Anti-inflammatory therapy to improve outcomes in patients with chronic pancreatitis undergoing total pancreatectomy islet autotransplantation

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Protocol Synopsis

Study Title	Anti-inflammatory therapy to improve outcomes in patients with chronic pancreatitis undergoing total pancreatectomy islet autotransplantation
Principal Investigator	Melena Bellin, MD
Participating Site	University of Minnesota
Accrual Objective	Up to 60 patients with chronic pancreatitis undergoing total pancreatectomy and islet autotransplant (IAT) will be enrolled for screening. Up to 48 participants will be randomized to treatment, with accrual goal of 45 completing 90 days of follow up.
Study Design	Single center randomized, open-label
Study Duration	8 years (2.5-3 years recruitment + 5 years follow up)
Study Objectives	**SPECIFIC AIM #1: To determine if anti-inflammatory treatment with etanercept or alpha-1 antitrypsin during the critical peri-transplant engraftment period improves islet engraftment and function after islet autotransplant**, as assessed by area under the curve for C-peptide on mixed meal tolerance testing (oral stimuli), acute insulin response to glucose-potentiated arginine stimulation (IV stimuli), and insulin requirements at day 90 post-transplant. The primary endpoint of the maximally stimulated acute insulin response to arginine (AIRmax) from GPAIS at day 90 will allow us to rapidly identify the most promising therapeutic agent for further development and testing in a large randomized trial. **SPECIFIC AIM #2: To gather pilot data on the long-term impact of etanercept or alpha-1 antitrypsin administered during the peri-transplant period by evaluating islet function at 1 and 2 years post-transplant,** as assessed by area under the curve for C-peptide on mixed meal tolerance testing (oral stimuli), acute insulin response to glucose-potentiated arginine stimulation (IV stimuli), and insulin requirements (units/kg/day) at 1 year post-TPIAT. In addition, insulin requirements and fasting/stimulated C-peptide by MMTT will be assessed at yearly intervals from year 3-5 post-transplant. **SPECIFIC AIM #3: To evaluate the mechanistic effects of etanercept and alpha-1 antitrypsin on inflammation, islet cell viability/survival, and beta cell apoptosis.** This will be assessed by circulating cytokine and chemokine levels and measurement of circulating unmethylated DNA (a recently validated marker of beta cell death in T1DM) in patient serum. The **goal** of the current research is to select the anti-inflammatory agent with the greatest potential to preserve islet mass early after TPIAT.
Study Endpoints	***Primary Endpoint:*** 1) AIRmax from the Day 90 visit (obtained from glucose potentiated arginine-induced insulin secretion): We have data from UMN that supports this as the strongest correlate with beta cell mass, so this measure best addresses our research question. The early endpoint is chosen to rapidly translate pilot results into randomized clinical trials. Patients are not expected to achieve insulin independence in this population before day 90. ***Key Secondary Endpoints:*** 1) Insulin independence: At day 365 and day 730. Defined as no insulin use for ≥14 days, with fasting blood sugar <126 mg/dL; 90 minute mixed meal glucose <180 mg/dL, and HbA1c ≤6.5% 2) Insulin dose (unit/day): At day 90, 365, and 730. 3) ACRmax, derived from GPAIS at days 90, 365, and 730 4) AIRglucose and ACRglucose, derived from the IVGTT at days 90, 365, and 730 5) AUC C-peptide from 2 hour MMTT, at days 90, 365, and 730 6) AUC glucose from 2 hour MMTT, at days 90, 365, and 730 7) Absence of severe hypoglycemic episodes (inclusive of day 90- 365) and HbA1c <7% at day 365 8) Absence of severe hypoglycemic episodes (inclusive of day 365-730) and HbA1c <7% at day 730

Inclusion Criteria	1) Age 18- 68 years. . 2) Scheduled for total pancreatectomy and IAT at UM. All patients who are approved for pancreatectomy and IAT at UM are reviewed by a multi-disciplinary committee including surgeons, gastroenterologists specializing in pancreatic disease, a pain specialist, psychologist, and endocrinologist to confirm the diagnosis of chronic pancreatitis and candidate suitability for surgery. 3) Able to provide informed consent
Exclusion Criteria	1) Pre-existing diagnosis of diabetes mellitus, fasting blood glucose >115 mg/dl, or hemoglobin A1c level >6.0% because these are all evidence of inadequate beta-cell mass. 2) Use of any of the following treatments in the 30 days prior to enrollment: insulin, metformin, sulfonylureas, glinides, thiazolidinediones, GLP-1 agonists, DPP-4 inhibitors, or amylin. 3) IgA deficiency (serum level <5 mg/dL), which has been associated with hypersensitivity to alpha-1 antitrypsin. 4) ALT or AST>2.5 times the upper limit of normal (ULN). Bilirubin >ULN, unless due to benign diagnosis such as Gilbert's. 5) Known history of HIV infection, hepatitis B (chronic), or hepatitis C (chronic). 6) History of tuberculosis (latent or active disease), or positive TB skin test. 7) History of symptomatic fungal lung infection. 8) History of multiple sclerosis, transverse myelitis, Guillain Barre, or other suspected demyelinating disease, due to risk of exacerbation of these conditions with use of etanercept; or prior history of systemic lupus erythematosus 9) Any of the following hematologic abnormalities: severe anemia (hgb <10 g/dL), thrombocytopenia (<150/mm3), or neutropenia (<1.0 x10^9/L). 10) Current use or expected use of oral or injected corticosteroids, or any mediation likely to affect glucose tolerance. However, use of hydrocortisone for physiologic replacement, or use of any topical, inhaled, or intranasal glucocorticoid is permitted. 11) Current or expected use of any other immunosuppressive agent. 12) Known hypersensitivity to etanercept or A1AT. 13) Any condition that is likely, in the opinion of the patient's medical providers, to necessitate use of TNF alpha therapeutically in the future (such as psoriatic arthritis). 14) Known coagulopathy, or need for anticoagulant therapy preoperatively (coumadin, enoxaparin), or any history of pulmonary embolism. 15) For females, plans to become pregnant or unwillingness to use birth control for the study duration. 16) Inability to comply with the study protocol. 17) Untreated psychiatric illness that may interfere with ability to give informed consent, or other developmental delay or neurocognitive disorder that impairs with a patient's ability to consent on their own behalf. ***18)*** Any other medical condition that, in the opinion of the investigator, may interfere with the patient's ability to successfully and safely complete the trial.
Treatment Description	Forty-five patients undergoing TP-IAT (day 0) at a single center will be randomized 1:1:1 to receive during the peri-operative period either: 1) **etanercept** (50 mg on day 0 SQ; 25 mg SQ on days 3, 7, 10, 14, and 21), 2) **alpha-1 antitrypsin** (90 mg/kg IV infusion on days -1, and +3, 7, 14, 21, and 28 post-transplant) or 3) **standard care**.
Study Procedures	Patients will be seen within 4 weeks prior to surgery (screening/baseline), and at day 90 (± 14 days), day 365 (± 28 days), and day 730 (± 56 days) post-transplant. These will occur over two days. The following metabolic testing will be performed at each visit: 1) 10 minute IV glucose tolerance test 2) Glucose potentiated arginine induced insulin secretion (GPAIS), performed on same morning as the IVGTT 3) Mixed meal tolerance test (2 hour, Boost HP) In addition, patients will have serum draws for mechanistic assessments and safety labs in the first month post-transplant as detailed in the protocol. The mechanistic assessments will include unmethylated insulin DNA and cytokine/chemokine profiles. For *long-term* follow up of participants, patients will receive routine care subsequent to the day 730 visit, which includes a HbA1c and mixed meal test at yearly intervals. Study investigators will review results from these annual labs and have a telephone follow up with the participant at years 3, 4 and 5 for insulin requirements, blood glucose review, and hypoglycemia history.

Study Abstract

Chronic pancreatitis is associated with incapacitating pain, frequent hospitalization and risk of narcotic dependence. If endoscopic procedures do not provide sufficient pain relief, total pancreatectomy (TP) may be considered. This results in certain diabetes unless the islets are returned with autologous islet transplantation (IAT). In this procedure, the patient's pancreas is completely removed, and the islets are isolated and infused into the portal vein. The islets engraft and potentially survive long term in the liver, releasing insulin in response to glucose. While 90% of patients have some function of the transplanted islet graft, only about 1/3rd come completely off insulin. The high prevalence of postsurgical diabetes is a major barrier for many patients and providers in considering TPIAT for treatment of chronic pancreatitis. The **long-term goal** of the proposed research is to develop new therapies that will increase the number of patients who are non-diabetic after TPIAT.

Following islet transplantation, the islets must acutely survive the stress of the procedure, and then they must engraft in the liver and establish a vascular supply. The greater the functional islet mass engrafted, the lower the risk of post-operative diabetes. It has been estimated that more than half of the islet mass may be lost in the early post-transplant period in islet transplant recipients. Beta cell apoptosis is common during the first month post-transplant and is upregulated in the presence of inflammatory cytokines such as TNF-α. Thus, a **major contributor to islet loss** is the inflammatory damage sustained by the transplanted islets in the early post-transplant period; *we propose to directly target this destructive process*.

Two promising anti-inflammatory therapies are available to address this problem: (1) the TNFα inhibitor etanercept and (2) alpha-1 antitrypsin. Both agents are clinically available and prime for clinical trials. **Etanercept** functions by binding TNFα, thus preventing its interaction with the TNFα receptor. Proof of principle is demonstrated in type 1 diabetic allotransplant recipients, in whom a 10 day course of etanercept early post-transplant significantly improved insulin independence rates up to 5 years after transplantation, postulated due to better survival of the transplanted beta cell mass in the engraftment period. **Alpha-1 antitrypsin (A1AT)** is a serine protease inhibitor. A1AT reduces inflammatory cytokines and islet cellular infiltrates, protects against cytokine-induced beta cell apoptosis, and prolongs islet graft survival in mice and non-human primates, notably including in syngeneic transplant in mice and marginal mass intraportal islet autotransplantation in monkeys, settings that closely replicate the TPIAT scenario.

