islet graft in the absence of a functioning kidney/liver graft. To ensure further that there is no detrimental impact on renal or liver graft function, the maintenance immunosuppression regimen already in place for the renal or liver graft will be continued without marked changes to benefit the islet transplant. This approach will avoid immunosuppressant drug changes that could potentially precipitate renal and/or liver allograft rejection. In addition, the induction immunosuppression regimen based on thymoglobulin is well studied and has been demonstrated to have a favorable safety profile in solid organ transplantation.

The islet and parathyroid gland preparation to be transplanted will be isolated from cadaveric donors thoroughly tested for transmissible infectious agents and donors free of recent high-risk behaviors. In addition, the islet product will be tested prior to infusion to verify a suitable level of purity, viability, mass, and absence of endotoxin.

Since gaining insulin independence in previous islet transplant studies in Type 1 diabetics was dependent on the recovery and infusion of a sufficient islet mass per recipient body weight, the trial has been limited to subjects < 100 kg in an attempt to maximize the likelihood that insulin independence will be achieved with one (or two) islet transplants. It is recognized that subjects ≥ 100 kg could benefit equally for this therapy should a sufficient islet mass be obtainable.

The informed consent process is carefully organized to minimize unrealistic expectations. We will also not enroll volunteer subjects who appear incapable of understanding and/or coping with the possibility of failure. We believe that our process leading to informed consent is purposefully organized so as to minimize psychological risk to the recipient.

Patients receiving induction immunosuppressive medications such as thymoglobulin, or basiliximab will be monitored in the UCSF Moffitt Hospital and will receive appropriate pre-medications to minimize side effects. Side effects of other medications will be monitored closely and substitutions will be made when appropriate in subjects who do not tolerate specific medications. Appropriate prophylactic antibiotics will be given to minimize the incidence of opportunistic infections in patients receiving immunosuppressive medications.

To minimize risks associated with the intramuscular transplant, all subjects will undergo continuous monitoring of vital signs and oxygen saturation and frequent laboratory tests during and after the procedure. Monitoring immediately after the procedure will take place in the recovery area of the Moffitt Post-op recovery suites at UCSF. Only patients who have completely recovered from the procedure will be transported back to 9th floor of Moffitt Hospital at Parnassus campus. Glucose monitoring will be performed at frequent intervals for the first 48 hours after the procedure to detect any episodes of hypoglycemia. Low blood glucose levels (<70mg/dl) will be treated with intravenous and/or oral glucose replacement therapy. Antibiotics will be administered at the time of the procedure to reduce the risk of infection.

The risks associated with blood draws and placement of IV cannulas will be minimized by using sterile technique and providing a secure environment for the procedure. Peripheral blood draws performed during these studies will not exceed 450 cc per eight-week period.

3.4. Potential benefits of the proposed research to the subjects and others

The potential benefits of islet transplantation include insulin independence, improved glycemic control, reduced incidence of hypoglycemic events, and possible reduction in diabetes-associated complications affecting the kidney and/or liver allograft.

The potential benefits of intramuscular islet transplantation (as compared with standard intraportal infusion) include a less invasive and safer approach to islet transplantation, better islet engraftment due to avoidance of the blood-mediated inflammatory response and improved immunologic monitoring of the graft with imaging and percutaneous biopsy options.

3.5. Importance of the knowledge to be gained

Given the drawbacks of conventional intraportal islet transplantation, there is a significant need to develop alternative anatomic sites that are safe and can improve islet allograft survival. Identification of such a site would represent a significant step forward in the field of islet transplantation since it would address the ongoing organ shortage problem and approximate the success rates obtained with solid organ pancreas transplantation. Of equal significance, the establishment of a site that will facilitate retrieval of transplanted islet tissue will be important for future clinical translation of stem-cell derived beta cell clusters as well as xenoislets.

4. OBJECTIVES

This is a single-center, non-randomized, open label safety and efficacy trial to evaluate islet and parathyroid gland co-transplantation intra-muscularly in people with Type 1 (c-peptide negative) diabetes with stable kidney and/or liver allografts. A total of 8 patients will be enrolled in the study and followed for a period of 1-2 years (minimum of 12 months) after the last islet transplant. The islet manufacturing protocol and patient treatment and monitoring procedures are adapted from those used in the NIH CIT 06 trial [6].

4.1. Study objectives

The primary objective is to test the hypothesis that islet co-transplantation with PTG in the IM in patients with Type 1 (c-peptide negative) diabetes with functioning kidney or liver transplants is safe, allows islet engraftment, and leads to insulin independence. The secondary objectives include defining the potential metabolic and improved glycemic benefits of islet transplantation in a novel extra-hepatic site.

4.2. Primary and secondary endpoints

The primary efficacy endpoint is the proportion of subjects who are insulin independent at one year after the first islet transplant. The primary safety endpoint is the incidence of adverse events related to the transplant procedure or immunosuppression, incidence of post- transplant infections and malignancies, incidence of *de novo* sensitization, effect on renal or liver function and risk of hyperparathyroid related complications including hypercalcemia and hypophosphatemia and their associated clinical manifestations.

Secondary endpoints include: 1) the proportion of subjects with an HbA1c <7.0% and free of severe hypoglycemic events from day 28 to 365 after the first and final islet transplant, 2) measures of metabolic control including c-peptide levels), differential c-peptide secretion from bilateral arms, BETA-2 score, response to mixed meal tolerance test, reduction in insulin requirements, Clarke survey and mean amplitude of glycemic excursions (MAGE), insulin sensitivity, and glycemic control as derived from the continuous glucose monitoring system (CGMS).

Budgetary constraints preclude inclusion of a control (intraportal) group into this safety and efficacy trial, and this is a potential weakness of the study. We plan to address this in part by comparing

outcomes with the ongoing CIT06 trial, since the both the protocol and endpoints of that trial are similar to those of the current proposal. In addition, the CITR database has approximately 65-70 islet-after-kidney recipients transplanted between 2007-2010 who were treated with a comparable induction protocol (T cell depletion and etanercept) and monitored for similar endpoints ([6,9]). These patients will also be used for further analysis (see statistical section).

5. SELECTION AND WITHDRAWAL OF SUBJECTS

5.1. Inclusion Criteria

Only subjects who meet ALL of the criteria are eligible for enrollment.

1. Male and female subjects age 18 or older.
2. Subjects who are able to provide written informed consent and to comply with study procedures.
3. Clinical history compatible with Type 1 diabetes (onset < 40 yrs old and insulin dependent for > 5 yrs at enrollment, c-peptide negative).
4. Recipients should have absent stimulated c-peptide (< 0.3 ng/mL) in response to a (Boost® 6 mL/kg BW to a maximum of 360 mL; another equivalent product), measured at 60 and 90 min after start of consumption.
5. Subjects who are > 6 months post-renal transplant or >6 months post-liver transplant who are taking appropriate calcineurin inhibitor (CNI) based maintenance immunosuppression ([tacrolimus alone or in conjunction with sirolimus, mycophenolate mofetil, myfortic, or azathioprine; or cyclosporine in conjunction with sirolimus, mycophenolate mofetil, or myfortic ± Prednisone ≤ 10 mg/day).
6. Stable renal function as defined by a creatinine of no more than one third greater than the average creatinine determination performed in the 6 previous months prior to islet transplant, as well as absence of a rejection episode in the 6 months prior to islet transplant
7. Stable liver function tests as defined by: SGOT (AST), SGPT (ALT), alkaline phosphatase values < 1.5, or total bilirubin < 1.5 times normal upper limits at time of study entry, as well as absence of a rejection episode in the 6 months prior to islet transplant

5.2. Exclusion Criteria

Subjects who meet ANY of the following criteria are NOT eligible for enrollment

1. Presence of donor specific anti-HLA antibodies detected by Luminex Single Antigen/specificity bead assay including weakly reactive antibodies that would not be detected by a flow cross match
2. Insulin requirement of >1.0 IU/kg/day
3. Weight more than 100 kg or body mass index (BMI) > 30 kg/m2.
4. Primary hyperparathyroidism OR secondary hyperparathyroidism
5. Untreated or unstable proliferative diabetic retinopathy.
6. Blood Pressure: SBP > 180 mmHg or DBP >100 mmHg despite treatment with antihypertensive agents.

7. Calculated GFR of ≤ 40 mL/min/1.73 m2 using the subject's measured serum creatinine and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation, as well as presence of a rejection episode in the 6 months prior to islet transplant
8. Elevated liver function tests as defined by: SGOT (AST), SGPT (ALT), alkaline phosphatase values > 1.5, or total bilirubin >1.5 times normal upper limits at time of study entry, as well as presence of a rejection episode in the 6 months prior to islet transplant
9. Proteinuria (albumin/creatinine ratio or ACr > 300mg/g) of new onset since kidney transplantation.
10. For female subjects: Positive pregnancy test, presently breast-feeding, or unwillingness to use effective contraceptive measures for the duration of the study and 4 months after discontinuation. For male subjects: intent to procreate during the duration of the study or within 4 months after discontinuation or unwillingness to use effective measures of contraception. Oral contraceptives, Norplant®, Depo-Provera®, and barrier devices with spermicide are acceptable contraceptive methods; condoms used alone are not acceptable.
11. Active infection including hepatitis B, hepatitis C, HIV, or TB. Quantiferon gold assay will be used to determine TB infection.
12. Invasive aspergillus, histoplasmosis, and coccidioidomycosis infection within 1 year prior to study entry.
13. Any history of malignancy following receiving either the kidney or liver transplant, except for completely resected squamous or basal cell carcinoma of the skin
14. Known active alcohol or substance abuse.
15. Severe co-existing cardiac disease, characterized by any one of these conditions:
    a) Recent MI (within past 6 months),
    b) Evidence of ischemia on functional cardiac exam within the last year, c) Left ventricular ejection fraction < 30%,
    d) Valvular disease requiring replacement with prosthetic valve.
16. Active infections (except mild skin and nail fungal infections).
17. Active peptic ulcer disease or gastritis, symptomatic gallstones, or portal hypertension.
18. Use of any investigational agents within 4 weeks of enrollment.
19. Administration of live attenuated vaccine(s) within 2 months of enrollment.
20. Any medical condition that, in the opinion of the investigator, will interfere with safe study completion.
21. Positive screen for BK viremia at time of screening.
22. Untreated hyperlipidemia – TC > 200 mg/dL, TGC > 200 mg/dL, LDL > 130 mg/dL

5.3. Patient selection and recruitment:

We plan to recruit patients with Type 1 diabetes with preexisting kidney or liver transplants into this trial for the following reasons: 1) subjects are already on chronic immunosuppression, so exposure to additional study-related immunosuppression will be limited to the brief period of induction therapy at the time of islet transplantation; 2) improved glycemic control resulting from the islet transplant may have beneficial renal and cardiovascular effects [73,74]; and 3) study costs can be significantly reduced since many of these patients are already on chronic immunosuppressive regimens, which will be unchanged in this protocol, and 4) a significant portion of long term

management is part of standard of care (SOC) follow-up for recipients of solid organ transplants. This ensures that the patients will have sufficient follow-up following the end of the study period.[75]

We are confident that we will be able to recruit the required number of patients during the proposed study period. UCSF is one of the busiest kidney transplant centers in the world, performing approximately 350 transplants annually. Additionally, UCSF is one of the busiest liver transplant centers in the US performing about 180 transplants annually. Of the kidney transplant patient cohort, approximately 5-8% have Type 1 diabetes as the cause of their renal failure, resulting in approximately 17-28 patients per year who could be screened for study eligibility. Additionally, we have a pre-identified cohort of Type 1 diabetic patients who have received liver grafts in the past who would be excellent candidates for extra-hepatic islet transplants.

Given that islet transplantation in the intramuscular position represents a relatively low additional risk and has the potential to significantly improve these patient's quality of life, the proposed protocol significantly expands the number of potential candidates by eliminating previously restrictive "hypoglycemic episode" inclusion criteria used in prior trials. We believe this protocol is not competing with other on-going islet trials at UCSF as there will be ample study patients whom can be recruited.

Obtaining sufficient pancreata to transplant the subjects recruited into this protocol will also be feasible. UCSF is the only active clinical islet transplant center on the west coast and routinely receives organ offers from California, Nevada, Utah, Arizona, New Mexico and Oregon. This translates to 5-6 deceased donor pancreas offers every month, and approximately 2-3 of these are potentially suitable for clinical islet transplantation. Once pancreata are accepted for islet transplantation, the likelihood of obtaining sufficient islets for transplantation at our institution exceeds 80%. This should secure sufficient numbers for this proposal even in the presence of other ongoing islet transplant trials. Similarly, Donor Network West (DNW) has been offering 8-10 deceased donors per month who have consented to research and donation of parathyroid tissue.

5.4. Donor Acceptance Criteria for Pancreas and Parathyroid Donation

We outline below our current donor selection criteria used for all potential deceased pancreas donors at UCSF for whole organ and islet transplantation.

5.4.1. Donor Exclusion Criteria

Table 3: Donor Acceptance Table- Inclusion Criteria

TEST	METHOD	REQUIREMENT
Identity	Visual Inspection	Container Label must specify Human Pancreas, and a UNOS number must be present.
Supplier	Visual Inspection	The Organ Procurement Organization (OPO) must be identified.

Review of Supplier's Records	Visual Inspection	**A. Donor Acceptance – Inclusion Criteria** 1. Preservation in (i) UW or (ii) HTK Solution(s) 2. Maximum 12 hr cold ischemia time 3. Donor age 15-65 years 4. Cause and circumstances of death acceptable to the transplant team

Table 4: Donor Acceptance Table- Exclusion Criteria

TEST	METHOD	REQUIREMENT
Review of Supplier's Records	Visual Inspection	**B. Donor Acceptance – Exclusion Criteria** Any of the following criteria is grounds for rejection of a potential donor: 1. History or biochemical evidence of Diabetes Mellitus Type 1 or 2 (Transplant teams may consider donor HbA1C > 6.1% in the absence of transfusions in the week prior to death as an indication for exclusion, with discretion for donors who have received transfusions.) 2. Non-heart-beating cardiac death 3. Malignancies as the cause of death, other than resected basal or squamous cell carcinoma or intracranial tumor. 4. Suspected or confirmed sepsis 5. Evidence of clinical or active viral Hepatitis (A, B, HBcAg, or C) (HBsAb+ is acceptable, if there is a history of vaccination) 6. Acquired Immunodeficiency Syndrome (AIDS) 7. HIV seropositivity (HIV-I or HIV-II), or HIV status unknown 8. HTLV-I or HTLV-II (optional) 9. Syphilis (RPR or VDRL positive)* 10. Active viral encephalitis or encephalitis of unknown origin 11. TSE or Creutzfeldt-Jacob Disease 12. Suspected rabies diagnosis 13. Treated or active tuberculosis 14. Individuals who have received pit-hGH (pituitary growth hormone) 15. Any medical condition that, in the opinion of the transplant team, precludes a reasonable possibility of a favorable outcome of the islet transplant procedure. 16. Clinical history and/or laboratory testing suggestive of West Nile Virus, vaccinia, or SARS The following behavioral profiles will also be considered as grounds for rejection of a potential donor: 17. High-risk sexual behavior within 5 years prior to time of death: Men who have had sex with men, individuals who have engaged in prostitution, and individuals whose sexual partners have engaged in high-risk sexual behavior 18. Non-medical intravenous, intramuscular, or subcutaneous drug use within the past five years 19. Persons with hemophilia or related clotting disorders who have received human derived clotting factor concentrates 20. Findings on history or physical examination consistent with an increased risk of HIV exposure. 21. Current inmates of correctional systems and individuals who have been incarcerated for more than 72 consecutive hours during the previous 12 months.