This 3-arm **drug-treatment clinical trial** will investigate the use of Etanercept and A1AT to improve IAT function at 90 days and 1 and 2 years post-transplant compared to standard care of TPIAT recipients. **We hypothesize** that either anti-inflammatory treatment will reduce post-transplant beta cell apoptosis and improve islet engraftment, and that this will be reflected in higher stimulated insulin and C-peptide secretion at day 90.

Forty-five patients undergoing TPIAT at a single center will be randomized 1:1:1 to receive either: 1) etanercept (50 mg on day 0; 25 mg on days 3, 7, 10, 14, and 21), 2) alpha-1 antitrypsin (90 mg/kg IV days -1, +3, 7, 14, 21, 28) or 3) standard care in the peri-operative period. Patients will have mechanistic assessments drawn in the early post-operative period including inflammatory cytokines and chemokines and measures of beta cell loss (circulating unmethylated insulin DNA, released by dying beta cells). Patients will return for sophisticated metabolic testing at 90 days, and (to gather preliminary long-term efficacy data) 1 and 2 years post-transplant. Because the number of islets cannot be directly counted after islet infusion, we will use stimulatory testing as a surrogate measure of islet mass. At 90, 365, and 730 days post-TPIAT, patients will undergo mixed meal tolerance testing, IV glucose tolerance testing, and glucose-potentiated arginine-induced insulin secretion (GPAIS). The latter measures the maximally stimulated acute insulin response, or AIRmax, which is the best estimate of islet mass and the **primary endpoint** (at day 90) for this study. Results will be used to select the most promising agent for further study in a randomized, blinded multi-center clinical trial.

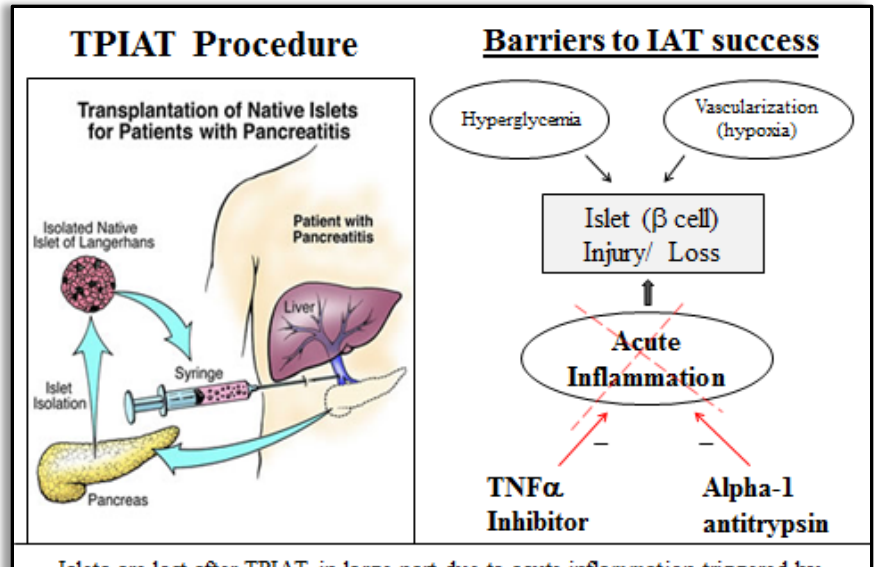
BACKGROUND

Chronic pancreatitis affects as many as 1 in every 2,500 persons and is associated with incapacitating pain, frequent hospitalization and risk of narcotic dependence (1). The health and economic costs of pancreatitis are great. Nearly $300 million dollars are spent on emergency department visits alone in the US each year (2). More than 90% of patients with chronic pancreatitis have been hospitalized, half use narcotic analgesics regularly, and one-fourth are on disability (3). The lifetime risk of diabetes mellitus is as high as 70% (4, 5). **Chronic pancreatitis is a significant health burden with few treatment options.**

While endoscopic procedures are often employed as a first-line therapy, if ERCP procedures do not provide sufficient pain relief, surgical therapy including total pancreatectomy (TP) may be considered to relieve the root cause of pain. TP results in certain diabetes unless the islets are returned with autologous islet transplantation (IAT). In this procedure, the patient's pancreas is completely removed, mechanically and enzymatically digested with collagenase, and the islets are isolated and infused into the portal vein. The islets engraft and potentially survive long term in the liver, releasing insulin in response to glucose (6). IAT results in complete insulin independence in 1/3rd of patients. Another 1/3rd require only small doses of insulin, while the remaining patients are completely insulin dependent (4).

The field of TPIAT is growing, with improved acceptance of this procedure in the medical GI community. This is reflected in growth of the number of centers performing the procedure and the number of procedures performed within the past decade. With no medical therapies available to halt the progression of the disease, TPIAT is likely to remain the only treatment option for many patients, particularly those with debilitating small duct chronic pancreatitis. However, the high prevalence of postsurgical diabetes is a major barrier for many patients and providers in considering TPIAT for treatment of chronic pancreatitis. **By reducing the risk of diabetes after TPIAT, we have the opportunity to substantially increase the applicability of TPIAT as a treatment option for patients with severe chronic pancreatitis.**

Following islet transplantation, the islets must acutely survive the stress of the procedure, and then they must engraft in the liver and establish a vascular supply. The greater the functional islet mass engrafted, the lower the risk of post-operative diabetes (6-10). Preventing early post-operative hyperglycemia reduces but does not prevent beta cell apoptosis. Even under conditions of adequate glycemic control, beta cell apoptosis is common during the first month post-transplant (11, 12) and is upregulated in the presence of inflammatory cytokines such as TNF-α. It has been estimated that more than half of the islet mass may be lost in the early post-transplant period (13). Thus, a **major contributor to islet loss** is the inflammatory damage sustained by the transplanted islets in the early post-transplant period; this in turn adversely impact long-term diabetes outcomes. ***We propose to directly target this destructive process***.



Islets are lost after TPIAT, in large part due to acute inflammation triggered by intraportal infusion of islets. Targeted anti-inflammatory therapy with TNFα inhibition or alpha 1- antitrypsin can directly target this damaging process.

Dramatic increases in beta cell apoptosis relative to that present in the native pancreas are observed *in vitro* in human islets and *in vivo* in animal models after islet isolation, increasing over the first 10 days post-transplant (12, 14), and worsened in the presence of inflammatory cytokines, such as TNFα (15). *In vitro* exposure of islets to TNFα alone or with IL-1 induces beta cell apoptosis (15). Expression of damaging inflammatory cytokines has been documented both in islet graft (16), and in total pancreatectomy islet autotransplant (TPIAT) recipients,

even under conditions of strict glycemic control (17, 18), presumably due to the stress of surgery. While inflammation may occur in the native pancreas due to recurrent acute and chronic pancreatitis prior to TPIAT, the isolated and intrahepatically transplanted islets are *uniquely and acutely vulnerable* to this inflammatory stress, because the islets are devascularized, hypoxic, and directly exposed to the intraportal blood after TPIAT. By developing therapies to protect beta cell mass from the damaging effects of inflammation during islet engraftment, we have the opportunity to increase the success of total pancreatectomy and islet autotransplant for patients with chronic pancreatitis.

These observations present an opportunity to intervene; blockade of the innate inflammatory response – which is directly damaging to beta cells— holds the potential to substantially improve the engrafted islet mass and thereby reduce diabetes risk after TPIAT. Two promising anti-inflammatory therapies are the TNFα inhibitor etanercept and alpha-1 antitrypsin. Both agents are clinically available and prime for clinical trials. There are reasons to believe that either drug may be more efficacious than the other. Etanercept is a potent anti-inflammatory drug which targets TNFα, a key central inflammatory mediator in beta loss after transplant. Alpha-1 antitrypsin has a more generalized anti-inflammatory effect and may directly inhibit beta cell apoptosis. However, there are currently not enough data available to determine the optimal therapeutic agent. To select the agent with the greatest potential for efficacy and favorable benefit-risk ratio, the two therapies must be compared head-to-head, as well as compared to standard care. This is the focus of this pilot study, and represents an **essential first-step** to select the most promising drug for clinical development.

The short-term goal is to perform a pilot study with etanercept and alpha-1 antitrypsin to determine which is the most promising anti-inflammatory drug in TPIAT, which will inform future clinical trials. To accomplish this goal, we will randomize 45 TPIAT patients to receive either: 1) standard care, 2) etanercept, or 3) alpha-1 antitrypsin in the early peri-operative period. Outcome assessments will focus on inflammation and islet loss in the perioperative period, and engrafted islet mass at 90 days post-transplant, with pilot data collected for long-term (1 and 2 year) diabetes outcomes.

The proposed agents have shown promise in preclinical trials. In addition, etanercept has shown clinical efficacy in allotransplantation and the survival of marginal mass autologous islet transplants in non-human primates—a situation nearly analogous to TPIAT—are dramatically improved under simultaneous short-term administration of A1AT. However, no controlled clinical trials to date have been conducted in TPIAT using these agents. We have a novel opportunity to test these agents formally in clinical trials in TPIAT, using the large volume of patients with pancreatitis seen for this procedure at our institution.

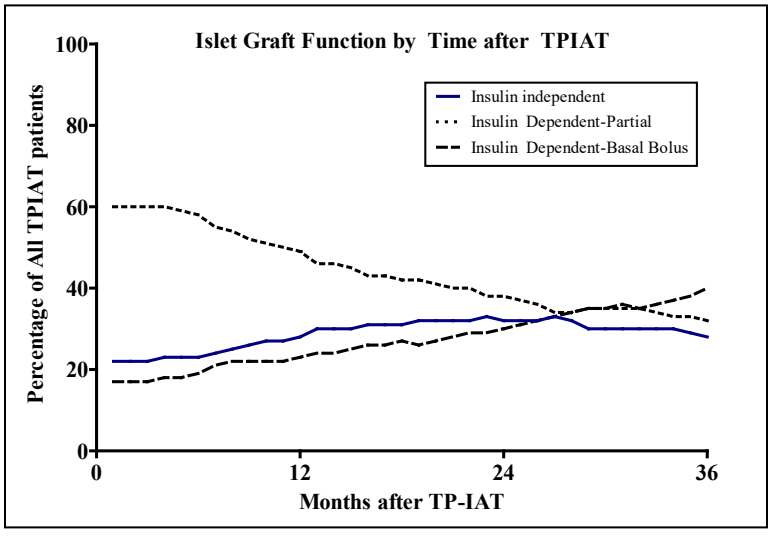
While the primary indication for total pancreatectomy and islet autotransplant is to relieve pain and improve health-related quality of life (HRQOL), preventing or minimizing diabetes is an important secondary outcome which benefits recipients' long-term health and HRQOL

Because TPIAT is a major surgical procedure, with risk of diabetes, patients with chronic pancreatitis are carefully considered for this procedure by a multi-disciplinary team, and selected based on clear diagnosis of pancreatic disease causing pain, narcotic dependence or severely impaired quality of life, and failure to respond to maximal medical/ endoscopic therapy (19). Patients at our institution since 2007 have been enrolled in a prospective cohort study to follow pain symptoms, narcotic use, and HRQOL, in addition to diabetes outcomes, after this procedure. At 1, 2, and 3 years after TPIAT, 84-86% of patients report pain as resolved or significantly improved compared to before pancreatectomy. Mean summary scores for HRQOL obtained from the SF-36—a standardized HRQOL questionnaire— are 2 standard deviations below the population average for physical functioning and 1 standard deviation below the population mean for mental functioning before surgery, reflecting the severity of disease in this population, but improve by $\geq$1 standard deviation at 1 year and 2 years after TPIAT (7).

Insulin independence is observed in approximately 30% of patients at any given time between 1-3 years post-transplant (figure, below). Thus, there is significant room for improvement in diabetes outcomes after the IAT procedure. About 90% of patients have functioning islets, but this function is insufficient to achieve total insulin independence in many cases. Twenty percent of patients are unable to maintain their hemoglobin A1c level <7%

(7), the glycemic goal recommended by the American Diabetes Association and shown by the Diabetes Control and Complications Trial to reduce risk of microvascular complications of diabetes (20).

While insulin dependent patients do exhibit improvement in overall HRQOL and self-report diabetes as a more manageable chronic disease than pancreatitis, there are important differences in HRQOL scores in the diabetic patients. On HRQOL subscales derived from the SF-36, insulin independent patients had higher scores for physical functioning, physical role, and vitality at 2 years compared to those patients requiring insulin therapy. Perhaps more importantly, failure of the islet autograft puts patients at risk for diabetes complications, including retinopathy, nephropathy, and neuropathy. Thus, improving the proportion of patients who have a successful diabetes outcome is critical to optimizing our management of this group of patients afflicted with intractable chronic pancreatitis.



Successful islet engraftment during the first few months after TPIAT is critical to long-term prevention of diabetes

The first hurdle to a successful islet transplant is the number of islets available, and thus the transplanted islet mass is an important predictor of subsequent diabetes risk (7-9, 21). However significant variability in diabetes outcomes is seen for any given transplanted islet mass. Approximately 70% of IAT recipients with >5000 islet equivalents (IEQ)/ kilogram body weight transplanted will achieve insulin independence, while 30% never come off insulin despite the large number of islets infused. In contrast, nearly 10% of those patients with a very low islet mass (<2500 IEQ/kg) will maintain insulin independence, sometimes for years (7). This variability can largely be explained by the difference between the *transplanted* islet mass and the *engrafted* islet mass. Beta cell apoptosis and loss occurs in the immediate post-operative period due to the stress of the procedure and transplant environment. High levels of beta cell apoptosis are observed, particularly in the 7 days after surgery, with apoptosis observed even at 30 days (11, 12). It has been estimated that more than half of the islet mass may be lost in this early post-transplant period in islet transplant recipients (13). The innate inflammatory response, triggered by islet infusion, hypoxia, and hyperglycemic stress, is an important contributor to beta cell loss. In alloislet transplant, successful engraftment of transplanted islets, as demonstrated by functional testing at 3 months, is strongly predictive of diabetes remission at 1 year, even more so than the initial number of islets transplanted (22). Thus, this is a critical early window in which to intervene to optimize engrafted islet mass.

Islets are damaged by inflammatory cytokines, which are expressed in the islet graft and upregulated in the TPIAT recipient immediately after surgery

Cytokines are expressed from the islet graft itself, induced by the stress of isolation and transplant. Montolio et al demonstrated that TNFα and IL-1 were both highly upregulated in islet tissue immediately after isolation and following a one day culture period, and increased after transplantation into streptozotocin-induced diabetic Lewis rats (day 1, 3, and 7 post-transplant) even when conditions of normoglycemia were maintained (17). When islets are infused into the intraportal environment, islet expression of tissue factor triggers an instant blood mediated inflammatory reaction (IBMIR), in which the complement and coagulation cascades are activated. That, in turn, exacerbates the non-specific inflammatory response, leading to neutrophil and macrophage infiltration into the graft, increased local cytokine expression, and ultimately islet loss (23-26). Cytokines, particularly TNFα and IL1β, are directly damaging to islets *in* vitro and are proposed to mediate similar damage *in vivo* post-transplantation (15, 16, 27, 28). Islet isolation itself induces high rates of beta cell apoptosis, due to the stress of

the isolation procedure, and this apoptosis is unfortunately exacerbated when islets are exposed post-isolation to TNFα (15). Thus, the immediate post-operative period is an opportunity for meaningful intervention.

Further contributing to a pro-inflammatory milieu is the major surgical procedure of total pancreatectomy and islet autotransplantation in patients with underlying history of chronic and/or recurrent acute pancreatitis. Multiple pro-inflammatory cytokine/ chemokine pathways are upregulated in the one week period following surgery in clinical TPIAT recipients (18). Itoh et al demonstrated that the "danger signal" high-mobility group box-1 (HMGB-1) was upregulated in islet grafts from clinical TPIAT recipients and that elevated HMGB-1 levels were directly correlated with greater post-transplant inflammation which in turn was negatively associated with islet graft outcomes, as measured by insulin independence (18). Pro-inflammatory cytokines are notably much following TPIAT with an adequate islet graft compared to pancreatectomy alone or TPIAT with very few islets, demonstrating that in fact this inflammatory response is elicited by the intraportal infusion of the islet graft and not simply a consequence of the major surgery (26).

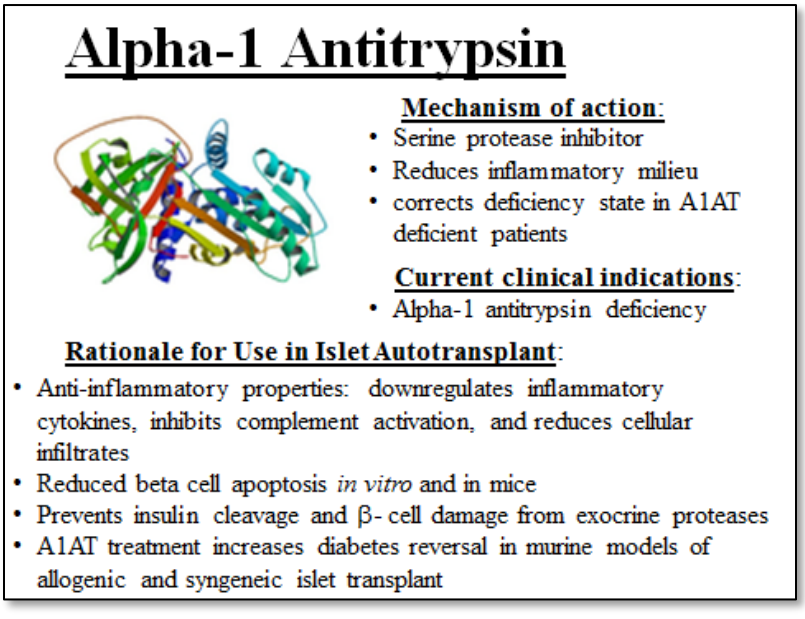
Promisingly, therapeutic agents that target damaging inflammation are clinically available

We have the opportunity to intervene by targeting this early inflammatory response with clinically available medications. Based on preclinical studies and/or experience in islet allotransplantation, two therapeutic agents are particularly promising—Alpha-1-antitrypsin (A1AT) and etanercept. Both agents are FDA-approved for indications other than IAT and have been used extensively in humans. A1AT is approved for use in adults with A1AT deficiency, and is currently under study as a beta-cell protective agent in type 1 diabetes; this agent has substantial preclinical data that suggest a preservation of islet mass and higher likelihood of diabetes cure when administered in the early post-operative period. Etanercept is FDA approved for use in both children and adults with particular forms of inflammatory arthritis or psoriasis. Etanercept has been studied in non-controlled clinical trials in islet allotransplantation and appears within this setting to convey substantial benefit in long-term engraftment and survival of islet mass, even when administered for a short duration (10 days post-transplant). We have the opportunity to study both agents in a head-to-head comparison, and against a standard care control arm, to determine utility of each agent in protecting early islet engraftment and to select which agent is most promising for further study and development for clinical use in TPIAT.

Alpha-1 antitrypsin

Alpha-1-antitrypsin is a safe, clinically available drug that may downregulate innate inflammation, and may have additional protective effects on transplanted islets

Alpha-1-antitrypsin (A1AT), also known as alpha$_1$-protease inhibitor, is a serine protease inhibitor. A1AT inhibits the enzymatic activity of neutrophil elastase, thrombin, cathepsin G, proteinase 3, trypsin, and chymotrypsin. A sterile, stable, lyophilized preparation is manufactured for clinical use by purification from human plasma, and is marketed by several manufacturers under different product names, including Aralast NP (Baxter, CA). The product is indicated for use in adults with lung disease due to alpha-1-antitrypsin deficiency but has been studied for other indications, including pain management in fibromyalgia and beta cell preservation in new onset type 1 diabetes mellitus (29-31). While preclinical data is promising, there are currently no clinical trials underway studying this agent in patients with pancreatitis undergoing TPIAT.