In the USA tests for transmissible disease, indications must be performed by CLIA-certified laboratories.
*Test results required by FDA regulation

5.4.2. Donor Pancreatic Islet Selection Criteria

Based on the previous experience of UCSF islet isolation clinical team and multivariate analysis performed as part of previous CIT trials, the following selection criteria will be used in accepting donor pancreata for islets in this trial. Ultimately, the decision will be multifactorial and at the discretion of the operating transplant surgeon and clinical principal investigator.

Table 5: Donor Selection Criteria for Pancreatic Islet Donation

Criteria	Pancreatic Islet Donation
Age	15 to 65 yrs
Body Mass Index*	$\geq$ 21
History	Absence of Type 1, Type 2 and gestational diabetes, pancreatic trauma, and sepsis. Donor HbA1C $\leq$ 6.1%
Circulation	Absence of relevant hypotensive episodes, i.e. episodes that are followed by persistent organ dysfunction (e.g., significant increase of creatinine, AST, ALT, or drop in urine output)
Blood Glucose	Minimum recorded blood glucose w/o insulin <200 mg/dL, i.e. at least one glucose < 200 throughout hospitalization
Sex/ABO	Any
HLA	Any
Serology	Negative for HIV, Hepatitis A, B, and C, Syphilis, encephalitis, TSE, Rabies, Tuberculosis, West Nile Virus, Vaccinia, or SARS
Cold Ischemia	< 12 hrs

*Body Mass Index (weight in kg/height in m^2)

5.4.3. Donor Parathyroid Selection Criteria:

Based on our pre-clinical PTG animal and stability studies, as well as accepted endocrine surgical practices in autotransplantation, the following selection criteria will be used in accepting donor parathyroid glands. Ultimately, the decision will be multifactorial and at the discretion of the operating transplant surgeon and clinical principal investigator.

Table 6: Donor Selection Criteria for Parathyroid Donation

Criteria	Parathyroid Donation
Age	15 to 65 yrs
Body Mass Index	$\geq$ 21

History	Absence of primary, secondary or tertiary hyperparathyroidism, parathyroid adenoma, parathyroiditis, parathyroid carcinoma or primary hypoparathyroidism. Absence of family history of MEN (Multiple Endocrine Neoplasia) any tyoe.
Circulation	Absence of relevant hypotensive episodes, i.e. episodes that are followed by persistent organ dysfunction (e.g., significant increase of creatinine, AST, ALT, or drop in urine output)
Blood Ca	Absence of serum Ca>10.5.
Sex/ABO	Any
HLA	Any
Serology	Negative for HIV, Hepatitis A, B, and C, Syphilis, encephalitis, TSE, Rabies, Tuberculosis, West Nile Virus, Vaccinia, or SARS
Cold Ischemia	< 12 hrs

6. STUDY DESIGN

This is a prospective, single-center, open label, non-randomized trial examining the safety and efficacy of co-transplantation of adult pancreatic islets and parathyroid gland in patients with Type 1 diabetes in the intramuscular site. Study subjects who have met the inclusion and exclusion criteria and completed all pre-transplant assessments will be recruited and transplanted during the initial 1.5 years of the trial and then followed for at least 2 years after the final islet and PTG co-transplant.

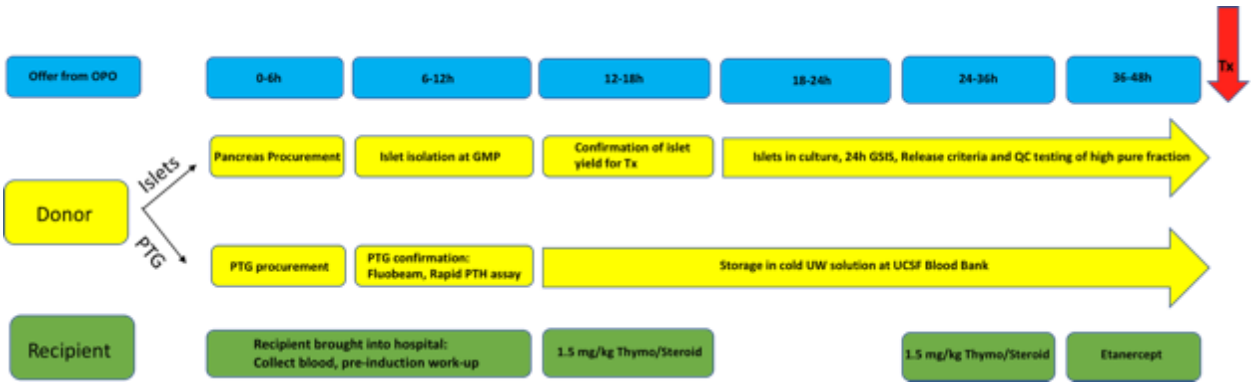


Figure 5: Schedule of events from donor offer to transplantation.

6.1. Formulation of Test and Control Products – Pancreatic Islets

6.1.1. Islet manufacturing:

The islet manufacturing process will closely follow protocols developed for the CIT trials. Briefly, pancreatic islets will be isolated from deceased donor pancreata and will be maintained in tissue culture for 24-48 hours prior to transplantation. Criteria for transplantation will include a yield of > 5,000 Islet Equivalents/kg recipient body weight, viability > 70%, purity >30%, glucose stimulated insulin release (GSIR) index > 1.0, endotoxin levels < 5.0 EU/kg recipient body

weight, and negative gram stain of the islet culture fluid [55,76]. Since the volume of the inoculum is important for the intramuscular injections, we will limit the settled tissue volume to <5 cc. This may decrease the total number of islets that are transplanted, but as shown in the table below, this effect will be relatively modest, since our pure fraction pellets which contain approximately 60-70% of the total islets have relatively small volumes (1.96+1.43 cc). The high purity aliquot will then be supplemented with the lower purity aliquot to achieve the 5 cc volume limit, and thus the final prep will contain approximately 80-90% of the initial islet mass. This will then be suspended in media and brought to the operating room.

6.1.2. Description of islet investigational product

Isolated human islets of Langerhans are used in this study. The development of a relatively non-toxic preparation method to isolate and purify islets is believed to be a key component in the success of the transplant procedure. The transplanted material, harvested from a cadaveric donor pancreas, needs to meet reasonable criteria for product composition, i.e., purity, quality, viability, and microbial contamination. The use of xenoprotein products (such as fetal- calf serum) is avoided during islet isolation and purification, and 25% human albumin is used instead. To minimize the risk of islet injury as a result of warm or cold ischemia, our protocol transplants cultured islets 48 hours after harvesting them.

A collection of standard procedures used at the University of California, San Francisco forms the basis for the methodology of preparing islet cells for transplant under this protocol. Section 6.1 is a description of the islet cell isolation methodologies and characteristics that will be used in all preparations for transplantation.

6.1.3. Method of preparation

The methods for islet cell preparation have been divided into four main sections. These include isolation of the donor pancreas and preparation for transfer to the laboratory, the automated method for pancreatic tissue dissociation, enzymatic digestion of the pancreas tissue and separation of the islet cells, purification of the islets and culture, and final preparation for transplantation. Detailed procedures prepared by NIH CIT Study and UCSF will be implemented.

6.1.4. Isolation of the pancreas and preparation for transfer

Pancreases are removed with minimal handling from brain-dead donors after informed consent has been obtained from the donors' relatives, and utilizing standard techniques previously developed for pancreas procurement for whole organ pancreas transplantation. Pancreata will be stored in chilled UW (University of Wisconsin solution, available from DuPont Pharma and or Barr Laboratories, Pomona, NY, and other sources) or comparable solution. Donor selection criteria are derived from the results of a multivariate analysis of factors that influence the success of islet isolation[77]. Details regarding donor criteria are provided in Section 5.4.2

All subsequent steps are performed under aseptic conditions in a Class II biological safety cabinet within a clean room facility. All solutions utilized in this procedure comprise of sterile components.

The UCSF Islet and Cellular Transplantation Facility is located on the 6th floor of the Mission Center building at 1855 Folsom Avenue, San Francisco. This 4,500 square foot clean room facility is dedicated to the manufacture of biological products following cGMP regulations and is operated by the University of California San Francisco– Diabetes Center. The Islet Isolation Suite, where the islet processing will take place is at the highest relative pressure. All areas of the facility are supplied by one pass HEPA filtered air. The rooms are also constructed to provide for unidirectional flow of both personnel and materials. Materials enter the space through a pass through in the staging area while personnel enter through the gowning room, into a clean corridor, and then enter the islet isolation suite.

The Islet Isolation Suite will be used solely for the processing of human donor islets and human donor parathyroid glands. This room is a class 10,000 (Federal Standard 209E) cleanroom. Particle counts from preliminary particle testing reveal values far below the standard for a class 10,000 cleanroom (the average particle count was 486 particles/ft) [78]. The Islet and Cellular Transplant Facility also includes class 10,000 cleanroom support areas (Quarantine Room, Release Materials Room, Material Preparation Area, Gowning, and Decontamination/Degowning room). A class 100 cleanroom is located on the sterile side of the sterilizing pass-through Autoclave. The Islet Isolation Suite consists of five class 100 biological safety cabinets (BSC), one dedicated for each of the following procedures: Dissection (trimming and cleaning the pancreas and cannulation of the pancreas duct), Distension (collagenase loading), Digestion (enzymatic and mechanical dissociation of the pancreas which is connected via a pass-through to another class 100 BSC used for recombination of the tissue suspension), and Purification and Culture (islet purification using a Cobe 2991 Cell Processors). The islet cultures will be placed into one of two Kendro Heracell 37°C CO_2 incubators or Hotpack 22°C CO_2 refrigerated incubator.

6.1.5. Enzymatic digestion of the pancreas to obtain free islets

Upon arrival, the pancreas transport box will be inspected for package integrity and then wiped down with an antimicrobial cleaner, placed into pass-through and then transferred to the islet isolation suite. In the BSC, the pancreas will be sterilely removed from its container and placed in a sterile tray with cold transport solution, extraneous fat and non-pancreatic tissue will be carefully dissected and discarded. The pancreas will then be placed in a topical antibiotic solution of 80 mg gentamicin, 1g Cefazolin, and 100 mg amphotericin-B solution of cold Hanks' Balanced Salt Solution (phenol red-free) (HBSS) or Trimming solution (Mediatech Inc./Corning, Manassas, VA or Protide Pharmaceuticals, Lake Zurich, IL). After a 5-minute incubation period, the pancreas will be rinsed in a beaker containing 200 mL of HBSS.

After cleaning and trimming the pancreas of fat and vessels, the pancreas is weighed and then transferred to a sterile tray containing fresh dissection solution or HBSS. The pancreatic duct of each half of the pancreas is cannulated with an angiocatheter (14-22 gauge). The pancreas is then transferred to the second BSC and perfused under controlled conditions using a collagenase perfusion solution (SERVA Electrophoresis GmbH, Heidelberg, Germany; Roche Diagnostics Corporation, Indianapolis, IN; VitaCyte LLC., Indianapolis, IN). A constant pressure of 60-80 mmHg is maintained for the first 4 minutes and 160-180 mmHg for the next 4-6 minutes. The perfusion solution consists of collagenase and neutral protease dissolved in Phase I Media (Mediatech Inc./Corning, Manassas, VA or Protide Pharmaceuticals, Lake Zurich, IL). After 8-10 minutes of cold perfusion, the pancreas will be further trimmed of the remaining capsule and cut into 3-5 cm^2 pieces.

The pancreas pieces are then transferred to the third BSC and placed in a sterile "Ricordi chamber" containing sterile nitrite marbles. Any collagenase solution that has "leaked" from the distended pancreas is added to the chamber along with DNAse (Genentech, South San Francisco, CA) and the remaining system volume filled with HBSS or Phase I Media Mediatech Inc./Corning, Manassas, VA or Protide Pharmaceuticals, Lake Zurich, IL). The collagenase solution is recirculated during Digestion Phase at 32°C to 37°C as the chamber is agitated. Samples are taken at regular intervals to monitor, via inverted microscope, the digestion of the pancreas and the liberation of islets. The "switch" from Digestion Phase to Dilution Phase occurs when there is an increase in the amount of tissue liberated from the chamber, most or all of the islets are free of the surrounding acinar tissue, intact islets are observed, and the acinar tissue becomes finer (small cell clusters).

Once the switch point is reached, the islet isolation is continued in a system in which the temperature is progressively decreased to 25-30°C and the collagenase is diluted with RPMI-1640 w/out phenol red (Mediatech Inc., Manassas, VA). The digest containing the free islets is collected first in four 1-liter Erlenmeyer flasks, pre-filled with RPMI-1640 and 25% human serum albumin, followed by four more 1-liter Erlenmeyer flasks, pre-filled with 50 mL of 25% human serum albumin. During the dilution phase, digest samples are taken and stained with dithizone (Sigma Chemical, St. Louis, MO); the percentage of free islets, the degree of fragmentation, and the condition of the acinar tissue are noted. The collected digest are transferred to conical tubes and centrifuged at 140 x g and 8°C for 4 minutes. The pellets are subsequently suspended in Cold-Storage Solution (Mediatech Inc./Corning, Manassas, VA or Protide Pharmaceuticals, Lake Zurich, IL), combined into a 1000 mL beaker and kept cold. If stringiness or clumping of tissue is observed, DNAse may be added to the RPMI-1640 or Cold-Storage wash.

Once the entire digest has been collected, the combined pellets are distributed into one to two 250 mL conical tubes. The tubes are centrifuged at 220 x g and 8°C for 3 minutes and the supernatant is discarded.