A1AT is directly relevant to the current proposal because of its anti-inflammatory effect.

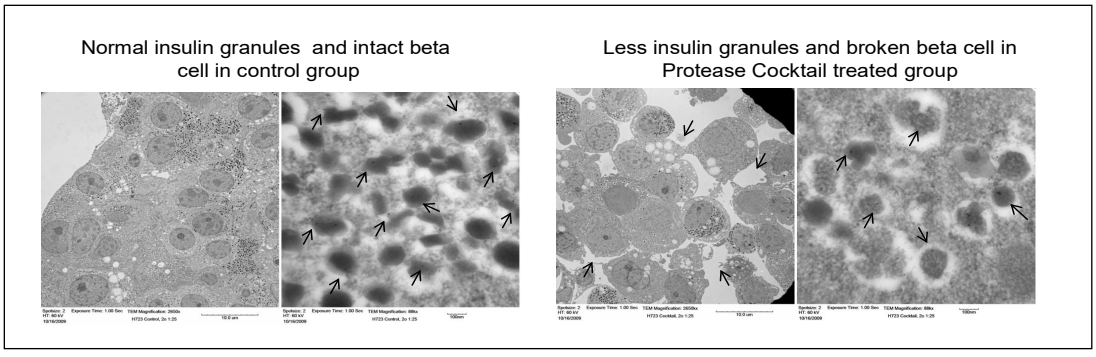
A1AT downregulates inflammatory cytokines, inhibits complement activation, and reduces cellular infiltrates, all of which mediate beta cell damage post-transplant (32-35). It has been demonstrated to reverse diabetes in NOD mice (36, 37), protect against streptozotocin- mediated beta cell toxicity (33), protect against cytokine-induced beta cell apoptosis (38, 39), prolong islet allograft survival in mice (33, 34, 36, 40), and preserve islet architecture when islets are co-cultured with acinar proteases (41). Importantly, A1AT has been shown to downregulate transcription of multiple inflammatory pathways that are activated in diabetes and islet transplant (42-44). TNFα is an important central mediator in the inflammatory pathways targeted by A1AT, but many other innate inflammatory cytokines (IL-6, IL-8) and chemokines are affected by A1AT; thus, A1AT may be advantageous compared to other immune agents in its ability to target the greater inflammatory milieu, rather than a single cytokine pathway. Treatment with A1AT in clinical islet autotransplant recipients has the potential to preserve peri-transplant beta cell mass by downregulating innate inflammatory cytokines. In addition, A1AT has been proposed to directly protect beta cells from apoptosis, and to protect transplanted islets against potentially damaging effects from co-transplanted exocrine tissue.

Alpha-1 antitrypsin has other appealing properties, including the ability to reverse diabetes in mice and inhibit beta cell apoptosis

Treatment with A1AT protects against both development of STZ-induced diabetes in mice and spontaneous development of autoimmune diabetes in NOD mice (33, 36, 39). Lower levels of A1AT have been observed in children with type 1 diabetes (45) and adults with type 2 diabetes (46). These findings suggest a general protective effect against islet damage in diabetes. Furthermore, A1AT may have a direct inhibitory effect on beta cell apoptosis, which is increased 10-fold after islet isolation and transplantation (12, 47), and exacerbated by inflammatory cytokines and hyperglycemia (12, 13). In a mouse insulinoma cell line exposed to TNFα *in vitro,* treatment with A1AT greatly reduced apoptosis and entirely abolished caspase 3 expression (39). In another study, beta cell apoptosis was reduced in A1AT-treated versus untreated cultured islets exposed to either TNFα or a three cytokine cocktail with TNFα /IL-1β/ INF-γ (38). Koulmanda et al demonstrated a 14-fold reduction in cleaved caspase 3 expression (a pro-apoptotic marker) at day 3 post-transplant in mice treated with A1AT receiving syngeneic islet grafts (43).

Alpha-1 antitrypsin protects transplanted islets from the exocrine proteases that are invariably co-transplanted with the islet product.

One of the less considered areas that could potentially influence human islet transplantation outcomes and functional capacity is the release of activated proteolytic enzymes (e.g., trypsin, chymotrypsin, and elastase) by dying acinar cells during the isolation process, culture, and post-transplantation. Invariably, some exocrine tissue is co-cultured in the case of allografts or cotransplanted in the case of auto- and allografts. Autologous islet preparations in particular often contain as much exocrine as endocrine tissue, because aggressive purification of islets must be avoided to maximize the islet mass available for transplant. This acinar tissue that accompanies the islets may have a detrimental impact on the islet graft in the peri-transplant period. Co-transplantation of islets with acinar tissue in impure preparations causes islets to be surrounded by exocrine cells in the portal vein capillaries. The active proteolytic enzymes released from the acinar tissue (trypsin, chymotrypsin, and elastase) continuously degrade insulin produced from the islet cells, which may negatively impact early glycemic control. More importantly, acinar proteases may be directly damaging to the transplanted beta cell. In a recent analysis of 110 islet after kidney transplantations performed within the Nordic Network for Clinical Islet Transplantation, for every milliliter of exocrine tissue transplanted, there was a reduction in islet graft function equivalent to a loss of 100,000 IEQ (48). Protease exposed human islets show insulin degranulation and microstructural damage on electron microscopy (41), with a reduction of insulin granules and broken beta cells compared to intact beta cells and insulin granules in untreated isolated islets (electron microscopy figure below).



Preclinical data in mice and non-human primates demonstrates dramatically increased rates of diabetes reversal with marginal mass islet grafts when alpha-1 antitrypsin therapy is administered; Pilot data in TPIAT recipients suggests a benefit when endogenous A1AT levels are elevated

Preclinical trials in which a short duration course of A1AT is administered in the immediate post-operative period demonstrate clear efficacy in both allogeneic and syngeneic murine transplant models (32-34, 40, 43). C57BL/6 mice that were rendered diabetic with streptozotocin exhibited prolonged islet allograft survival when treated with human A1AT compared to an untreated control group, lasting until the time of development of human A1AT antibodies. Reduced neutrophilic inflammation was observed in the islet grafts of A1AT treated mice at 24 and 48 hours after transplant compared to controls (33). A second study confirmed these findings—streptozotocin-induced diabetic mice that were treated with human A1AT exhibit superior islet allograft survival, in a dose-dependent fashion, with mice treated for 30 days having superior outcomes (100% 30 day graft survival) compared to those treated for 14 days (50% diabetes reversal) compared to no diabetes reversal in untreated (34). Grafts of treated mice had low levels of expression of pro-inflammatory mediators/cytokines (34). Streptozotocin-induced diabetic mice genetically programmed to express human A1AT exhibited prolonged survival (>100 days) of islet allografts whereas control animals and those treated with inactive truncated A1AT rejected islets within 10 days, supporting a role for the active A1AT (32).

Notably, these findings have been confirmed in a *syngeneic non-autoimmune* model (43) and in non-human primate autografts (49). C75BL/6 mice receiving syngeneic marginal mass islet grafts— a scenario analogous to clinical islet autograft recipients, who lack immunogenicity and often receive marginal mass islet grafts---exhibited superior islet graft survival when treated with A1AT (Aralast). Of those treated with A1AT, 100% had islet graft function at day 3, compared to 10% in the placebo group, and 70% retained graft function for 60 days, compared to none in the placebo group. Mechanistic analysis revealed downregulation of multiple pro-inflammatory pathways including reduced transcription of chemokines, chemokine receptors, and innate inflammatory cytokines including IL-6 and IL-8. Islet grafts from treated animals had superior survival of intact islets at day 3, with highly reduced apoptosis (cleaved caspase 3 detected in only 5% of the islets of A1AT treated vs 70% of control islets). More critically, these results were replicated in non-human primates receiving marginal mass islet autografts infused intraportally following subtotal pancreatectomy and streptozotocin treatment (49). Because these are autografts, and the infusion is intraportal, the clinical TPIAT scenario is closely replicated. In this study, all 5 monkeys treated with 4 doses of A1AT over 2 weeks (day -1 to 14 days post-transplant at 60 mg/kg IV) maintained normoglycemia off insulin for >1-3 years (no failures to date of publication), whereas all 4 untreated monkeys all had graft failure by day 180 post-transplant.

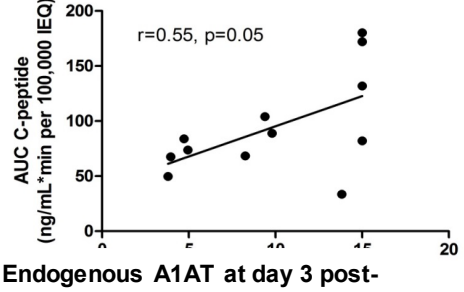


Figure: Endogenous A1AT at day 3 post-IAT and C-peptide on MMTT

In a small cohort of patients at our institution, endogenous alpha-1 antitrypsin levels were higher in those patients who subsequently demonstrated superior islet graft function. Islet graft function was measured at 3 months by C-peptide production from mixed meal tolerance testing. Those patients with high C-peptide levels (above the median) at 3 months versus low C-peptide levels (below the median) had higher endogenous alpha-1 antitrypsin levels. Notably, there was

significant correlation between the day 3 alpha-1 antitrypsin level and graft function at 3 months, as measured by area under the curve for C-peptide.

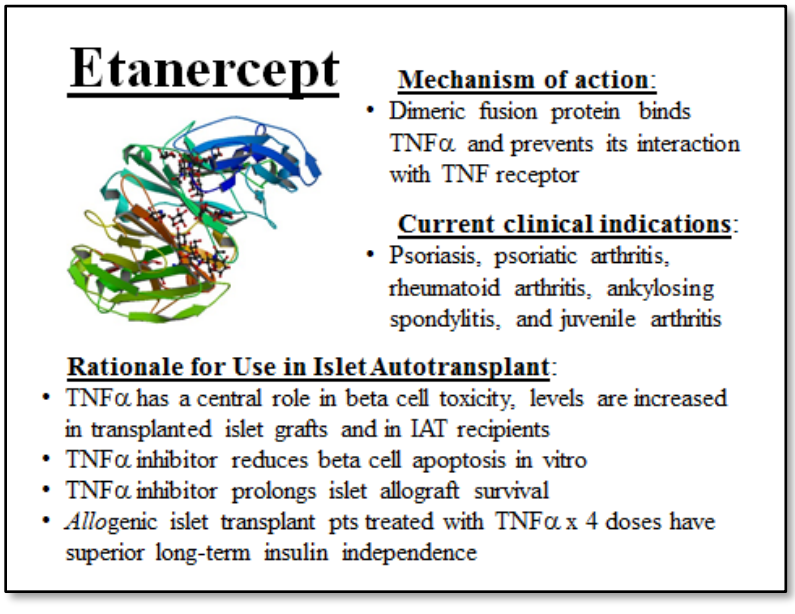
Alpha-1 antitrypsin is safe and well-tolerated in clinical use and clinical trials for other indications

The product is indicated for use in adults with lung disease due to alpha-1-antitrypsin deficiency and is generally well-tolerated in this population. Commonly reported side effects include increased liver enzymes, cough or upper respiratory symptoms, chest discomfort, and dizziness (50). Transmission of serious viral infection has not been reported. The conventional replacement dose of A1AT studied in patients with pulmonary disease related to deficiency state is 60 mg/kg body weight infused intravenously once weekly. However, higher doses have been studied, up to 250 mg/kg for 2 consecutive weeks, with more potent suppression of neutrophil-mediated proteolysis (marker of lung inflammation) at the higher dose (51). ClinicalTrials.gov cites 5 clinical trials evaluating A1AT in new onset type 1 diabetes, at doses as high as 90 mg/kg IV weekly, and in patients as young as 8 years of age (31).

Etanercept

Etanercept, a TNFα inhibitor, targets this key inflammatory cytokine implicated in β-cell apoptosis, with strong clinical evidence for efficacy based on studies in islet allotransplantation for type 1 diabetes

Etanercept is a dimeric fusion protein containing an extracellular ligand-binding portion of the TNF receptor linked to the Fc portion of human IgG. Etanercept functions by binding TNFα, thus preventing its interaction with the TNFα receptor.



TNFα is a particularly appealing therapeutic target in TPIAT recipients for several reasons. Islet grafts themselves release TNFα in response to the stress of islet isolation (17, 23), in addition to the pro-inflammatory milieu of the post-operative patient. TNFα is directly toxic to islets, and potentiates the effect of toxicity induced by other circulating cytokines, including IL-1, as demonstrated by *in vitro* data (15, 27, 28). *In vitro,* the addition of anti-TNFα inhibitor to islets in standard culture media reduced TNFα secretion by >60%, reduced beta cell apoptosis by 26%, and increased stimulated insulin secretion (16). TNFα inhibitor therapy has been studied in murine models of islet transplantation and in non-controlled studies of islet allotransplantation, but these findings have never been translated to TPIAT recipients.

In murine models, administration of a TNFα inhibitor prolonged islet allograft survival, shifted the circulating cytokines towards an anti-inflammatory profile, and increased expression of anti-apoptotic pathways within the islet graft (52). In a separate study, when human islets were transduced via an adenoviral vector to express a soluble type 1 tumor necrosis factor receptor (TNFR) immunoglobulin-Fc fusion transgene (TNFR-Ig), which blocks TNF signaling, these TNFR-Ig expressing islets were protected from cytokine-induced apoptosis *in vitro,* and when these islets were transplanted into a diabetic mouse model, insulin independence was successfully restored for longer durations than untreated islets (53). Etanercept therapy has been administered in several clinical trials of alloislet transplantation for type 1 diabetic recipients, in addition to standard immunosuppression (54-56). Recipients were given a 10 day course of etanercept early post-transplant (50 mg on day 0, and 25 mg on days 3, 7, and 10). In recent analyses conducted by our institution and the Collaborative Islet Transplant registry, patients who received etanercept had significantly improved insulin independence rates up to 5 years after transplantation compared to those who had not. Because the drug was given early in the early post-operative

stage, we postulated that TNFα inhibition led to better engraftment and survival of the transplanted beta cell mass, thus allowing longer duration of insulin independence over conventional immunosuppression alone (54, 57). In a preliminary analysis of 12 TPIAT recipients at our institution, higher levels of TNFα at 3 days post-transplant were associated with lower C-peptide levels at 3 months (r= -0.38, p=0.2).

Etanercept is FDA approved for treatment of ankylosing spondylitis, rheumatoid arthritis, and psoriatic arthritis and moderate to severe plaque psoriasis in adults. The standard adult dose for arthritic conditions is 50 mg once weekly, although doses as high as 50 mg twice weekly for 3 months are approved for treatment of severe plaque psoriasis. Etanercept is FDA approved for the treatment of juvenile idiopathic arthritis in children 2 years of age or older at a dose of 0.8 mg/kg weekly (50). Clinical trials with etanercept have focused on these conditions and other immune, rheumatic, or skin disorders, in children and adults (58-68). The most common adverse events are injection site reactions. Other potential safety concerns include risk for opportunistic infection, demyelinating disorder, or malignancy. However, current long-term follow up studies suggest a favorable safety profile, even with prolonged use (60, 69), In 58 children treated with etanercept for JIA for up to 8 years under an open-label extension study, the overall rate of serious adverse events was low at 0.12 SAE per patient-year, with no cases of tuberculosis, opportunistic infection, lymphoma, other malignancy, or demyelinating disease observed over the study period (64). Similar findings are reported in 380 adults treated for rheumatoid arthritis and followed for 5 years (69)-- the most common adverse event was upper respiratory tract infection; serious adverse events included one case of tuberculosis but no other opportunistic infections, blood dyscrasias, or demyelinating disease.

Etanercept has been studied in new onset diabetes mellitus as a strategy to preserve beta cell mass. In a cohort of 18 children with new onset type 1 diabetes randomized to receive etanercept at a dose of 0.4 mg/kg twice weekly (maximum 25 mg twice weekly) for 24 weeks, hemoglobin A1c was lower in treated subjects, and area under the curve for C-peptide from mixed meal tolerance testing increased by 39% at 24 weeks compared to a 20% decrease in C-peptide in the placebo group, suggesting a beta cell-protective effect of etanercept (70).

Measures of islet function serve as surrogate measures of islet mass

In order to adequately assess the impact of any therapy on islet engraftment, it is important to have endpoints that reflect islet mass. Because it is not possible to directly quantify the number of islets that engraft and survive in the liver post-transplant, we have carefully selected surrogate endpoints that reflect beta cell function and mass. We will assess several previously validated measures: 1) C-peptide response to a mixed meal tolerance test (MMTT), 2) the acute C-peptide and insulin response to intravenous glucose tolerance (IVGTT), and glucose potentiated arginine-induced insulin secretion (GPAIS).

The oral stimulus used most often in the TPIAT setting and in trials of new onset type 1 diabetes mellitus is the mixed meal tolerance test, in which the subject ingests a standard amount of Boost High Protein or a similar beverage. The MMTT is an accepted measure of beta cell *function* in islet transplant and diabetes studies (7, 71, 72), and it is simple to perform in the clinic setting. However, it may be less useful than the intravenous stimuli as a direct measure of islet *mass* (73). Intravenous stimuli—dextrose and/or arginine—have been shown to correlate with islet mass in animal and clinical transplant models (74-76). Both the acute insulin response to glucose (AIRglu) derived from the IVGTT and the maximal acute insulin response to arginine (AIRmax) measured from GPAIS correlate strongly with transplanted islet mass in patients who maintain euglycemia long-term (74). While transplanted islet mass is not the same as engrafted islet mass, these studies were performed in patients who were specially selected from those with optimal long-term beta cell function, and so were likely the recipients with the best engraftment. Thus, they appear to be reasonable surrogate measure for engrafted beta cell mass. Both measures were superior to a simple arginine stimulation test (22). In allotransplant patients, the AIRglu and AIRmax at 3 months post-transplant predicted insulin independence at 1 year. Thus, this test performed early after transplant was predictive of key long-term clinical outcomes (22).

Measures of islet loss

Another approach to measuring engraftment is to use surrogate measures of islet loss. Recently, a novel assay developed by Herold et al, measuring circulating levels of unmethylated insulin DNA as a measure of beta cell death, was validated in type 1 diabetes (77). When studied in 80 diabetic patients (37 treated with teplizumab), researchers found that the levels of unmethylated insulin DNA were higher in new onset diabetics than in healthy controls, due to accelerated beta cell turnover. Those type 1 diabetic patients treated with teplizumab had lower levels of unmethylated DNA than the untreated patients, consistent with the observation that the teplizumab group preserved beta cell mass and function (based on C-peptide secretion from MMTT). In collaboration with Dr. Herold, we have a novel opportunity to measure this marker in the early post-transplant period as a measure of islet loss.

Summary of significance

Approximately 4 in every 10,000 persons in the U.S. suffer from chronic pancreatitis, with few treatment options available (1). Chronic pancreatitis can result from genetic or obstructive factors, trauma, or alcohol use, or, often, unknown causes; it is a disabling disease that affects many young adults with an otherwise long lifespan ahead of them.