6.1.6. Purification of islets by centrifugation using a continuous density gradient

Oxidixianol continuous gradient (Optiprep, Axix-Shield, Oslo, Norway) and Cold-storage Solution (Mediatech Inc./Corning, Manassas, VA or Protide Pharmaceuticals, Lake Zurich, IL) is prepared in a dual chamber gradient maker using a light density gradient (1.075-1.085 g/cm^3) and a heavy density gradient (1.09-1.105 g/cm^3), which is pumped onto a 2991 Cobe cell processor spinning at 1800 rpm. The suspended pancreas tissue is pumped slowly onto the continuous gradient followed by a capping layer (density 1.040 g/cm^3). After spinning at 1800 rpm for 3 minutes, 25 mL fractions are collected and screened for the presence of islets. Fractions with islet purities (percentage of dithizone positive cells) > 70% will be combined for culture. Fractions with islet purities between 65% and 40% will be combined and cultured separately.

6.1.7. Islet Culture

The islet suspension is cultured free-floating in an atmosphere of 95% air and 5% CO2 in 175 cm2 tissue culture flasks in CMRL 1066 (Mediatech Inc./Corning, Manassas, VA or Protide Pharmaceuticals, Lake Zurich, IL) and 0.5% final concentration of 25% human serum albumin. Islet preparations with purity >70% will be cultured overnight at 37^{O}C and for an additional 24 to 48 hours at 22^{O}C. Islet preparations with purity < 70% are diluted appropriately to give the same tissue concentration as pure preparations and will be cultured at 22°C until transplant.

6.1.8. Preparation of purified islets for transplantation

Prior to transplantation, the islets are collected from the tissue culture flasks, washed in sterile transplant medium [phenol-red free CMRL-1066 (Lonza, Basel, Switzerland) supplemented with 2.5% human serum albumin to remove cellular debris, tissue culture media and soluble proteolytic activity. The islets are then suspended in 200 milliliters of transplant medium supplemented with heparin at 70 U/kg recipient body weight and collected into a 600-mL Fenwal Transfer Pack Container. The container is labeled as Allogeneic Islets of Langerhans and will also include recipient information, reference number of the allogeneic islet preparation, and processing time and date of the allogeneic islet preparation. Final islet product will be labeled as followed:


University of California – HICTF & GMP Facility
1855 Folsom Ave. San Francisco, CA 94103
FDA Registration #: FEI3005404215

HUMAN ISLETS, Allogeneic

IND # _____

Date of Manufacture: _____ Lot#:CHIP -_____

Donor UNOS I.D. # _____

Donor ABO/Rh Blood Type: _____

Recipient Name: _____

Recipient Medical Record #:_____

Recipient ABO/Rh Blood Type: _____

ISLET INFORMATION

Date / Time of Islet Release: _____ am / pm

Expiration Time (6 hrs after release): _____ am / pm

TOTAL Volume: _____mL

Total Tissue Volume: _____ mL

Total IE/bag: _____

Total Heparin (U/bag): _____ Cipro? Yes / No

Sterility Testing has not been completed

BIOHAZARD warning: *Human Tissue*

Caution: New Drug - Limited by Federal (or United States) law to investigational use

STORE AT ROOM TEMPERATURE

Bag # ____of ____

6.1.9. Resuspension of islets prior to transplant

When the islet product arrives in the operating room, the islets are allowed to settle by gravity in the transport container. These settled islets are then transferred into a 10 - 20 ml syringe, in a volume less than 5cc, in preparation for mixing with PTG and ultimately intramuscular transplant.

6.1.10. Product testing

For the clinical trial, all islet preparations under this protocol are tested in the same manner. Adventitious agents (Sterility Testing) of islet cultures will be conducted by UCSF Microbiology and all quality control assays will be performed at the UCSF Islet and Cellular Transplantation Facility on the final islet product.

6.1.11. Quality control testing during islet cell preparation

The in-process quality tests are listed in **Table 7**. The minimum number of islets that will be infused into a recipient will be titrated on the basis of the recipient body weight. A minimum of 5,000 IEQ/kg will be infused for the first transplant. A second infusion of islets, if necessary, will ensure that each recipient receives a minimum of 10,000 IEQ/kg cumulative total islet transplanted mass.

Table 7. In-process quality testing for pancreatic islet product

Test Sample	Parameter and Test Method	Specification
Digested material during digestion (repeated during process until desired quality reached)	Determination of acinar-free (not trapped) islet cells using visual examination by qualified personnel	A target proportion of >50% acinar-free islets is desired, but this will depend on the rate of digestion and islet fragmentation
Cell suspension before continuous gradient separation	Islet quantification using diphenylthiocarbazone (DTZ) and visual examination by qualified personnel	> 250,000 IEQ
Cell suspension after gradient separation	Islet quantification using DTZ and visual examination by qualified personnel	To provide >5,000 IEQ/kg recipient body weight for initial transplant, and cumulative total of 10,000 IEQ/kg following the second transplant

6.1.12. Quality control testing of the final product.

All final product tests are listed in the following **Table 8** (tests completed prior to transplant) and **Table 9** (tests performed following transplant). As above, the islet quantification value of > 250,000 IEQ is a minimum that should be adjusted according to the recipient body weight and the target infusion of at least 4,000 IEQ/kg per transplant (5,000 IEQ/kg for initial transplant)[79].

Table 8. Final pancreatic islet product quality testing reported prior to transplant

Test	Test Method	Criteria
Purity	DTZ-staining and enumeration using light microscopy with visual examination by qualified personnel	> 30%
Viability	Fluorescent dye method using fluorescein diacetate (FDA) and propidium iodide (PI) with visual examination by qualified personnel	> 70%
Islet yield (Cell number)	DTZ-staining and enumeration using light microscopy with visual examination by qualified personnel	To provide >5,000 IEQ/kg recipient body weight for initial transplant, and cumulative total of 10,000 IEQ/kg following the second transplant
Tissue transplant volume (volume, cc or mL)	Measure settled tissue volume in conical tube	Not to exceed 5cc settled volume
Microbiological assessment	Gram stain on 100 µL smear with microscopic examination by qualified personnel	No intact organism staining observed

Table 9. Final pancreatic islet product quality testing reported after transplant

Test	Test Method	Criteria
Insulin secretion (potency stimulation index)	In vitro insulin release in 2.8 and 20mM glucose	>1.0
Endotoxin unit (EU) content	USP or equivalent method	<5% EU/kg body weight
Microbial contamination	Culture for aerobes, anaerobes, and fungi	No growth by culture*

* Growth of any organism from the final implanted islet cell preparation will be further evaluated and the subject treated as medically indicated. Cultures will be incubated for 14 days for aerobic/anaerobic bacteria and 28-30 days for fungus. All testing will be conducted by the UCSF Microbiology department.

6.1.13. Quality control procedures:

Package inserts and certificates of analysis for all of the media and supplements used will be filed. Catalog and lot numbers will be recorded in the batch production records. Solutions are prepared in compliance with FDA's Good Manufacturing Practices (GMPs) regulations as custom-made solutions for islet preparations. No reagents containing xenoproteins are used in the preparation of the islets. Currently, HBSS, H-Phase I and Dissection solutions, RPMI-1640 and CRML-1066 are prepared by Mediatech, Inc and Protide Pharmaceuticals. Collagenase,

neutral protease, and thermolysine are prepared by SERVA Electrophoresis GmbH, Roche Pharmaceuticals, and Vitacyte LLC. With respect to Human Serum Albumin, we are in close contact with the UCSF pharmacy and we will be notified regarding a recalled lot of albumin. Removal of a recalled lot occurs immediately upon notification by the manufacturer. All liquids are handled using sterile techniques and transplant infusion media prepared by Lonza Walkersville, Inc. is filtered before use using 0.22 µm sterile filtration systems.

6.1.14. UCSF islet isolation results

Qualifying preparations will meet all the above quality criteria including endotoxin levels and tests for microbial contamination, and all must meet a minimum yield of > 5,000 IEQ/Kg recipient weight. **Table 10** demonstrates our ability to isolate and purify donor human islets.

Table 10. Settled pellet volumes of the most recent clinically used islet preparations at UCSF

		BAG 1 (High Purity)			BAG 2 (Lower Purity)		
Isolation #	**Pt Wt (kg)**	**Pellet Vol (cc)**	**IEQ/Bag**	**IEQ/kg**	**Pellet Vol (cc)**	**IEQ/Bag**	**IEQ/kg**
cHIP 70	69.3	2.3	602,620	8,696	7.8	221,670	3,199
cHIP 69	92	3.6	578,460	6,288	7	125,180	1,361
cHIP 68	72	1	423,470	5,882	10	163,070	2,265
cHIP 67	70	1.9	506,780	7,240	5	196,550	2,808
cHIP 66	59	0.6	399,490	6,771	6.2	152,570	2,586
cHIP 65	68	5.7	513,330	7,549	8.8	217,970	3,205
cHIP 64	50.5	1	400,370	7,928	10	112,880	2,235
cHIP 63	63	0.8	383,047	6,080	3.2	212,181	3,368
cHIP 62	72	0.6	235,800	3,275	12.2	459,393	6,380
cHIP 61	77.7	0.5	206,873	2,662	7.2	238,581	3,071
cHIP 60	50.5	0.7	398,854	7,898	1.2	89,180	1,766
cHIP 59	68.5	0.75	273,390	3,991	3	269,823	3,939
cHIP 57	60.2	3.3	754,527	12,534	0	0	0
cHIP 55	47	3.4	771,340	16,411	0	0	0
cHIP 54	49	2	426,920	8,713	0	0	0
cHIP 53	73.3	3.4	493,600	6,734	0	0	0
cHIP 52	96.3	1.8	512,210	5,319	7	209,850	2,179
cHIP 51	77	2	676,500	8,786	5.5	308,800	4,010
Average	**67.52**	**1.96**	**475,421**	**7,375**	**6.72**	**212,693**	**3,027**
±SD	**13.71**	**1.43**	**160,514**	**3,208**	**3.02**	**93,382**	**1,233**

6.2. Formulation of Test and Control Products – Parathyroid Gland

6.2.1. Isolation of the parathyroid and preparation for transfer

The parathyroid tissue will be isolated from the same deceased donor as the pancreas used for islet isolation as described above. Parathyroid glands are removed with minimal handling from deceased donors after informed consent has been obtained from the donors' and/or donor relatives utilizing standard parathyroidectomy operative techniques (**Figure 6**)[80]. We will use intra-operative near infared real time imaging (FluOptics ® Fluobeam 800) to further confirm identification and location of parathyroid gland in addition to anatomical and intra-operative tissue characteristics [80-85].

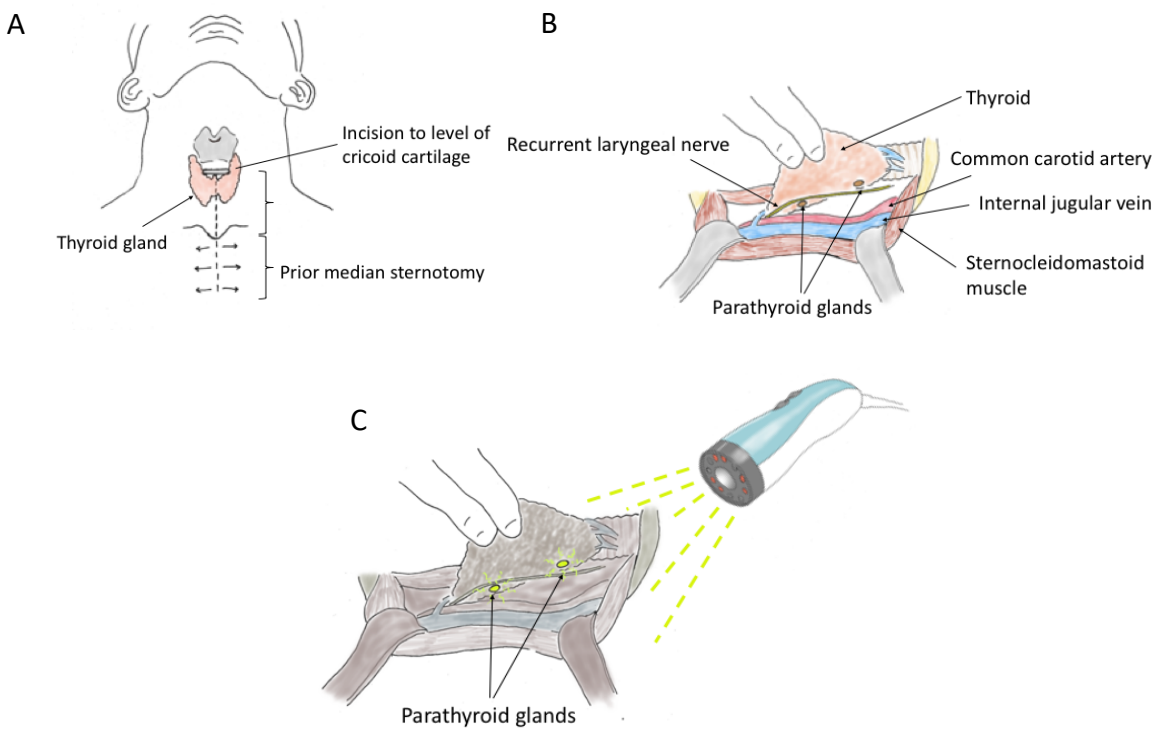
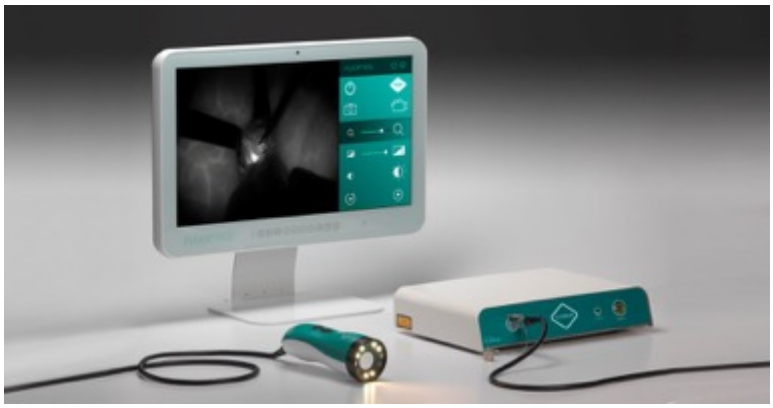


Figure 6: Deceased donor parathyroidectomy operation. **A.** The standard donor sternotomy incision will be extended cephalad to the level of the cricothyroid cartilage. The overlying strap muscles with be divided exposing the anterior portion of the thyroid. **B.** The superior pole of the thyroid will be taken down sharply to allow medial and lateral rotation of the thyroid to expose the parathyroid glands. **C.** After visual identification of presumed PTG's, the Fluobeam camera will be used to further confirm identification of the PTG's via autofluorescence of PTG at near infra-red excitation. (Images courtesy of Dr. James Gardner, MD, PhD; UCSF)

6.2.2. Fluobeam® (Fluoptics, Grenoble, France) assistance in parathyroid gland identification



The Fluobeam® (Fluoptics, Grenoble, France) will be used in this study to help in detecting parathyroid glands. This device, CE marked, and FDA approved, is intended for intraoperative visual assessment of blood vessels and related tissue perfusion while Indocyanine Green (ICG) is injected intravenously into patients. This device has been proved to be able to image parathyroid glands in autofluorescence (no injection). The laser provides an irradiance of 5mW/cm2 at 750nm and the emitted fluorescence is detected in the range 800-900nm. The data were acquired in the

High Sensitivity mode with an integration time of 164ms. Auto-fluorescence requires operating lights to be turned off. The white lights provided by the Fluobeam® (5000 lux, 3000°K, CRI > 90) were turned ON during the experiments allowing the surgeon to clearly see the surgical field. The white light does not interfere with autofluorescence signal.