While TPIAT can provide pain relief and restore quality of life, application of this procedure is limited by the high rate of postsurgical diabetes mellitus (7, 19). Success of the IAT is hampered by early islet loss. In fact, insulin independence has been observed in low islet mass recipients (<2500 IEQ/kg) (7), suggesting a high success rate could be achieved with a much lower number of islets if engraftment is optimized. Islet engraftment and surviving beta cell mass is compromised by the innate inflammation that occurs following intraportal islet infusion. We have the opportunity to test the efficacy of two anti-inflammatory therapies, A1AT and etanercept, in patients undergoing TPIAT. This study will pave the way for a larger, definitive multicenter trial by helping to define the appropriate therapeutic agent. While our study is focused on patients with pancreatitis at risk for surgical diabetes after TPIAT, findings would also be applicable to the field of islet *allo*transplantation for type 1 diabetes mellitus.

OBJECTIVES AND SPECIFIC AIMS

This **drug-treatment clinical trial** will investigate the use of etanercept and A1AT to improve IAT beta cell function at 90 days post-transplant compared to standard care of TP-IAT recipients. **Our overall hypothesis is that one or both anti-inflammatory drugs will reduce post-transplant beta cell apoptosis, improve islet engraftment, and that this will be reflected in higher stimulated insulin and C-peptide secretion at day 90.** Mechanistic data from the early post-transplant period and graft function at day 90 will be used to select the most promising agent for further clinical development.

There are reasons to believe that one strategy may be more impactful than the other. Alpha-1 antitrypsin has a generalized anti-inflammatory effect and directly inhibits beta cell apoptosis, which makes this agent appealing. Etanercept is a more potent anti-inflammatory that directly targets a central mediator in post-transplant beta cell damage—TNFα—and thus may be more effective. The most efficient approach to select the best therapy is to compare these two agents head-to-head. Thus, we propose the following sub-hypothesis for the study:

- Sub-hypothesis #1: The efficacy of etanercept will be superior to that of A1AT because it specifically targets the cytokine most clearly implicated in beta cell apoptosis.
- Sub-hypothesis #2: The efficacy of A1AT will be superior to that of etanercept because it both inhibits cytokine production and it blocks protease activity.

Specific Aims

Forty-five patients undergoing TP-IAT (day 0) at a single center will be randomized 1:1:1 to receive during the peri-operative period either: 1) **etanercept** (50 mg SQ on day 0; 25 mg SQ on days 3, 7, 10, 14, and 21), 2) **alpha-1 antitrypsin** (90 mg/kg IV infusion on days -1, and +3, 7, 14, 21, and 28 post-transplant) or 3) **standard care**.

SPECIFIC AIM #1: To determine if anti-inflammatory treatment with etanercept or alpha-1 antitrypsin during the critical peri-transplant engraftment period improves islet engraftment and function after islet autotransplant, as assessed by area under the curve for C-peptide on mixed meal tolerance testing (oral stimuli), acute insulin response to glucose-potentiated arginine stimulation (IV stimuli), and insulin requirements at day 90 post-transplant. The primary endpoint of the maximally stimulated acute insulin response to arginine (AIRmax) from GPAIS at day 90 will allow us to rapidly identify the most promising therapeutic agent for further development and testing in a large randomized trial.

SPECIFIC AIM #2: To gather pilot data on the long-term impact of etanercept or alpha-1 antitrypsin administered during the peri-transplant period by evaluating islet function at 1 and 2 years post-transplant, as assessed by area under the curve for C-peptide on mixed meal tolerance testing (oral stimuli), acute insulin response to glucose-potentiated arginine stimulation (IV stimuli), and insulin requirements (units/kg/day) at 1 year post-TPIAT. In addition, insulin requirements and fasting/stimulated C-peptide by MMTT will be assessed at yearly intervals from year 3-5 post-transplant.

SPECIFIC AIM #3: To evaluate the mechanistic effects of etanercept and alpha-1 antitrypsin on inflammation, islet cell viability/survival, and beta cell apoptosis. This will be assessed by circulating cytokine and chemokine levels and measurement of circulating unmethylated DNA (a recently validated marker of beta cell death in T1DM) in patient serum.

The **goal** of the current research is to select the anti-inflammatory agent with the greatest potential to preserve islet mass early after TPIAT.

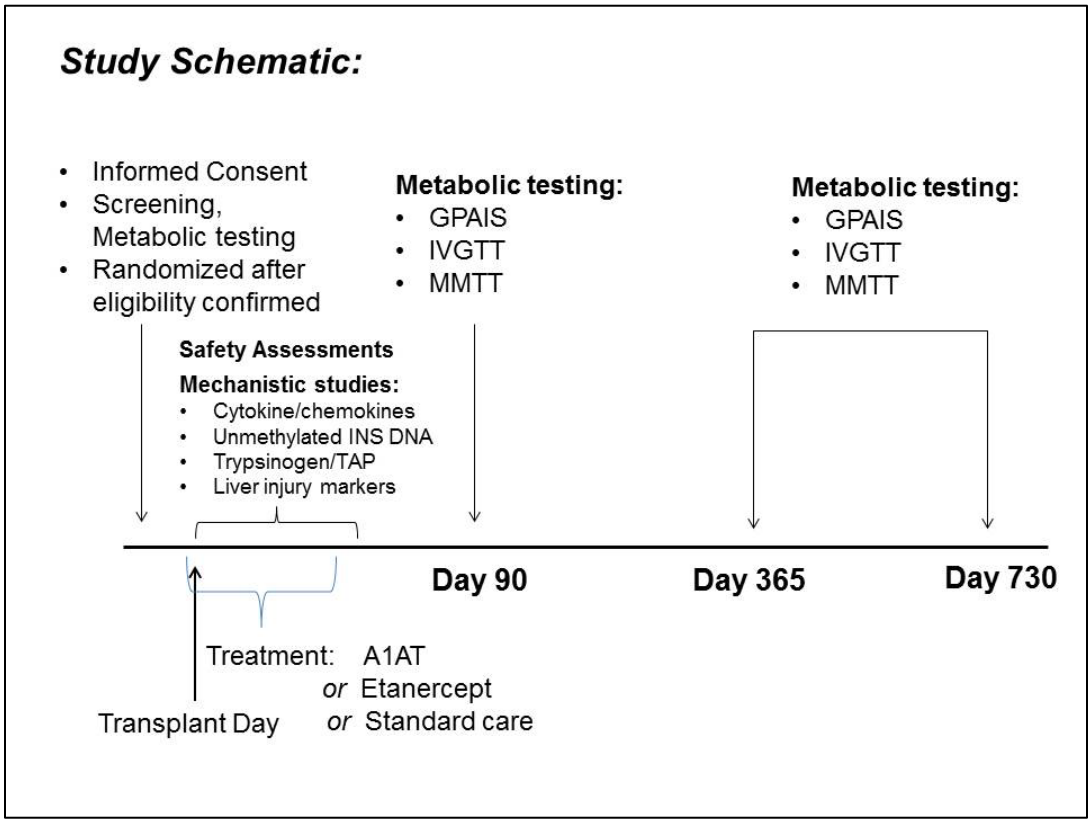
STUDY DESIGN OVERVIEW

Overview of Clinical Trial Design for All Aims

In this randomized, controlled non-masked trial, 45 patients age 18-65 years with chronic pancreatitis who are accepted for TPIAT procedure based on standard clinical criteria will be enrolled and randomized 1:1:1 to three study arms:

1) Alpha-1 antitrypsin (A1AT), 90 mg/kg/week IV, administered days -1, and +3, 7, 14, 21, and 28.
2) Etanercept administered as 50 mg SQ prior to the islet infusion (day 0) and 25 mg SQ on days 3, 7, 10, 14, 21 post-transplant.
3) Standard TPIAT care

Patients will be seen in follow up at 90 days and 1 year post-transplant for metabolic testing. The maximally stimulated acute insulin response (AIRmax) obtained from glucose potentiated arginine-induced insulin secretion (GPAIS) at 90 days post-transplant is the primary endpoint for the study. AIRmax has previously been demonstrated to correlate best with islet mass in human and animal models; by assessing AIRmax at 90 days post-transplant, we will be able to rapidly evaluate the therapeutic impact of these agents and select the agent most promising for further study and development. Mechanistic studies will be performed frequently throughout the early transplant period. Inflammatory cytokines will be measured by repeated serum sampling in the first 1 week post-transplant. Unmethylated insulin DNA levels will be monitored in the first 1 month post-transplant. Insulin requirements and fingerstick blood glucoses will be monitored throughout the study duration. Key secondary endpoints include the area under the curve (AUC) for C-peptide from mixed meal tolerance testing, the acute insulin response (AIRglu) on IV glucose tolerance test, the acute C-peptide response to glucose (ACRglu) on IVGTT, and the maximally stimulated C-peptide response (ACRmax) from GPAIS at day 90 and 1 and 2 years, and the AIRmax at 1 and 2 years, as well as average daily insulin requirements at each time point. As an exploratory component, we will continue to collect the basic clinically available measures of insulin use/dose, HbA1c, and simple fasting and stimulated glucose and C-peptide from a mixed meal test annually for years 3-5 post-transplant, all part of the patients' routine cares.



Study Treatment

Study drugs: The two investigational agents administered in this study are as follows:

1) Alpha-1 antitrypsin (Aralast NP) intravenously at a dose of 90 mg/kg IV weekly. Treatment will be started 1 day prior to surgery and on days +3, 7, 14, 21, and 28 after infusion. This is infused as a rate of no more than 0.2 mL/kg body weight/minute, and may be slowed depending on patient tolerability.
2) Etanercept: dosing in adult recipients is 50 mg SQ on day 0, and 25 mg SQ on days 3, 7, 10, 14, 21.

All medication doses will be administered in the hospital (during post-operative hospitalization) or in the research center by an RN.

Randomization Patients will be randomized to one of the three arms in a 1:1:1 ratio to A1AT, or etanercept, or standard care. Randomization will be stratified on BMI (< or ≥27 kg/m2). The investigational pharmacist will dispense study medication according to the randomization schedule provided by the biostatistician. Because of the nature of the drugs, given intravenously and subcutaneously, this initial study will not be blinded, to minimize the burden on participants.