We have utilized Fluobeam® assistance in the confirmation of 13 glands from four consecutive PTG research donors with autofluorescence confirmed ex-vivo via near infared imaging with Fluobeam® assistance. Of note, example images below highlight the autofluorescence difference between PTG and surrounding soft tissue and cervical lymph nodes (**Table 11**).

Donor ID	Donor Age/Sex	Date of Procurement	Fluobeam Confirmation
AFDH231	44 M	4/10/2018	
AFEF171	16 M	5/9/2018	
AFFV282	49 M	6/24/2018	
AFHT135	40M	7/21/2018	

Table 11. **Parathyroid glands confirmed by autofluorescence**. 13 glands from four consecutive PTG research donors with autofluorescence confirmed ex-vivo via near infared imaging with Fluobeam® assistance. Autofluorescence difference between PTG and surrounding soft tissue and cervical lymph nodes (marked by white arrows).

6.2.3. Preparation of parathyroid glands for transplantation

Parathyroid glands will be immediately washed with saline after removal to limit any possible contamination from upper airway dissection if lung procurement is performed concurrently. All Fluobeam confirmed glands will be stored independently in chilled UW (University of Wisconsin solution, available from DuPont Pharma and or Barr Laboratories, Pomona, NY, and other sources) or comparable solution in separate specimen containers. Details regarding donor selection criteria are provided in Section 5.4.3. The parathyroid glands will be individually triple wrapped and labeled with UNOS ID with gland identifier number and transported on ice. The parathyroid glands will be stored sterile on ice in UW solution for 24-48hrs while pancreatic islet isolation occurs.

Rapid parathyroid hormone assay for additional confirmation

We will further confirm the identity of PTG by utilizing a rapid IOPTH assay from the prospective Fluobeam confirmed glands at the donor hospital. The rapid IOPTH assay is performed routinely in the operating room by the UCSF Endocrine Surgery Division and the UCSF Parnassus Chemistry Laboratory.

The IOPTH assay is performed as follows: under direct vision, a 5 cc syringe with a 1.5 inch (3.8 cm) 23-gauge needle was used to aspirate suspected in situ or ex vivo parathyroid tissue. Two or three passes were used to obtain a sufficient sample. Samples were collected by aspiration of the tissue in question with a syringe containing 2 ml of saline or UW solution and then injecting the saline into ethylenediaminetetraacetic acid (EDTA) tubes for analysis. The vials will be placed on ice and transferred to the chemistry laboratory. This technique is adapted from Perrier et al's description.[86]

The aspirate will be collected prior to packaging the prospective PTG's and placed on ice. The aspirate will be labeled with corresponding UNOS ID and gland identifier that corresponds to the gland the aspirate was taken from. Sample will be run at UCSF Parnassus Chemistry lab according to standard operating procedure. We will utilize cutoff of >1500 pg/ml shown previously to have 100% sensitivity and specificity. Prospective glands that do not meet this cutoff value will be discarded at the transplant surgeon's discretion.

The confirmed parathyroid glands will be stored in a controlled refrigerator in the UCSF Blood Bank; the current site of donor blood vessel and cold perfused pump solid organs awaiting transplantation. There is already in place a secure log-in and sign-out of donor organs and regularly scheduled checks of all storage appliances. Parathyroid gland storage pre-operatively will follow in accordance with these policies and standard operating procedures.

6.2.4. Stability of PTG Activity after 48 hours in cold UW solution storage [See Study Report SR-i-005]

6.2.5. Final Product Quality Testing

All final product tests are listed in the following **Table 12.** Samples will be collected in operating room just prior to preparation of PTG for co-transplantation.

Table 12. **Final product quality testing reported after transplant**

Test	Test Method	Criteria
Endotoxin unit (EU) content	USP or equivalent method	<5% EU/kg body weight
Microbial contamination	Culture for aerobes, anaerobes, and fungi	No growth by culture*

* Growth of any organism from the final implanted PTG preparation will be further evaluated and the subject treated as medically indicated. Cultures will be incubated for 14 days for aerobic/anaerobic bacteria and 28-30 days for fungus. All testing will be conducted by the UCSF Microbiology department.

6.3. Ultrasound Guided Axillary Brachial Plexus Block

The axillary approach to brachial plexus blockade (including musculocutaneous nerve) results in anesthesia of the upper limb from the midarm distal to and including the hand. This procedure is commonly used for arteriovenous fistula, wrist fracture repair and reconstruction cases in forearm and hand plastic surgery cases routinely at our institution. Using ultrasound guidance, the axillary brachial plexus block is a safe minimally invasive procedure and provides appropriate anesthesia for the proposed islet and PTG co-transplantation in the brachioradialis muscle of the forearm.

6.3.1. Regional block technique

With the patient in the proper abducted arm position, the skin is disinfected and the ultrasound transducer is positioned in the short axis orientation to identify the axillary artery about 1 to 3 cm from the skin surface. The needle is inserted in-plane from the cephalad aspect and directed toward the posterior aspect of the axillary artery.

As nerves and vessels are positioned together in the neurovascular bundle by adjacent musculature, advancement of the needle through the axilla may require careful hydro-dissection with a small amount of local anesthetic or other injectate. This technique involves the injection of 0.5 to 2 mL, which 'peels apart' the plane in which the needle tip is continuously inserted. The needle is then advanced a few millimeters and more injectate is administered.

Local anesthetic should be deposited posterior to the artery first, to avoid displacing the structures of interest deeper and obscuring the nerves, which is often the case if the median or ulnar nerves are injected first. Once 5 to 10 mL is administered, the needle is withdrawn almost to the level of the skin, redirected toward the median and ulnar nerves, and a further 5 to 10 mL is injected in these areas to complete the circle around the artery.

Finally, the needle is once again withdrawn to the biceps and redirected toward the musculocutaneous nerve. Once adjacent to the nerve (stimulation will result in elbow flexion), 5 to 7 mL of local anesthetic is deposited.

In an adult patient, 20 to 25 mL of local anesthetic is usually adequate for successful blockade. Complete spread around the artery is necessary for success but infrequently seen with a single injection. Two to three redirections and injections are usually necessary for reliable blockade, as well as a separate injection to block the musculocutaneous nerve.

6.4. Preparation of islet and PTG for co-transplantation

Purified allogeneic islets will be transferred to the OR, donor and recipient identification will be checked per standard operating room protocol. Allogeneic islets will be allowed to settle to the bottom of the 200 ml bag. After settling, a sterile 60 ml syringe will be added to 3-way stopcock and islets will be slowly aspirated from bag until islets are all removed. Syringe will be kept upright to allow islets to settle to bottom and extra media will be returned back into the bag to concentrate islets into 1-5ml concentrated pellet (**Figure 8)**.

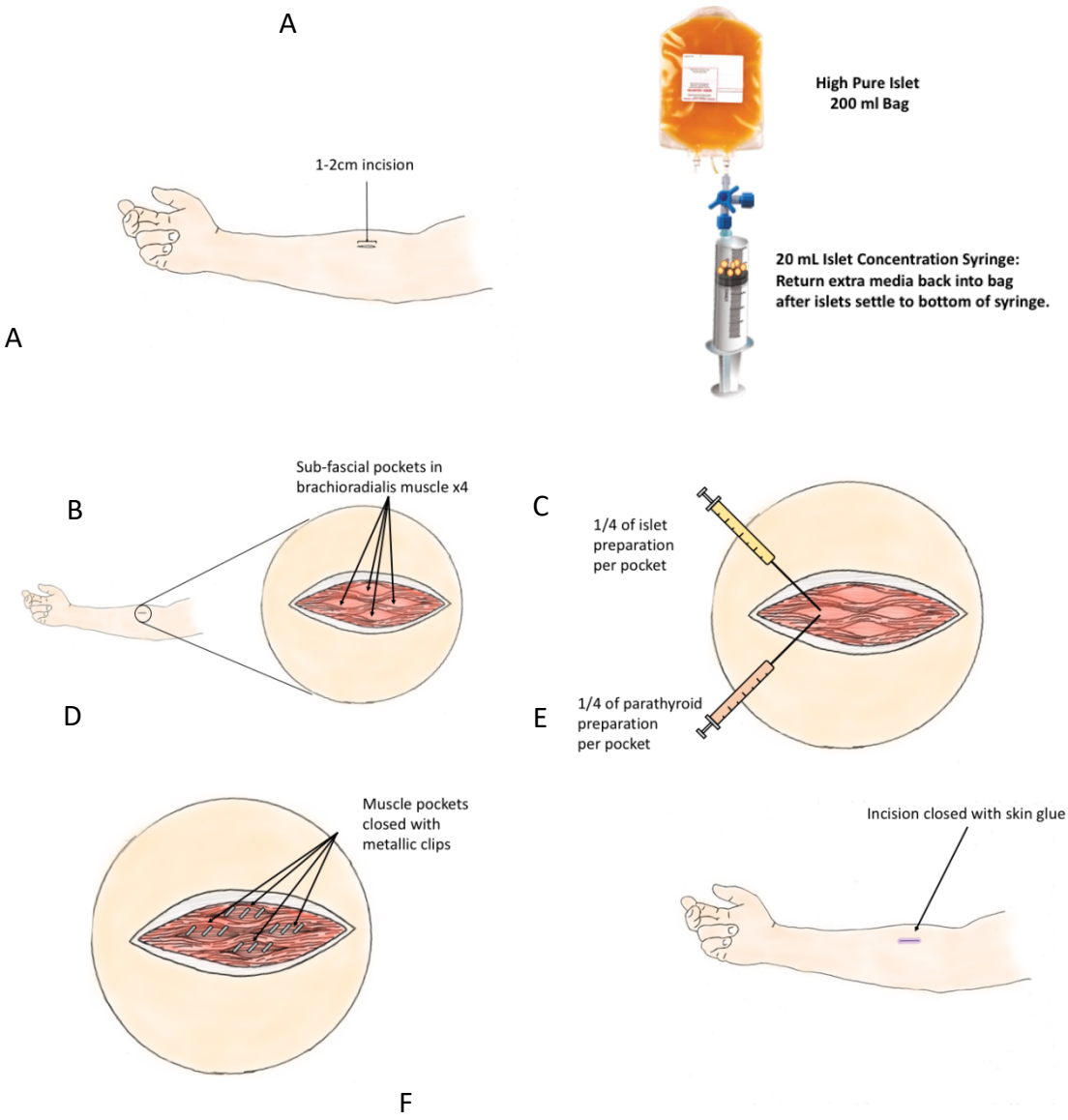


Figure 8: Pancreatic islet loading and islet and parathyroid co-transplantation operative procedure. The pancreatic islet product will be brought from GMP processing center to operating room and operating surgeon will perform islet transfer into 20ml syringe **(A)**. After regional block is performed by anesthesiology colleagues, a 1-2 cm incision will be made over brachioradialis muscle, and overlying fascia will be excised to make 4 pockets for islet and PTG co-transplantation **(B-D).** Following completion of co-transplantation, the pockets will be closed with metallic clips for future identification and skin will be closed in layers with final skin glue administration **(E-F).**

Allogeneic PTG will be brought to the operating room at this time. donor and recipient identification will be checked per standard operating room protocol. The individual PTG will be removed from each sterile container. Any remaining fat or fibrous tissue will be trimmed away leaving only PTG and capsule. The PTG capsule will be incised and the PTG will be finely

chopped with #10 blade scalpel into at least eight fine pieces (~1 mm x 1mm) making sure to break capsule apart. One 1mm x 1mm piece of PTG will be sent for permanent pathology.

PTG and islet co-transplantation operative procedure
Anesthesiologists will perform regional block of brachial plexus in the operative arm as described in section 6.3.1. The arm will be sterilely prepped and draped. Standard transplant pre-operative timeout confirming patient identity, donor/recipient tissue matching and laterality will be performed by operative team. A small 2 cm incision will be made over the brachioradialis muscle of the forearm. The fascia will be excised to expose the underlying brachioradialis muscle fibers. Using blunt dissection, 4 small pockets in each cardinal direction will be made to accommodate the ~2-5 ml islet and parathyroid mixture. If four parathyroid glands are available, each pocket will receive the chopped pieces of one gland and ¼ of the total islet pellet. If four parathyroid glands are not available, the total number of pieces of chopped PTG available will be distributed evenly among the 4 pockets.

Regarding islet placement into the pocket, a 14 Ga angiocath will be placed into the muscle pockets (operative protocol adapted from Bertuzzi et. al.[20]). The islets will be spread evenly into the muscle fibers of pre-formed PTG lined IM pockets.

After completion of the infusion, the muscle pocket will be closed with a permanent stitch and clip to help in locating pocket later (if needed). The skin will be reapproximated with a running 4-0 Monocryl subcuticular suture. Dermabond or equivalent skin glue will help approximate the incision completely and a sterile dressing will be applied.

This procedure is expected to last approximately 30-45 minutes, after which the patient is brought to the recovery suite. To prevent hypoglycemia in the post-transplant period, all patients will receive a dextrose-containing intravenous solution. During the infusion and for the first 3 hours after transplant, glucose measurements will be done at least every 30 minutes, and then every 15-60 minutes depending on the lability of the glucose levels. Insulin will be delivered as an intravenous infusion during this time to further minimize blood glucose fluctuations and support islet engraftment peri-operatively.

Subjects will be discharged approximately 1-2 days after transplant and will be expected to return to the CRC for periodic follow-up visits that will include assessment of adverse events, blood draws, review of blood glucose logs, and performance of metabolic studies. Discharge criteria requires: 1. Pain control on oral medication; and 2. Ability to maintain glycemic control with subcutaneous insulin management. The study visits and tests are outlined in **Appendix 1**. Subjects will also be seen on the CRC for unplanned visits if deemed necessary by study physicians.

6.5. Immunosuppression:

6.5.1. Induction Immunosuppression

Induction immunosuppression for the first transplant will consist of thymoglobulin and will be initiated 24-48 hours prior to islet transplantation. A total of 3 mg/kg thymoglobulin will be given as an IV infusion on days –1 and day 0. The dose will be 1.5 mg /kg on day –1 and 1.5 mg/kg on day 0. The doses will be administered as directed on the package insert.

Premedications will be used as follows:
#1: Acetaminophen (Tylenol®) 650 mg PO/PR 1⁄2 hr before and midway through ATG infusion
#2: Diphenhydramine (Benadryl®) 50 mg PO 1⁄2 hr before and midway through ATG infusion
#3: Methylprednisolone (Solu-Medrol®) 2 mg/kg IV one hour prior to dose on Day -1 and 1mg/kg mg IV one hour prior to dose on Day 0.

If the subject is admitted when the vascular access team is not available or at a time when the placement of a PICC could delay the first rabbit ATG dose it may be administered IV via a peripheral line as follows:

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

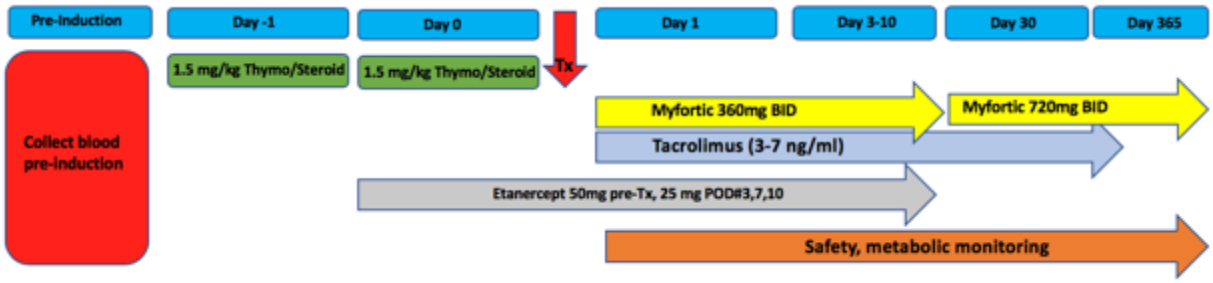


Figure 9: Schematic of pre-transplant, induction and maintenance immunosuppression plan.

6.5.2. Subsequent Transplants

If a second or third transplant is necessary to achieve independence , induction immunosuppression will consist of the IL-2 receptor antagonist basiliximab instead of thymoglobulin. If basiliximab is administered, 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 3 or 4 after the transplant.

6.5.3. Maintenance Immunosuppression

During and after induction therapy, subjects will remain on a calcineurin inhibitor-based maintenance immunosuppression regimen that they were on pre-transplant. This can consist of tacrolimus alone or in conjunction with sirolimus, mycophenolic acid, or azathioprine; or cyclosporine in conjunction with sirolimus or mycophenolic acid. Up to 10 mg/day of steroids are also acceptable in conjunction with either regimen. If patients were being maintained on a steroid free regimen for their solid organ transplant, prednisone will be tapered off following thymoglobulin induction over the course of one week. If patients were maintained on prednisone at 5 mg/day for their solid organ transplant, prednisone will be tapered down to 5 mg/day following thymoglobulin induction. For subjects on tacrolimus plus sirolimus, tacrolimus levels should be maintained between 5-8 ng/mL, and sirolimus levels between 3-7 ng/ml. For subjects receiving tacrolimus plus mycophenolic acid, tacrolimus levels should target 5-8 ng/ml and the mycophenolic acid dose should be Cellcept® 500 -1000 mg po BID or Myfortic® 540-720 mg po BID). For subjects on CsA plus either sirolimus or mycophenolic acid , combined trough levels should target 100-300 ng/mL or the 2 hour level should target 350-500 ng/mL for the first 3 months post-transplant and 200-350 ng/mL thereafter. Drug target levels can be adjusted at the discretion of the treating transplant physician based on subject care needs including graft rejection, drug-related graft injury, drug- related side effects, or infection.

6.5.4. Concomitant Medications:

6.5.4.1. TNF alpha inhibitors

Etanercept is an engineered soluble tumor necrosis factor (TNF) receptor-Fc that blocks TNF binding and reduces inflammation. It is approved for use in severe rheumatoid arthritis, juvenile arthritis, ankylosing spondylitis, and psoriatic arthritis. It will be administered 50 mg subcutaneous (SQ) prior to transplant on Day 0 and 25 mg subcutaneous (SQ) on days +3, +7, and +10 post-transplant for each islet transplant

6.5.4.2. Broad spectrum antimicrobial prophylaxis

Should be administered preoperatively according to site-specific standards, or as the Transplant Infectious Disease consultant recommends.

TRIMETHOPRIM/SULFAMETHOXAZOLE (BACTRIM SS® OR SEPTRA SS®) will be administered at a dose of 80 mg/400 mg PO QD starting on Day +1 for 12 months after islet transplantation for prevention of Pneumocystis carinii pneumonia (PCP). 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. Side effects of Bactrim include allergic reactions, nausea, vomiting, diarrhea, fulminant hepatic necrosis, and blood dyscrasias (leucopenia, agranulocytosis, aplastic anemia, and hemolytic anemia). Subjects who are allergic to sulfa drugs will receive pentamidine. 300mg of pentamidine will be given via nebulizer once a month after the transplant to prevent PCP. Pentamidine aerosol has the side- effects of metallic taste, fatigue, and decreased appetite.

FLUCONAZOLE will be administered as 100 mg PO Q week starting on day –2 relative to initial transplant, day -1 for subsequent transplants, to be continued for 3 months after transplantation.

VALGANCICLOVIR (VALCYTE®) is an antiviral drug which will be given to prevent CMV infection starting on Day --1 for islet transplants at a dose of 450 mg PO QD, increasing to 900 mg QD by Day 12 and continuing for 14 weeks posttransplant. If the CMV status of the donor and recipient are both negative, then valganciclovir administration may be adjusted or eliminated. Valganciclovir has the possible side effects of neutropenia and thrombocytopenia, with related risks of infection and bleeding. Frequent cell counts will be performed and the valganciclovir dose adjusted accordingly. Other infrequent (~2%) side effects include low red blood cell count, fever, rash, and an increase in liver enzymes.

6.5.4.3. Insulin

Insulin (e.g., Regular®, Lispro®, NPH®, or Glargine®) will be administered as needed to maintain glucose levels between 80-120 mg/dL. The subject will test BG five times per day (AM fasting, before lunch, 2 hours after lunch, before supper, and at bedtime). The subject's daily BG levels will be reviewed by a study nurse and/or one of the investigators three times per week during the first two weeks after discharge, and then weekly during the next month. Exogenous insulin will be withdrawn or adjusted as needed. Subjects will be considered insulin independent according to the definition of insulin independence 7.8.1.

6.5.4.4. Proton pump inhibitors

Proton pump inhibitors including but not limited to omeprazole, lansoprazole and pantoprazole will be administered according to standard of care for the duration of the study.

6.5.4.5. Anti-hypertensive, anti-hyperlipidemia, and other approved 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.

6.5.4.6. Prohibited medications

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

- Any medications in the macrolide antibiotic class other than Zithromax
- Other investigational products
- Immunomodulatory agents
- Dapsone
- > 10 mg Prednisone (nasal or ophthalmic steroids are allowed)

6.6. Modification of Standard Immunosuppression:

6.6.1. Islets or PTG are unsuitable

Induction immunosuppression will not be initiated until it is certain that the islet product meets release criteria. Should the combined product subsequently become unsuitable for transplantation and the patient has already received some induction immunosuppression, the subject will remain on maintenance therapy required for their renal or liver transplant. A request will be placed through UNOS and the local organ procurement organization that the next available pancreas for islet transplantation is directed to the transplant site.

6.6.2. Graft Failure

Subjects who experience islet graft failure will be maintained on their current immunosuppressive regimen for their renal or liver graft. If/when it is determined that a subject will not receive a subsequent islet transplant, the subject will move to the reduced follow-up schedule.

6.6.3. 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 the subject is contraindicated, the subject will receive the basiliximab - based induction immunosuppressive regimen as described above.

6.6.4. Intolerance of Protocol Medications

In the event that protocol-regulated concomitant medications are not tolerated, the subject will continue taking the immunosuppressive therapy in order to protect the islet and renal or liver grafts. In the event that the immunosuppression regimen is not tolerated, the PI may elect to prescribe an alternative immunosuppression regimen. The intent would be for the alternative regimen to be temporary in nature where possible and any such decision made with the primary intent of maintaining the function of the renal or liver allograft. Any non-protocol directed study treatment modification that the site PI determines is necessary should be reported as a protocol deviation.

6.6.5. 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.

6.6.6. 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 (Solu-Medrol, 30 mg minimal pretreatment dose), 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.

6.6.7. 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 A TG by 50%.
- Test for CMV and if negative hold valganciclovir.
- Reduce trimethoprim/sulfamethoxazole to 80 mg/400 mg 3 times per week or hold trimethoprim/sulfamethoxazole.
- Review and obtain current sirolimus trough levels and consider dosage adjustment if trough levels are > 12 ng/dL (please refer to Appendix 1, SOE, for additional information).
- If subject is using mycophenolate mofetil or mycophenolate sodium in lieu of sirolimus, consider dose reduction.
- Consider administration of G-CSF.
- Monitor temperature BID.
- Follow-up within 48-72 hours to obtain repeat 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.
- Obtain CMV antigenemia or PCR for CMV.
- Hold rabbit ATG.
- Hold valganciclovir and trimethoprim/sulfamethoxazole.
- Review and obtain current sirolimus trough levels and consider dosage adjustment if trough levels are > 12 ng/dL
- If subject is using mycophenolate mofetil or mycophenolate sodium in lieu of sirolimus, consider dose reduction.
- Administer G-CSF.

- Monitor temperature BID.
- Follow-up within 48-72 hours to obtain repeat CBC with differential, subject symptoms, and measured temperatures.

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

- Hold rabbit ATG.
- Hold administration of trimethoprim/sulfamethoxazole and/or valganciclovir.
- Review and obtain current sirolimus trough levels and hold dose if trough levels are > 12 ng/dL
- If subject is using mycophenolate mofetil or mycophenolate sodium in lieu of sirolimus, consider holding dose.
- Obtain CMV antigenemia or PCR for CMV.
- Consider fluoroquinolones in afebrile subjects.
- Consider clotrimazole.
- Administer G-CSF.
- Monitor temperature BID.
- Follow up within 24 hours to obtain repeat CBC with differential, subject symptoms, and measured temperatures.

If a subject's absolute neutrophil count is 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 an Infectious Disease/Hematology consult will be obtained.
- Hold rabbit ATG.
- Hold administration of trimethoprim/sulfamethoxazole and/or valganciclovir.
- Review and obtain current sirolimus trough levels and hold dose if trough levels are > 12 ng/dL
- If subject is using mycophenolate mofetil or mycophenolate sodium in lieu of sirolimus, consider holding dose.
- Obtain CMV antigenemia or PCR for CMV.
- Administer G-CSF.

6.6.8. Thrombocytopenia

If the subject is found to have a platelet count (PLT) of < 50 x 109/L, rabbit ATG will be withheld until PLT > 50 x 109/L, then resume at a 50% reduced dose. If the PLT is < 50 x 109/L, sirolimus will be withheld for 24 hours, then resume at a 50% reduced dose. If PLT fails to return to > 50 x 109/L within one week, sirolimus is to be withheld until PLT > 50 x 109/L, after which sirolimus is resumed at 50% of the dose that preceded the drop in PLT to < 50 x 109/L. If the PLT is between 50 and 75 x 109/L, reduce rabbit ATG dose by 50% until PLT is > 75 x 109/L.

6.6.9. Nephrotoxicity

A sustained 33% increase in SCr warrants a prompt referral to a nephrologist for evaluation. Additionally, significant changes in renal function should be reported to the patient's physician managing the renal transplant. Similarly, a 1.5-fold increase in transaminase level, alkaline phosphatase or bilirubin should prompt an immediate referral to the liver transplant surgeon/hepatologist. Since both the kidney and liver transplants were performed at UCSF, the transplant team will immediately be contacted with any evidence of renal or liver allograft dysfunction. If it is thought that the decrease in renal function is attributable to CNI immunosuppressive therapy, the physician managing the renal transplant should consider ONE of the therapeutic alternatives listed in **Table 13** below:

Table 13. CNI Alternatives

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

These therapeutic adjustments are standard of care in the management of immunosuppressive complications utilized in solid organ transplants.

6.6.10. Anti-hypertensives and anti-hyperlipidemics

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

6.6.11. TNF alpha inhibitor- Etanercept

Etanercept is a dimeric soluble form of the p75 TNFR receptor that blocks TNF binding and reduces inflammation. In the United States, it is FDA-approved for use in severe rheumatoid arthritis, juvenile arthritis, ankylosing spondylitis, and psoriatic arthritis[87]. In controlled trials, approximately 37% of subjects treated with etanercept developed injection site reactions (see Enbrel® package insert). All injection site reactions were described as mild to moderate (erythema and or itching, pain or swelling) and generally did not necessitate drug discontinuation. In placebo-controlled trials, there was no increase in the incidence of serious infections. The observed rates and incidence of malignancies were similar to those expected for the population studied. However, the incidence of TB has been shown to be statistically higher in anti-TNF-alpha-treated patients [88], and based on post-marketing studies a warnings have

been issued about the following conditions, which have been reported with the use of Enbrel®: serious infections and sepsis, including fatalities; an increased risk of lymphoma and other malignancies in children and adolescents; and leukemia. Many of the serious infections occurred in patients on concomitant immunosuppressive therapy.

Experience with anti-TNF alpha therapies in clinical and experimental islet transplantation has been limited. Farney et al described a beneficial role of etanercept in promoting engraftment of marginal mass islet grafts in mice[41]. Hering et al used etanercept in a recent trial of 8 T1D subjects receiving single donor islet transplants, and all 8 achieved insulin independence suggesting a beneficial role for anti-TNF therapy in clinical islet transplantation[28].