Rationale for study drug dosing regimen

Alpha-1 antitrypsin doses of 90 mg/kg/week are currently administered in 8-35 year old participants in the Research Trial of Aralast in New Onset Diabetes (RETAIN), sponsored by the NIAID, Immune Tolerance Network, and Juvenile Diabetes Research Foundation. This is a dose that has been administered without serious adverse events to adults and children (down to 8 years of age) in the RETAIN trial (personal communications, Drs. Kevan Herold and Eva Tsalikian). Although there are multiple clinical grade A1AT products, Aralast was selected for this trial based on: 1) pre-clinical studies which have primarily used Aralast; and 2) use of Aralast NP in clinical trials in new onset type 1 diabetes. Treatment with A1AT for 14-30 days post-transplant has been observed to be efficacious in murine models of islet transplantation, with benefits sustained beyond the period of treatment, and even 14 days of treatment (4 doses) was effective in permitting long-term survival of marginal islet mass grafts in non-human primates (49). Thus, we have focused therapy specifically in this early post-islet infusion period where it is likely to yield benefit, in the first one month after islet infusion. The first 1-3 months post-transplant in clinical TPIAT recipients are critical to long-term outcomes. Clinically, C-peptide to glucose ratios (SUITO index) at 1 month after transplant and the acute insulin response to glucose at 3 months after transplant appear to predict achievement of insulin independence in auto and allo-islet transplantation (22, 78, 79). Thus, establishing early engraftment is key to improving later success. The short-term post-operative treatment duration proposed for this clinical trial targets this critical period of early islet engraftment—by protecting engrafted islet mass with short-term peri-transplant treatment, we expect to achieve superior long-term metabolic outcomes.

Etanercept has been administered as part of multiple clinical trials in allotransplantation. The dosing regimen is extrapolated from the experience with islet allotransplantation, in which doses are administered on day 0, 3, 7, and 10. Although this short duration of treatment appears efficacious in islet allotransplantation, because TPIAT patients undergo a more significant surgical procedure than those patients who receive a cadaveric donor alloislet infusion for type 1 diabetes (a relatively minor procedure with rapid recovery), we have extended the treatment duration in the TPIAT trial to 21 days.

PRIMARY AND KEY SECONDARY ENDPOINTS FOR THE STUDY

Primary Endpoint:

1) AIRmax from the Day 90 visit (obtained from glucose potentiated arginine-induced insulin secretion): We have data from UMN that supports this as the strongest correlate with beta cell mass, so this measure best addresses our research question. The early endpoint is chosen to rapidly translate pilot results into randomized clinical trials. Patients are not expected to achieve insulin independence in this population before day 90.

Key Secondary Endpoints:

1) Insulin independence: At day 365 and day 730. Defined as no insulin use for ≥14 days, with fasting blood sugar <126 mg/dL; 90 minute mixed meal glucose <180 mg/dL, and HbA1c ≤6.5%
2) Insulin dose (unit/day): At day 90, 365, and 730.
3) ACRmax, derived from GPAIS at days 90, 365, and 730
4) AIRglucose and ACRglucose, derived from the IVGTT at days 90, 365, and 730
5) AUC C-peptide from 2 hour MMTT, at days 90, 365, and 730
6) AUC glucose from 2 hour MMTT, at days 90, 365, and 730
7) Absence of severe hypoglycemic episodes (inclusive of day 90- 365) and HbA1c <7% at day 365
8) Absence of severe hypoglycemic episodes (inclusive of day 365-730) and HbA1c <7% at day 730

Key exploratory and safety endpoints:
1*)* Mean HbA1c by study group over the duration of the study
2) Number of patients experiencing at least 1 hypoglycemic event; and the incidence of SHE (events per subject-years) over the course of the study by study group

STUDY SUBJECTS

Study Subjects: inclusion and exclusion criteria to select suitable candidates for study

Inclusion Criteria

1) Age 18- 68 years. .
2) Scheduled for total pancreatectomy and IAT at UM. All patients who are approved for pancreatectomy and IAT at UM are reviewed by a multi-disciplinary committee including surgeons, gastroenterologists specializing in pancreatic disease, a pain specialist, psychologist, and endocrinologist to confirm the diagnosis of chronic pancreatitis and candidate suitability for surgery.
3) Able to provide informed consent

Exclusion Criteria

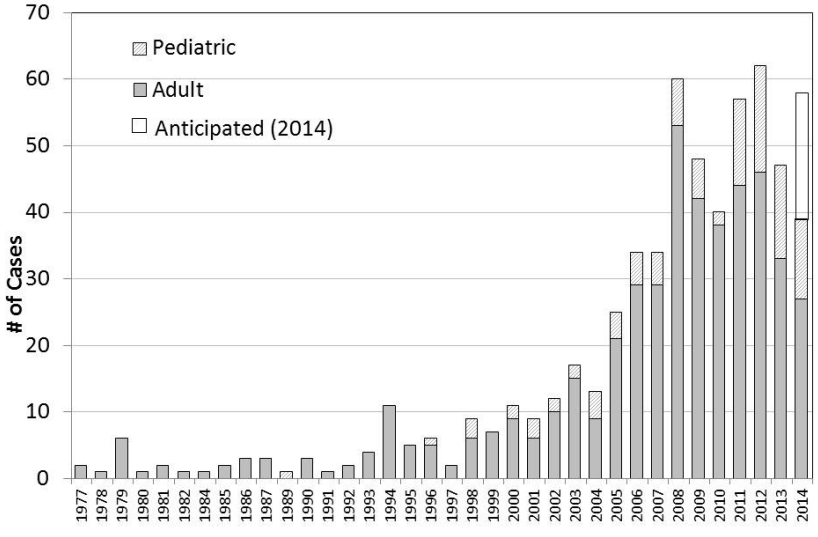
1) Pre-existing diagnosis of diabetes mellitus, fasting blood glucose >115 mg/dl, or hemoglobin A1c level >6.0% because these are all evidence of inadequate beta-cell mass.
2) Use of any of the following treatments in the 30 days prior to enrollment: insulin, metformin, sulfonylureas, glinides, thiazolidinediones, GLP-1 agonists, DPP-4 inhibitors, or amylin.
3) IgA deficiency (serum level <5 mg/dL), which has been associated with hypersensitivity to alpha-1 antitrypsin.
4) ALT or AST>2.5 times the upper limit of normal (ULN). Bilirubin >ULN, unless due to benign diagnosis such as Gilbert's.
5) Known history of HIV infection, hepatitis B (chronic), or hepatitis C (chronic).
6) History of tuberculosis (latent or active disease), or positive TB skin test.
7) History of symptomatic fungal lung infection.
8) History of multiple sclerosis, transverse myelitis, Guillain Barre, or other suspected demyelinating disease, due to risk of exacerbation of these conditions with use of etanercept; or prior history of systemic lupus erythematosus
9) Any of the following hematologic abnormalities: severe anemia (hgb <10 g/dL), thrombocytopenia (<150/mm3), or neutropenia (<1.0 x10^9/L).
10) Current use or expected use of oral or injected corticosteroids, or any mediation likely to affect glucose tolerance. However, use of hydrocortisone for physiologic replacement, or use of any topical, inhaled, or intranasal glucocorticoid is permitted.
11) Current or expected use of any other immunosuppressive agent.
12) Known hypersensitivity to etanercept or A1AT.
13) Any condition that is likely, in the opinion of the patient's medical providers, to necessitate use of TNF alpha therapeutically in the future (such as psoriatic arthritis).
14) Known coagulopathy, or need for anticoagulant therapy preoperatively (coumadin, enoxaparin), or any history of pulmonary embolism.

15) For females, plans to become pregnant or unwillingness to use birth control for the study duration.
16) Inability to comply with the study protocol.
17) Untreated psychiatric illness that may interfere with ability to give informed consent, or other developmental delay or neurocognitive disorder that impairs with a patient's ability to consent on their own behalf.
18) Any other medical condition that, in the opinion of the investigator, may interfere with the patient's ability to successfully and safely complete the trial.

SUBJECT RECRUITMENT AND INFORMED CONSENT PROCESSES

Study subject recruitment and rationale for study site

Subjects will be recruited from patients who are scheduled to undergo TPIAT at the University of Minnesota. Importantly, all patients are screened for TPIAT procedure on clinical grounds, after review by a multi-disciplinary committee to confirm the diagnosis of pancreatitis, maximal medical management, and impaired quality of life and/or narcotic dependence, as previously described (19).



The University of Minnesota (UMN) performs the greatest number of islet autotransplants per year of any institution in the United States, making it an ideal site for a clinical trial in this population. Since 1977, over 550 islet autografts have been performed, with an anticipated patient volume of 55-60 patients/year. The patient volume by year through 2014 is displayed in the graphic below.

We expect at least 60% of our TPIAT population, or ~33subjects per year will meet eligibility criteria for the proposed trial. We anticipate enrolling 45 participants over 2.5- 3 years based on our prior experience with drug-treatment clinical trials in adults (25- 30 patients enrolled per year). Patient interest in study participation has been very high. Patients from this population are interested in clinical trials for several reasons, including an altruistic desire to help others who have suffered with similar chronic disease.

The multidisciplinary UMN team has complementary expertise in medical and surgical management of chronic pancreatitis, diabetes, metabolic testing of islet mass and function, and manufacturing and testing of human islet products. It is in a unique position to help improve islet transplant protocols for both auto- and alloislet transplant.