6.7. Assessment of Compliance with Study Treatment

Assessment of subject compliance will be determined by the completion of the scheduled study visits and required documentation that the specific subject is responsible for (e.g., Blood Sugar Records, AEs, and Insulin Use recording) as well as their willingness to comply with the recommendations of the study investigators. Any aberration of trough levels of immunosuppressive agents that could indicate nonadherence, lack of compliance that poses a significant clinical risk and or derangement of protocol data collection will be documented.

6.7.1. Premature Discontinuation of Study Treatment (Transition to "Reduced Follow-up" Treatment)

Study treatment will begin at the time of the first dose of induction antibody therapy for an islet/PTG co-transplant. 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. The renal or liver allograft is lost and the subject elects to terminate chronic immunosuppression.
4. Graft Failure: Failure to detect c-peptide in recipients with Type 1 diabetes.
5. An unexpected related SAE. The agent(s) to which the event is attributed will be discontinued.

Subjects who prematurely discontinue study treatment will remain in the study until normal termination, for the purpose of monitoring safety and efficacy parameters and will enter the reduced follow-up schedule outlined -. Data from these subjects will be used in the intent-to-treat analysis.

6.7.2. Criteria for premature termination of the study

In general, subjects may be prematurely terminated from the study for the following reasons:

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

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

6.7.3. Protocol Suspension and Review

Study enrollment will be suspended pending expedited review of all pertinent data by the institutional review board (IRB) or the Data Safety Monitoring Board (DSMB), if any one of the following occurs:

1. The Medical Monitor finds any unexpected fatal or life-threatening AE possibly related to the use of the test therapy.
2. Primary non-function (PNF) occurs in 3 or more consecutive subjects
3. Any event(s) that in the opinion of the Protocol Chair indicates the need for DSMB review.
4. The DSMB recommends termination of protocol enrollment and further transplants on a study-wide basis based on a review of the data and finding evidence that such action is necessary.

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

7. STUDY PROCEDURES

7.1. Enrollment and Screening

At the screening visit, the screening procedures will be discussed in lay terms to each potential research subject. If the potential subject remains interested, she/he will review the screening informed consent document with the coordinator, and an investigator will join the discussion and answer any questions. Once satisfied that all questions have been answered, the potential subject will either decline to participate or sign the screening informed consent document. The potential subject will sign an informed consent form before undergoing any screening study procedures. This may occur at a subsequent visit if the potential subject desires, in order to

think further about what participation means and/or to consult with family and/or friends. If at any time the suitability of diabetes care is questioned, the potential subject will be referred for further assessment and management. Once informed consent has been obtained, eligibility will be confirmed through the performance of the screening visit procedures detailed in **Appendix 1**, SOE, and from additional reports required from each subject's diabetologist and retinologist. A psychosocial evaluation may be requested if either a coordinator or investigator is unsure whether a potential subject may be mentally unfit to undergo the procedure or to determine whether a psychosocial problem may be responsible for the instability of diabetes; such an evaluation would be performed by an experienced transplant social worker and/or psychiatrist. More than one visit may be necessary to complete all of the screening procedures. Patients who enroll in this trial may have had some of the required screening tests done prior to signing the screening consent document as part of their routine diabetes care or a previous assessment for standard islet and/or pancreas transplantation. Results from assessments completed prior to signing informed consent must be current within the windows stated in **Table 14** below.

Table 14. Timeframes for local screening assessments

Local Screening Assessments	Allowable timeframe prior to the date of consent
EBV IgG	No limit.
Retinopathy evaluation; Physical exam; electrocardiogram (ECG); Pre-operative cardiac clearance; purified protein derivative (PPD); PTH; Serology; Coagulation; CMV IgG/IgM (if previously negative)	Within one year
CBC; Chemistry	Within 6 months
CXR	Within 1 month

The screening pregnancy test, first morning spot urine, and blood draws for all central laboratory assessments must be done at the study site after informed consent has been signed. Pregnancy and blood transfusion history will be collected and provided to the immunogenetics lab for Alloantibody analysis. Once eligibility has been confirmed, a subject will be placed on the wait list.

7.2. Waitlist/Baseline

Waitlist assessments will be repeated at pre-defined intervals as detailed in Appendix 1. Results from repeat assessments done closest to transplantation will be used as the subject's baseline values. During this period when subjects are awaiting their first transplant, and CGMS - should be completed as time allows. All one-time baseline assessments should be completed on Day -2, whenever possible, but always prior to the start of induction immunosuppression. As in any other transplant situation, medical conditions that arise (e.g., new serious infections, malignancy, compliance issues, etc.) will automatically trigger a re-evaluation to determine if the subject remains qualified for the protocol. Only qualified subjects may proceed to donor organ matching and transplant.

7.3. Islet Transplant and Study Treatment Visits

Blood group compatible and crossmatch negative subjects selected for islet transplantation will be invited to the study center upon determination of preliminary islet suitability based on count and quality. The virtual cross match versus physical cross match will be used as per the standard algorithm used for cross-matching kidney/pancreas recipients at UCSF. The subject will be admitted, and after preliminary testing detailed in **Appendix 1**, SOE (- 2 days relative to transplant), the subject will begin induction therapy with rabbit ATG. This can be administered via central vein catheter, PICC line, or peripheral IV. A period of in vitro culture is considered essential to the protocol because of the use of rabbit ATG induction immunosuppression may cause a transient cytokine release associated with the first dose. The period of in vitro culture will allow time for any cytokine release to dissipate after treatment of the subject with ATG and will also permit time for microbiological and potency assessment of the islet and PTG preparation prior to transplant. Following induction therapy, subjects will remain on a calcineurin-based maintenance immunosuppression regimen as outlined in section 6.5. If a patient is receiving belatacept as part of their maintenance immunosuppression for the kidney transplant, one dose of the drug will be administered immediately prior to the islet transplant at a dose based on the usual dose and interval from last dose.

7.4. Follow-up Visits

Islet transplant recipients will undergo a 1-2 year follow-up period following the last islet transplant to include time points relevant to the initial transplant. The timing of all follow-up assessments will "reset" with additional transplants; i.e., the day of the 2nd or 3rd transplant becomes day 0 and the subsequent assessments are conducted in relation to this day. Please refer to the **Appendix 1**, SOE, for the clinical time points of specific follow-up study procedures. All patients at the discontinuation of this study's follow-up will continued to be followed up in the pancreas and islet transplant clinic as well as their respective pre-existing solid organ clinic.

7.5. Criteria for second and third islet transplant

Islet recipients who have partial graft function but who do not meet the insulin independence criteria will be considered for a second intramuscular islet transplant. Partial function will be defined by basal or stimulated c-peptide >0.3 ng/ml. To be eligible for a second transplant, the following requirements must be met:

1. Subject received >5,000 IEQq/kg with the first transplant but failed to achieve or maintain insulin independence
2. Subject has been compliant with study medications and monitoring
3. No evidence of serious infections, AE, or other conditions that preclude repeat transplantation
4. No unresolved SAEs
5. No evidence of PTLD, requiring complete withdrawal from immunosuppressive therapy.
6. No evidence of hypersensitization, allergic responses, or other potentially serious drug

reactions to medications required by the protocol.
7. Stable renal function as defined as being a creatinine of no more than one third greater than the average creatinine determination performed in the 3 previous months, until rejection, obstruction or infection are ruled out. For liver transplant recipients, stable liver function as defined by <20% increases in transaminases, bilirubin, and alkaline phosphatase.
8. No medical condition(s) that, in the opinion of the investigator, will interfere with a safe and successful second islet transplant.
9. No evidence of peptic ulcer disease, gastritis, or other mucosal abnormalities on upper endoscopy.

Patients with partial graft function after the second transplant may be considered for a third intramuscular transplant if the above criteria are met.

7.6. Enrollment timeline and criteria for protocol suspension and review

Enrollment will be limited to 4 subjects with Type 1 diabetes in the 1^{st} year of the trial to allow close monitoring of the effects of intramuscular islet and PTG co-transplantation. These subjects will be followed for a period of 6 months from their initial transplant before recruitment of additional subjects can occur. Additionally, after the first two transplants are performed, an intentional 6-week delay following the transplant will be enacted in order to assess for possible hyperparathyroid and/or local side effects of islet transplantation into muscle of forearm before proceeding to transplanting the remaining patients.

If any of the following occur in the pilot group during the follow-up period, no additional subjects will be enrolled and the study will be suspended pending expedited review by the UCSF Institutional Review Board (IRB), the Data Safety Monitoring Board (DSMB), and by the funding agency:

1. Any unexpected fatal or life-threatening AE possibly related to the test therapy in any of the subjects.
2. New-onset renal or liver allograft rejection in 3 of 6 subjects within the follow-up period.
3. Evidence of significant allosensitization as defined by *de novo* development of high risk donor-specific antibodies in all 3 subjects. At UCSF, high risk antibodies are defined as having mean fluorescence intensities (MFI) greater than 8500 using the LABScreen Single Antigen® assay.
4. Graft failure (as defined by basal and MMTT-stimulated c-peptide <0.3ng/mL) compared to contralateral arm at the 75 day metabolic assessment in all 6 subjects.
5. Any other event which in the opinion of the DSMB necessitates suspension and review of the study.

7.7. Safety assessments

Clinical safety will be assessed according to the schedule of events **(Appendix 1)** by physical examination, measurement of vital signs (supine and standing blood pressure, heart rate, oral

temperature and respiratory rate), body weight, incidence of opportunistic infection, and adverse events.

Laboratory Safety Assessments will include the following:

- Frequent blood glucose measurements, particularly in the immediate post-transplant period - Fasting blood chemistry: blood urea nitrogen (BUN), creatinine, electrolytes
- Liver panel: Albumin, total bilirubin, ALT, AST, alkaline phosphatase, GGT, total protein - Hematology: complete blood count with differential and platelet count
-Coagulation parameters: PT/INR, PTT
-Calculated glomerular filtration rate (CKD-EPI and urinary protein measurements -EBV viral load
-JC Virus serum levels by PCR (ViaCor) – only for patients already on belatacept -Pregnancy test (urine) for all female subjects of childbearing potential
-Tacrolimus, cyclosporine, and sirolimus concentrations.
-Degree of immune sensitization as determined by the presence of anti-HLA antibodies

7.8. Efficacy assessments

7.8.1. Insulin independence

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

- HbA1c < 6.5% or a > 2.5% decrease from baseline;
- Daily fasting capillary glucose should not exceed 140 mg/dL (7.8 mmol/L) more than 3 times in the past week; - 2-hour post-prandial capillary glucose should not exceed 180 mg/dl (10.0 mmol/L) more than three times in the past week;
- Fasting plasma glucose < 126 mg/dL (7.0 mmol/L); if the fasting plasma glucose is > 126 mg/dL (7.0 mmol/L), it must be confirmed in an additional one out of two measurements;
- Evidence of endogenous insulin production defined as fasting or stimulated C-peptide >0.5 ng/mL (0.16 nmol/L).

7.8.2. Glycemic control

Glycemic control will be assessed by measuring HbA1c using high-performance liquid chromatography. The range of the assay is 4.8-6.7% for normoglycemic subjects in our clinical laboratory.

7.8.3. Glycemic lability

Glycemic lability will be assessed with the Mean Amplitude of Glycemic Excursions (MAGE) test which requires 14-16 capillary blood glucose measurements obtained over two consecutive days. (66) Glycemic excursions are calculated as the absolute difference in peak and subsequent nadir glucose values, and all excursions > 1 S.D. are summed and divided by the number of qualified excursions to give the MAGE in mmol/L (or mg/dl) glucose. A MAGE > 11.1 mmol/L (200 mg/dl) is indicative of marked glycemic lability.

7.8.4. Hypoglycemic episodes

The Clarke survey and HYPO score will be used to assess the frequency and severity of hypoglycemic episodes[89,90]. The Clarke survey involves subject completion of eight questions scored by the investigator according to an answer key that gives a total score between 0 and 7 (most severe), where scores of 4 or more indicate reduced awareness of hypoglycemia and increased risk for severe hypoglycemic events. The HYPO score involves subject recording of BG readings and hypoglycemic events (BG < 3.0 mmol/L [54 mg/dL]) over a 4-week period and recall of all severe hypoglycemic episodes in the previous 12 months. A HYPO score greater than or equal to the 90th percentile (1047) of values derived from an unselected group of T1D subjects indicates severe problems with hypoglycemia.

7.8.5. Mixed Meal Tolerance Test (MMTT)

Subjects will have basal plasma glucose and C-peptide levels drawn and then will receive 6 ml/kg body weight (to a maximum of 360 ml) of Boost® High Protein Drink (or a nutritionally equivalent substitute) to consume in 5 minutes starting at time = 0. At 90 and 120 minutes, stimulated plasma glucose and C-peptide levels will again be drawn.

7.8.6. β-Score: A composite index of post-transplant graft function

The β-score will be determined from the HbA1c, insulin requirements, fasting (basal) serum glucose, and basal or stimulated c-peptide as developed by Ryan, et al. [91]. The score may range from 0 (no graft function) to 8, with all subjects reported with a score of 8 also having 90-minute serum glucose levels during a MMTT that are ≤ 10.0 mmol/L (180 mg/dL), indicative of excellent graft function.

7.8.7. C-peptide to Glucose, Creatinine Ratio

The c-peptide to glucose, creatinine ratio (CPGCR) will be determined using the fasting (basal) serum glucose and c- peptide, and a simultaneous SCr. This measure accounts for both the dependence of c-peptide secretion on the ambient glucose concentration and the dependence of c-peptide clearance on kidney function. The CPGCR is calculated as [c- peptide (ng/mL) * 100]/[glucose (mg/dL) * creatinine (mg/dL)]. An index of islet graft function, this measure correlates well with both the 90-minute serum glucose levels during a MMTT and with the β-score [92].

7.8.8. Differential C-peptide secretion in forearm

Due to the ability to venous sample selectively in the draining veins of the forearm where islet and PTG co-transplant has been performed, we are afforded the ability to differentially monitor c-peptide secretion between non-transplanted and transplanted arm prior to cardiac recirculation. We will perform venous samples in the antecubital fossa of both arms at the same time in response to unstimulated and stimulated glucose tolerance tests. This information will be used to develop potential models for early rejection monitoring and islet function and engraftment.

7.8.9. Continuous glucose monitoring system (CGMS)

Subjects will be asked to wear a continuous glucose sensor if available (MiniMed Continuous Glucose Sensor, MiniMed, Downers Grove, IL) for one 3 day period on days 75 and 180 post initial and subsequent transplants, at 12 and 18 months post final transplant, and annually after 1^{st} and last transplant. CGMS may also be done at suspected graft dysfunction.

7.8.10. Renal function measurements

Serum creatinine, calculated glomerular filtration rate (CK-EPI), and urinary protein/albumin excretion will be determined before and at intervals after islet transplantation.[93]

7.9. Sample collection and banking

Peripheral blood samples will be collected according to the schedule outline in **Appendix 1**, SOE. In patients receiving a 2^{nd} transplant, the sample collection will switch to a similar schedule relative to the second transplant.

For-cause biopsies will also be performed in any patient who loses insulin independence or shows worsening glucose control (>20% increase in insulin requirement, >50% reduction in fasting or stimulated c-peptide levels, and a >25% increase in CGMS readings that are >140mg/dL if fasting or >180mg/dL if postprandial)). Samples will be split in two equal parts, half embedded in paraffin and half embedded in OTC and cryopreserved at -80°C.

8. DATA AND SAFETY MONITORING PLAN (DSMP)

Adverse Events (AEs) that are classified as serious according to the definition set forth by the health authorities must be reported promptly to CIRM, health authorities, PIs, and IRBs. This section defines the types of AEs and outlines the procedures for appropriately collecting, grading, recording, and reporting them. Information in this section complies with *International*

Conference on Harmonization (ICH). Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting and ICH E6: Guideline for Good Clinical Practice has been adapted to use the standards set forth in the Terminology Criteria for Adverse Events in Trials of Adult Pancreatic Islet Transplantation (CITTCAE). This document, created by the Clinical Islet Transplant (CIT) Consortium, modifies the National Cancer Institute (NCI), Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 (August 3, 2011), to ensure applicability in the setting of Islet Transplantation**.** (Additional information and a current version of the CIT-TCAE is available on the CIT website: http://isletstudy.org)

8.1. Definitions

8.1.1. Adverse Event

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

8.1.2. Serious Adverse Event

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

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

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

8.1.3. Unexpected Adverse Event

An AE is considered “unexpected” when its nature (specificity) or severity is not consistent with available product information, provided in the package insert, the protocol, or the investigator’s brochure.

8.2. Adverse Events

8.2.1. Collecting Procedure

AEs that are associated with a protocol mandated procedure which is not part of the normal standard of care for the participant and hypoglycemic events will be collected beginning at the time of admission for 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 from the study, whichever comes first.

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

8.2.2. Recording Procedure

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

8.2.3. Grading and Attribution

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

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

Grade 2 = Moderate AE.
Grade 3 = Severe and undesirable AE. Grade 4 = Life-threatening or disabling AE. Grade 5 = Death.

AEs, not included in the *CIT-TCAE* listing, should be recorded and their severity graded from 1 to 5 according to the General Grade Definition provided below (**Table 15**).

Table 15. General Severity Definition of Adverse Event

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

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

The relatedness, or attribution, of an AE to islet transplantation, which includes the transplant procedure and/or the islet product, or to the immunosuppression and/or infection prophylaxis will be determined by the PI or designee. The PI or designee will also record the determination of attribution on the appropriate CRF and/or SAE report form. The relationship of an AE (attribution of AE) to islet transplantation (islets or transplant procedure) or immunosuppression/infection prophylaxis will be defined by using the descriptors provided below **(Table 16)**.

Table 16. Adverse Event Attribution

Code	Descriptor	Definition
Unrelated Category		
1	Unrelated	The AE is clearly not related to allogeneic islets/PTG, the islet/PTG transplant procedure, immunosuppression or infection prophylaxis
Related Category		
2	Unlikely	The AE is doubtfully related to allogeneic islets/PTG, the islet/PTG transplant procedure, immunosuppression or infection prophylaxis
3	Probable	The AE may be related to allogeneic islets/PTG, the islet/PTG transplant procedure, immunosuppression or infection prophylaxis
4	Probable	The AE is likely related to allogeneic islets/PTG, the islet/PTG transplant procedure, immunosuppression or infection prophylaxis
5	Definite	The AE is clearly related to allogeneic islets/PTG, the islet/PTG transplant procedure, immunosuppression or infection prophylaxis

8.3. Serious Adverse Events

8.3.1. Collecting Procedure

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

8.3.2. Recording Procedure.

SAEs will be recorded on the AE CRF.

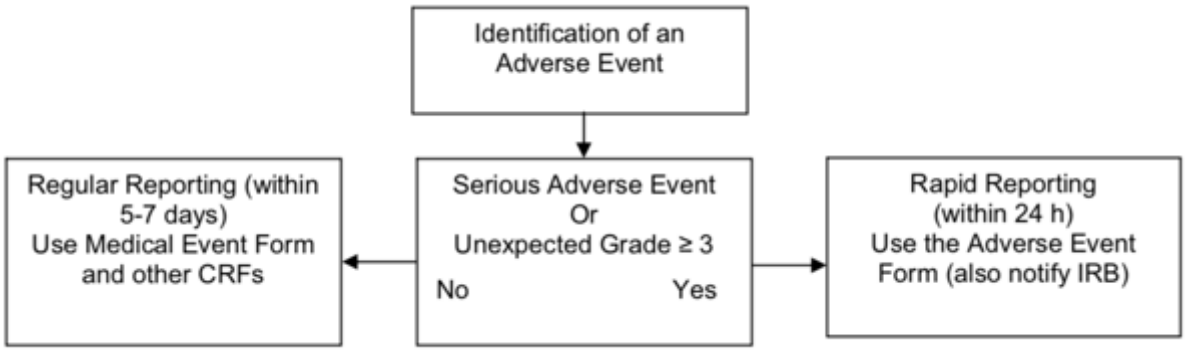
8.3.3. Reporting Procedure

In order to determine the urgency of serious adverse event reporting to the sponsor (regular reporting within 5-7 days versus rapid reporting within 24 hours), the protocol distinguishes between common adverse events (e.g., toxicities and low grade medical events) and serious adverse events depending upon the grade of event or toxicity. Regular reporting is permitted for any expected adverse event or toxicity through Grade 3. Rapid reporting by the study personnel is required for identified and suspected serious adverse events defined above (including death and life-threatening events) and those unexpected toxicities of Grade 3 or more.

All adverse events occurring during the study, whether or not attributed to the test therapy, will be recorded during the study. Serious events will be followed until resolved or considered stable. The following attributes must be assigned: description, date of onset and resolution (if known when reported), severity, assessment of relatedness to test therapy, and action taken.

All events associated with the trial must be recorded. The schema depicted below (**Figure 10**) will assist to determine whether an event requires regular or rapid reporting to the sponsor.

Figure 10: Reporting Decisions for Adverse Events



The sponsor will review the available information and request additional information as needed to determine if the serious adverse event is (a) serious, (b) expected or unexpected, (c) related or not to the study drugs. Three possible reporting scenarios (to the appropriate health authorities including the FDA as detailed in 21 CFR 312.32) could arise after assessment of the event:

1. No requirement to report. This would occur if the adverse event is deemed not serious.
2. Standard reporting is required. This would occur if the adverse event were classified as one of the following: (a) serious, expected and drug related; (b) serious, expected and not drug related; or (c) serious, unexpected and not drug related.
3. Expedited reporting is required. This would occur if the adverse event is considered serious, unexpected and drug related. These events must be reported to the appropriate health authorities and pharmaceutical manufacturers that provide study medications within 15 days unless the event is fatal or life threatening, the latter must be reported within 7 days by facsimile.

Other adverse events, toxicities, and other medical events, regardless of severity, will also be recorded and submitted as part of the annual report. The DSMB will review reported serious adverse events within 30 days of receipt of report and will routinely review cumulative reports of other study events, toxicities, and study results.

The site investigator must apply their clinical judgment whether or not an adverse event is of sufficient severity to require that the subject should immediately be removed from treatment. If necessary, an investigator must suspend any trial treatments and institute the necessary medical therapy to protect a subject from any immediate dangers. Subsequent review by the UCSF CRC, DSMB, IRB, the sponsor(s), or the FDA or relevant local regulatory authorities may also suspend further trial treatments. The FDA, study sponsor(s) and DSMB retain the authority to suspend additional enrollment and treatments for the entire study as applicable.

A subject may also voluntarily withdraw from treatment due to what he/she perceives as an intolerable adverse event, or for any other reason. If voluntary withdrawal is requested, the subject should be asked to continue (at least limited) scheduled evaluations, complete an end-

of-study evaluation, and be given appropriate care under medical supervision until the symptoms of any adverse event resolve or their condition becomes stable.

8.4. Reporting of Pregnancy

Subjects of childbearing potential must use adequate contraception and express intent not to become pregnant while participating in this study. Under this protocol, investigators should recommend contraception methods using either a) hormonal and barrier method combination, or b) abstinence (for both male and female subjects).

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

Pregnancy occurring while the subject is participating in this study will be reportable to regulatory bodies (IRB, CRC, DSMB, and the FDA). Pregnancy will also be reported to pharmaceutical manufacturers that provide study medications.

8.5. Data and Safety Monitoring Board (DSMB)

An independent DSMB will be assembled to monitor study progress. The responsibilities of the DSMB will include the following:

-Assure the safety of participants and the validity and integrity of the data.
-Achieve familiarity with the protocols.
-Review interim analyses of outcome data and cumulative toxicity data.
-Determine if trial should continue as originally designed, be changed, or terminated based on these data. -Ensure confidentiality of the trial data and results monitoring.

-Review reports of related studies for relevant indications.
-Achieve familiarity with major protocol modifications.
-Report on the safety and scientific progress of the trial to the PI and IRB.

8.5.1. Membership on the DSMB

Voting Members

The membership will be comprised of a transplant nephrologist, transplant surgeon, gastroenterologist, endocrinologist, and two lay persons. Members will be completely independent of the above-mentioned clinical trials and will have no financial interest in the outcomes of any study reviewed by the DSMB. Written documentation attesting to absence of conflict of interest will be required of all members as detailed below. A committee chairperson will be selected by a majority vote of the voting members of the DSMB.

Nonvoting Members

Program Staff, e.g. CRC Research Nurses involved with the study protocols will be encouraged to attend open sessions of the DSMB meetings.

8.5.2. Meetings

The DSMB will meet annually. Additional meetings will be scheduled when necessary for adequate monitoring. In the event of any unexpected fatal or life-threatening adverse event possibly related to the islet transplant or the use of any protocol-regulated treatment product, the DSMB will confer in emergency session, and advise the regulatory bodies on the appropriate course of action. Meetings will be convened in person whenever possible, external members may join via a conference call. All meetings will be closed to the public. The DSMB meetings may consist of both open and closed sessions.

The open sessions may be attended by investigators, and institution staff, but will always include the PI. Issues to be discussed at open sessions will include conduct and progress of the study including patient accrual, compliance with protocol, and problems encountered. Patient-specific data and treatment group data may not be presented in the open session.

The closed session will be attended only by voting DSMB members, but others may be requested to attend by the DSMB as considered appropriate. All safety and efficacy data must be presented at this session. The material presented and discussions during the closed session will be strictly confidential (procedures detailed later in this document). Should the DSMB decide to issue a termination recommendation, full vote of the board will be required. In the event of a split vote, majority vote will rule and a minority report will be appended. Such a recommendation should be transmitted to the PI, business official of the grantee institution, the IRB, and the FDA (if appropriate) as rapidly as possible, by immediate telephone and telefax if sufficiently urgent.

8.5.3. Temporary-Hatl Procedure Oversight by DSMB

We propose a 6-week delay following the transplant of each of the first two patients to assess for possible hyperparathyroidism and/or local side effects of islet transplantation into forearm muscle before proceeding with subsequent transplantation of remaining patients. The DSMB will be responsible for overseeing the temporary-halt procedure at this 6 week time-point. Criteria for continued enrollment include:

- Absence of severe adverse events related to intramuscular transplantation of human pancreatic islets in the muscle.
- Absence of hyperparathyroidism, specifically hypercalcemia, related effects secondary to allogeneic parathyroid transplantation.
- Absence of unexpected severe adverse events not foreseen by the clinical investigators prior to human parathyroid and islet co-transplantation.

8.5.4. Reports

1. Interim Reports

Interim reports will be prepared under the direction of the PI in cooperation with the study statistician(s) and distributed to the DSMB at seven (7) days prior to a scheduled DSMB meeting.

2. Serious Adverse Event Reports

Serious adverse events must be reported in writing to voting members of the DSMB within 10 working days. Serious adverse event reports will also be provided under the direction of the P.I. to the FDA and IRB within 10 working days.

3. Reports from the DSMB

The DSMB will provide written, confidential reports to the PI and the IRB after each meeting. Each report should conclude with a recommendation to continue, temporarily hold, or terminate the study. No information communicated to the DSMB is exempt from IRB review and all findings from the DSMB must be reported to the IRB as they are discovered.

8.5.5. Confidentiality Procedures for DSMB members

No communication, either written or oral, of the deliberations or recommendations of the DSMB will be made outside the DSMB except the report to the PI, IRB and federal regulatory authorities as required. Outcome results are strictly confidential and must not be divulged to any non-member of the DSMB until a recommendation to release the results has been accepted and implemented. Each member of the DSMB, including non-voting members, must sign a statement of confidentiality.

8.5.6. Conflict of Interest

DSMB members must disclose any potential conflicts of interest, whether real or perceived. Conflict of interest can include personal, professional, financial or proprietary interest. Potential conflicts that develop during the member's tenure on the DSMB must also be disclosed. Disclosure is a key factor in protecting one’s reputation and career from potentially embarrassing or harmful allegations of inappropriate behavior. Such disclosure will also serve to protect the integrity of the DSMB and its role in monitoring and oversight of clinical studies. Conflict of Interest Disclosure Forms must be signed prior to the first meeting of the DSMB. Failure to disclose a conflict of interest for administrative review and response will lead to removal from the DSMB.

8.6. Data analysis

8.6.1. Safety and efficacy analyses

Safety will be assessed by the summary of adverse events, laboratory test results, and vital signs. Descriptive statistics (mean, standard deviation, mean change from baseline) of laboratory values and vital signs will be summarized for each patient in the study. These safety summaries will be produced separately for pre-transplantation and post-transplantation periods. The post-transplantation period starts immediately after transplantation and lasts until the end of the 4-year study period. Since this study is a pilot non-randomized safety and efficacy trial with patient enrollment limited by budgetary constraints, no direct statistical significance tests can be performed. Efficacy of islet transplantation will be assessed by following changes in C-peptide levels, insulin requirements, HgbA1c levels as well as glucose tolerance testing at various time points after transplant as described. Time-to-event analysis (time to insulin independence, time to graft failure (return to insulin therapy) will be based on the log-rank test. Event-free rates will be calculated using the Kaplan-Meier method.

8.6.2. Sample size calculations

To provide some information about the clinical utility of the intramuscular site, we will perform several comparative analyses with historical data obtained from the CITR collaborative registry, since many of our study endpoints correspond with endpoints collected in the registry. While this may not be as rigorous as a direct comparison between intraportal and intramuscular routes, we believe that it will provide some useful data that can provide the basis for larger, randomized trials. The 75-day, 6-month and 1-year insulin independence rates for 65 intraportal islet transplants in patients with Type 1 diabetes with kidney allografts performed between 2007 and 2009 were 15%, 8% and 5%, respectively (CITR 6th Annual Report) (75). A sample size of 8 as proposed for this trial will allow us to sufficiently detect a 5%(75-day), 2% (6-month) and 1.5% (1-year) difference in insulin independence rates compared with intraportal infusion. Based on these assumptions, a test comparing the observed insulin independence rates to the reference rate using a one-group chi square test with a 0.05, two-sided Type 1 error will have 80% power to detect such a difference when the sample size is 8.

8.6.3. Comparative analyses

All outcomes listed in specific aim one will be calculated and described with two-sided 95% confidence intervals. If the confidence interval includes the historical outcome reference rate, the outcome will not be considered significantly different from standard therapy. If on the other hand the upper bound is lower than the reference rate that will support the conclusion that the rate is significantly lower than with standard therapy. In contrast, if the lower bound is higher than reference rate, the conclusion that the rate is significantly higher than with standard therapy will be supported. For example, the proportion of participants re-Infused prior to follow-up in reference group was 63%. If 3 of the 10 subjects in our study group re-Infused prior to follow-up, then the estimated rate will be 0.3, and a 95% confidence interval

will be 0.11 to 0.6. That is, we are 95% confident that the true rate is at least 11% and no more than 60%. The confidence interval rules out any rate less than 11% or greater than 60%.

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APPENDIX 1: SCHEDULE OF EVENTS

"Y" visits are relative to first Islet Transplant. All other post-transplant visits are relative to last transplant.

TIMEPOINT	Ev[1]	WL[2]	Day -2	Day -1*	Day 0	Day +1 hr	Day +1	Day +2	Day +3	Day +7	Day 14	Day 21	Day 28	Day 56	Day 75	Day 120	Day 180	Day 270	Day 365	Y1	Mon 18	Y2	For Cause	D+7 post treatment or rejection
Visit Window (in days)			NA	NA	NA		NA	NA	+1	±3	±3	±3	±3	±7	±7	±7	±7	±14	±14	±14	±14	±90		
GENERAL																								
Consent to participate in study	X																							
Incl/Exclusion criteria	X	X[6]																						
Medical and DM Hx	X																							
Routine vital signs, wt, Ht[7]	X	X	X[8]	X	X		X	X	X				X		X		X	X	X	X	X	X		
History & Physical Exam	X	QYr	X[8]					X	X				X		X		X	X	X	X	X	X		
Review meds & insulin use	X	X	X[8]	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Adverse events			X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Hypoglycemia history	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Endocrinologist's verification	X																							
QOL survey (SF36)		Q3m													X		X		X	X		X		
Record all changes in medication/dose/frequency for immunosuppression medications on log	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
LABORATORY ASSESSMENTS	Ev[1]	WL[2]	Day -2	Day -1	Day 0	Day +1 hr	Day +1	Day +2	Day +3	Day +7	Day 14	Day 21	Day 28	Day 56	Day 75	Day 120	Day 180	Day 270	Day 365	Y1	Mon 18	Y2		
CBC with diff	X	Q6m	X[8]	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
PT/INR, PTT	X	X	X																					
BUN, Cr, Na, K, Cl, CO_2, Glu,	X	Q6m	X[8]	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Calcium, Phos, Mag	X	Q6m	X				X	X	X	X	X		X	X	X	X	X	X	X		X	X		
GFR by CKD-EPI	X	X	X																X	X		X		
Glucose immediately after Tx					X																			
ALT, AST, T Bili, Alk Phos, Alb, GGT	X	Q6m	X[8]					X		X			X		X		X		X	X	X	X		
Fasting Lipid panel (T Chol, TG, LDL, HDL)	X	QYr													X		X		X	X	X	X		
Amylase, Lipase	X		X[6]																					
PTH (bilateral starting Day -1)	X	QYr		X						X	X	X	X	X	X	X	X	X	X	X	X	X		
HBsAg, HBcAb, HBsAb and/or Hep B DNA, Quantitative by PCR[9]	X	QYr																	X[7]			X[7]		
HCV Ab and/or Hepatitis C RNA, Quantitative by PCR[10]	X	QYr																	X			X		

TIMEPOINT	Ev[1]	WL[2]	Day -2	Day -1*	Day 0	Day +1 hr	Day +1	Day +2	Day +3	Day +7	Day 14	Day 21	Day 28	Day 56	Day 75	Day 120	Day 180	Day 270	Day 365	Y1	Mon 18	Y2	For Cause	D+7 post treatment or rejection
Visit Window (in days)			NA	NA	NA		NA	NA	+1	±3	±3	±3	±3	±7	±7	±7	±7	±14	±14	±14	±14	±90		
HIV Ab I/II	X	QYr																	X			X		
CMV IgG[11]	X	QYr																	X[9]			X[9]		
CMV quantitative by PCR		QYr	X[8]												X		X							
EBV IgM anti-VCA & Anti-EBNA[12]	X																							
EBV quantitative by PCR		QYr	X[6]																					
JCV quant by PCR (if pt on Belatacept)[13]	X	QYr	X[6]																X			X		
BKV quant by PCR [14]	X	QYr	X[6]												X		X	X	X		X	X		
Urinalysis, (w/culture if needed)	X	QYr																						
First morning spot Urine (alb/cr)[15]	X	QYr											X		X				X	X	X	X		
Pregnancy test for females[16]	X		X																					
Rapid PTH- from donor PTG			X																					
DIAGNOSTIC ASSESSMENTS	**Ev[1]**	**WL[2]**	**Day -2**	**Day -1**	**Day 0**	**Day +1 hr**	**Day +1**	**Day +2**	**Day +3**	**Day +7**	**Day 14**	**Day 21**	**Day 28**	**Day 56**	**Day 75**	**Day 120**	**Day 180**	**Day 270**	**Day 365**	**Y1**	**Mon 18**	**Y2**		
PPD and/or Quantiferon Gold[17]	X	QYr																	X			X		
EKG	X	QYr	X[8]																X			X		
Stress echocardiogram or other cardiac test	X																							
Cardiac cath (if evidence of ischemia)	X																							
Intramuscular co-transplant of PTG and islets					X																			
Chest X-ray PA & LAT	X		X[18]																X			X		
Mammogram (women)	X	Q2Yr																						
Record Results of Retinopathy exam Per Standard Care	X	QYr																	X			X		
METABOLIC ASSESSMENTS	**Ev**	**WL**	**Day -2**	**Day -1**	**Day 0**	**Day +1 hr**	**Day +1**	**Day +2**	**Day +3**	**Day +7**	**Day 14**	**Day 21**	**Day 28**	**Day 56**	**Day 75**	**Day 120**	**Day 180**	**Day 270**	**Day 365**	**Y1**	**Mon 18**	**Y2**		
Collect pt's blood glucose logs daily taken at least 4 X/day, plus insulin use[19]	X	X							X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Continuous Glucose monitor		X													X		X		X	X	X	X		
2 Hr Mixed Meal Tolerance Test	X														X		X	X	X	X	X	X		
Fasting c-peptide and Glucose (Forearm Sampling in both Tx arm and Un-Tx arm);)Day 0 = pre-implant)					X				X	X	X	X	X	X		X				X		X		

TIMEPOINT	Ev[1]	WL[2]	Day -2	Day -1*	Day 0	Day +1 hr	Day +1	Day +2	Day +3	Day +7	Day 14	Day 21	Day 28	Day 56	Day 75	Day 120	Day 180	Day 270	Day 365	Y1	Mon 18	Y2	For Cause	D+7 post treatment or rejection
Visit Window (in days)			NA	NA	NA		NA	NA	+1	±3	±3	±3	±3	±7	±7	±7	±7	±14	±14	±14	±14	±90		
2 hr post prandial C-Peptide and Glucose (Forearm Sampling in both Tx arm and Un-Tx arm)									X	X														
HgbA1c	X	Q3m													X		X	X	X	X	X	X		
CALCULATIONS																								
MAGE, LI (Calculations)		X													X		X	X	X	X				
HYPO Score (survey & calculation)		X																		X				
Clarke Survey (survey & calc)	X	Q6m															X		X	X	X	X		
Beta Score (Calculation)		X													X		X	X	X	X	X	X		
C-pep/glu/Cr Ratio (CPGCR: calc)	X	X											X	X	X	X	X	X	X	X	X	X		
IMMUNOLOGY ASSESSESSMENTS	Ev	WL	Day -2	Day -1	Day 0	Day +1 hr	Day +1	Day +2	Day +3	Day +7	Day 14	Day 21	Day 28	Day 56	Day 75	Day 120	Day 180	Day 270	Day 365	Y1	Mon 18	Y2		
ABO Blood typing	X	X[20]																						
HLA Typing		X[21]																						
Tray sample for XMs with donors		Q3m																						
Class I/II Single Antigen testing	X	Q3m													X	X	X	X	X	X	X	X		
MECHANISTIC STUDIES	Ev	WL	Day -2	Day -1	Day 0	Day +1 hr	Day +1	Day +2	Day +3	Day +7	Day 14	Day 21	Day 28	Day 56	Day 75	Day 120	Day 180	Day 270	Day 365	Y1	Mon 18	Y2		
Cf DNA (Methylated DNA)			X[8]		X	X	X		X	X	X		X										X	X
LMX-Ang (Angiogenesis)			X[8]				X		X	X	X		X											
LMX-SF (Survival Factors)			X[8]				X		X	X	X		X											
LMX-CCK (Cytokines & Chemokines)			X[8]				X		X	X	X		X										X	X
IMM (GAD Tetramer Testing)			X[8]										X		X		X		X			X	X	
AutoAb (ICA 512,GAD, ZnT8)			X[8]														X		X			X	X	
MODY Genetic Panel (only if AutoAb negative)																	X							

*** Subsequent transplants restarts at day -1**

[1] **During screening for islets, the following labs are acceptable if done within 3 months of study enrollment: CBC, lytes, BUN, Cr, liver function labs, and lipids. All other tests listed in the chart above under "laboratory Assessments" and "Diagnostic Assessments" must have been done within one year of enrollment in the study. In addition, all screening tests in these two sections of the chart above can be done by outside providers.**

[2] **All waitlist assessments in the chart above listed under "laboratory Assessments" and "Diagnostic Assessments" can be performed by outside providers. WL assessments that repeat screening assessments must be timed based on when the test was last done for screening. All other WL assessments should be done as soon as possible. ABO, repeat HLA typing (if needed), and specific antigen testing must be completed before subject can be added to UNOS waitlist.**

[3-5] **Deleted in Protocol Version 4.0**

[6] **Inclusion/Exclusion criteria must be verified after each set of WL lab results are available and immediately before pancreas accepted for transplant. Pt must be inactivated on or removed from WL if these criteria are not met.**

[7] **Height to be collected at first visit only. During transplant admission, weight must be done before immunosuppression orders are written, then is not required for study until first outpt visit.**

[8] **Collect/perform prior to start of induction immunosuppression.**

[9] **If HBsAg positive, pt not eligible for islets. If HBcAb positive, must have undetectable viral load to be eligible for islets. Hep B serologies not needed at Day 365, Month 24, or Month 36 if subject was HBcAb negative and HBsAb positive prior to islet transplant.**

[10] **If HCV Ab pos, must have undetectable viral load to be eligible for islets.**

[11] **If CMV IgG was positive prior to islet evaluation, CMV IgG (antibody) does not need to be repeated. For Day 365, Month 24, and Month 36, CMV IgG is only needed if previous result was negative.**

[12] **If EBV IgG (anti-EBNA) was positive prior to islet evaluation, EBV antibody testing does not need to be repeated.**

[13] **If JCV by PCR is positive, subject is ineligible for islets. Notify kidney transplant team immediately.**

[14] **BKV quantitative by PCR: If serum positive before first islet transplant, pt is not eligible for islets. BKV quantitative by PCR will be followed every three months after transplantation and transplant team will treat per standard of care if patient is found to convert to a positive screen.**

[15] **First morning spot Urine (alb/cr) should be at additional visits as clinically indicated, especially if subject is on sirolimus.**

[16] **Pregnancy test must be done on all subjects born female. Screening pregnancy test can be either urine or serum, but if urine is positive, serum testing must be done to confirm. Pre-transplant pregnancy test should be done with serum. If results not back before induction immunosuppression needs to start, a urine pregnancy test should be done before induction started. If urine is positive and/or there is any chance subject could be pregnant, subject cannot continue with induction or transplant until serum result is back and PI has approved proceeding.**

[17] **While both PPD and Quantiferon Gold may be obtained to determine exposure to TB, Quantiferon Gold testing is preferred with this patient population because PPD can be inconclusive and/or not accurate if administered without controls in this immunosuppressed patient population.**

[18] **CXR on Day -2 may just be one view (such as when a portable film is done post PICC placement) if last CXR was done within 6 months of admission.**

[19] **Recorded daily (except during admission) and sent to UCSF for data entry**

[20] **Must have two results from separate blood draws before pt can be waitlisted. Previous ABO typing done for kidney may be used for one of the two tests.**

[21] **Perform HLA typing only if recommended by ITL. Otherwise, pre-kidney transplant HLA typing is acceptable.**

APPENDIX 2: REDUCED FOLLOW-UP SCHEDULE OF EVENTS

Subjects prematurely discontinued from study treatment according to the criteria in section 6.5.1 will remain in the study until normal termination. For the purpose of monitoring safety and efficacy parameters, subjects should be followed according to the reduced follow-up schedule. The day on which study treatment is discontinued is considered "Day 0."

Days Post entry into Reduced Follow-up	**28**	**56**	**75**	**180**	**270**	**365**	**year post final transplan**	**2 years post final transplant**	**3 years post final transplant**
Visit Window (in days)	**+/- 7**						**+/- 14**		
Assess SAEs and hypoglycemic events	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Alloantibody				**X**			**X**	**X**	**X**
Hgb A1c		**X**					**X**	**X**	**X**
Serum Cr							**X**	**X**	**X**
LFTs (ALT, AST, Alk Phos, Tbili, albumin)									
QOL questionnaires							**X**	**X**	**X**