Description of the recruitment process

Recruitment plan

Patients will be recruited from the pool of eligible participants scheduled for total pancreatectomy and islet autotransplant at the University of Minnesota (UMN). These patients are known to the PI, as a member of the TPIAT team. Potential surgical patients undergo a comprehensive multi-disciplinary evaluation at UMN at an initial consult visit. Participants will be informed of the study by the patient's physician (medical doctor on the IAT team) or the transplant coordinator for the TPIAT program at the time of the initial consult. Patients will be offered a brief descriptive hand out for more information regarding the study. It will be clearly emphasized that participation in not required to have TPIAT surgery at the UMN. Those interested in the study will be contacted by the primary investigator to review the study protocol, including risks and benefits of participation. Patients will be provided with the informed consent document in advance of the initial visit to obtain informed consent. Once the patients have reviewed the written description of the study, and after telephone conversation with the PI

to answer questions, if patients remain interested in study participation, they will be scheduled in the research center for an informed consent visit.

Travel reimbursement and compensation plan

Seventy-five percent of the TPIAT patients seen at the University of Minnesota are referred from out of state, and must travel by airplane or prolonged car trip, and stay overnight at a hotel, to participate in the study. Thus, it is not possible to reliably get patients back for study visits without providing some compensation for travel expenses. The study will reimburse patients who are from more than 60 miles away from the study center for mileage and/or airfare, airport transportation, meals ($25/day), and hotel up to a total maximum reimbursement for travel of $750 per visit at the day 90, 365, and 730 follow up visits (when the extended metabolic testing is performed). Screening can be coordinated during the patient's preoperative visit in the week prior to surgery; however, because this will often require one additional night in Minnesota, we will provide reimbursement for one night's hotel, up to $250. In addition, because the metabolic tests are cumbersome, time consuming, and require a morning off of work, both local patients and those who travel will be given a stipend of $50 per study day ($100 total for 2 days) for completing the metabolic testing at each follow up interval. This is consistent with standard stipends provided to participants in diabetes studies at our institution.

Recruitment and advertisement materials

The study will include a one page informational handout which briefly describes the purpose of the study and the medications being tested. This hand out will include an "opt out" phone number that the patients can call if they do not wish to be contacted with any additional information. In addition, as per standard guidelines for clinical trials, the study will be posted on clinicaltrials.gov. It will be clearly emphasized that participation in a study is not required to have TPIAT surgery.

Description of the informed consent process

As detailed in the preceding section, interested patients will be mailed a copy of the informed consent document to review prior to the informed consent visit. Informed consent will occur when the patient returns for the screening study visit. Informed consent will be obtained by the primary investigator, or one of the co-investigators. Because this is a treatment study, a physician will always be involved in the initial informed consent process. Following review of the study protocol and the informed consent document, patients' understanding of the study will be assessed with directed questions, and informed consent will be confirmed and documented in the patient's record. The informed consent process will take place before any protocol-driven screening studies are done, and within 4 weeks of the patient's surgery date. The informed consent process will occur in the clinical research center within the University of Minnesota CTSI.

Adults that are unable to consent for themselves due to developmental delay or severe psychiatric illness will not be recruited for this study.

Because patients are sent the informed consent document prior to the informed consent visit, they are provided opportunity to review the study protocol including risks and benefits, in writing, prior to the consent visit. Patients are encouraged to discuss the study details and informed consent with significant others, family, and their local physicians.

Any significant changes in the risk profile of study medications, other risks, study medication administration, or testing/labs would prompt a change in the informed consent document. Updated informed consent will be obtained at the patient's next study visit, before any study testing is done.

STUDY VISITS AND ASSESSMENTS

Outline of study visits and endpoint assessments

Patients will be seen prior to surgery ("Baseline"), during the treatment phase for medication administration and safety labs , at 90 days (± 15 days; "Day 90 visit"), at 365 days (± 28 days, "Year 1"), and at 730 days (±56 days, "Year 2"). The year 1 and year 2 study visits will provide detailed pilot data on the long-term function of the islet grafts in treated and untreated patients.

A local follow up with telephone or face-to-face encounter to review insulin requirements, blood glucose logs, and obtain local mixed meal tolerance test results (part of standard clinical care) will occur at years 3, 4, and 5 (all ± 2 months) for exploratory data on long-term efficacy. This information will be readily available, as it is part of the standard routine care of all TPIAT recipients at UM.

Subject screening visit

Subjects' medical history will be reviewed from the electronic medical record and from history obtained from the patient to confirm eligibility. In addition, the following screening assessments will be performed at a screening visit within 4 weeks prior to the scheduled surgery date, after informed consent has been obtained:

- Complete metabolic battery (includes hepatic panel, creatinine, fasting glucose)
- CBC with differential and platelets
- Urine pregnancy test in women with child-bearing potential (if ≥7 days since screen, repeat prior to first dose of study drug administration; UPT not necessary in females s/p hysterectomy procedure)
- Hepatitis C and hepatitis B serology, HIV ELISA
- IgA level
- Mantoux skin test or Quantiferon (serum) for TB screening
- Hemoglobin A1c level
- Mixed Meal Tolerance Test
- IV glucose tolerance test
- Glucose potentiated arginine-induced insulin secretion

Table. Study Flowchart (year 0-2)

	screening	**Days 0-7**	**Days 7, 14, 21, 28**	**Day 60**	**Day 90**	**Day 365 (Year 1)**	**Day 730 (Year 2)**
Study Visit Assessments (abbreviated)							
Insulin Use/Dose (u/kg/day, endpoint)			X		X	X	X
Mixed Meal Tolerance Test (AUC C-	X				X	X	X
IVGTT (AIRglu, ACRglu)	X				X	X	X
GPAIS (AIRmax, ACRmax)	X				X	X	X
HbA1c level	X				X	X	X
iPro2 (6 day CGM)					X	X	X
Complete metabolic panel, CBC	X		X*		X	X	X
Random morning C-peptide and glucose			Day 7, 14				
Fasting glucose, C-peptide, creatinine	X		Day 28		X	X	X
Drug safety assessments (PE, hx, labs)		X	X				
Inflammatory cytokines (see text)**		X					
Unmethylated insulin DNA levels**		X	X				
Safety assessment (phone visit for symptoms)				X			
Islet Assessments (see text)		Day 0					

* **Years 3, 4, and 5:** Telephone or face-to-face review of insulin use/dose; labs (HbA1c, clinical MMTT)

** Drawn at multiple time points, as detailed in the methods for Specific Aim #3.

Design and Methods to Evaluate Specific Aim #1

SPECIFIC AIM #1: To determine if anti-inflammatory treatment with etanercept or alpha-1 antitrypsin during the critical peri-transplant engraftment period improves islet engraftment and function after islet autotransplant, as assessed by area under the curve for C-peptide (AUC C-pep) on mixed meal tolerance testing (oral stimuli), acute insulin response to glucose-potentiated arginine stimulation (AIRmax, IV stimuli), and insulin requirements at day 90 post-transplant.

Hypothesis 1: *Etanercept and A1AT groups will have higher AIRmax per islet transplanted than the control group.*

Hypothesis 2: *Etanercept and A1AT groups will have higher acute insulin response to glucose (AIRglu), higher acute C-peptide response to glucose (ACRglu) and greater AUC C-pep than the control group.*

Hypothesis 3: *A1AT group will have superior AIRmax, AIRglu, and AUC C-pep compared to etanercept group, because A1AT has a more generalized effect on the inflammatory milieu.*

Hypothesis 4: *Conversely, etanercept group will have superior AIRmax, AIRglu, and AUC C-pep compared to A1AT group, because of the central role of TNFα in beta cell loss.*

There are reasons to believe that either of A1AT or etanercept may be superior to the other (hypotheses 3 and 4), in view of their relative mechanisms. Thus it is important to compare these two agents head-to-head, in order to select the more promising therapy.

Definitions and procedures for metabolic assessments

For this aim, patients will be asked to return for testing at day 90 +/- 15 days after TPIAT. Patients will continue their basal insulin analog (if applicable) but will be instructed to withhold any rapid acting insulin in the 4 hours prior to the start of testing. The following tests will be performed over 2 mornings, following a 10 hour fast:

1. Two Hour Mixed Meal Tolerance Test (MMTT): Glucose and C-peptide are drawn at baseline and every half hour for 2 hours. The patient is given Boost HP 6 cc/kg (max 360 ml), ingested within 5 min, after the time 0 blood draw. Area under the curve for glucose (**AUC glucose**) and C-peptide (**AUC C-peptide**) will be evaluated. AUC C-peptide is the standard endpoint for assessment of beta cell function in the trial net studies for type 1 diabetes, and in the alloislet transplant trials in the Clinical Islet Transplant consortium. Scheduled on a separate morning from the IVGTT and GPAIS testing. An additional 2 mL plasma will be collected from each time point in a collection tube including protease and DPP-4 inhibitors; these samples will be stored for potential later analyses of incretin hormones and glucagon (which require the special inhibitor cocktail to avoid sample degradation).
2. Simple intravenous glucose tolerance test (IVGTT): A bolus of 0.3 grams/kg of dextrose is administered at time 0, and insulin, C-peptide, and glucose are sampled at times -10, -5, -1, and 1, 2, 3, 4, 5, 7, and 10 minutes. The AUC for the 10 minute insulin values minus baseline are used to calculate the acute insulin response to glucose (**AIRglu**) and acute C-peptide response to glucose (**ACRglu**). Glucose levels will be drawn additionally at +12, 14, 16, 18, and 20 minutes to calculate the glucose disposal rate (Kg=ln(glucose)/min x 100).
3. * Glucose potentiated arginine stimulation (GPAIS): Immediately following the IVGTT (at time +20 minutes), dextrose (D20%) is infused at a variable rate to maintain blood glucose at ~230 mg/dL until completion of the test. Glucose levels are drawn and measured by bedside autoanalyzer every 5 minutes to maintain glucose in target range. Following at least 30 minutes of continuous D20% infusion at target BG (~230 mg/dL), baseline samples for glucose, insulin, and C-peptide are drawn (three samples over 10 minutes), a bolus of 5 grams arginine is administered (time 60 minutes), and samples for glucose, insulin, and C-peptide are obtained at 2, 3, 4, 5, 7 and 10 minutes after the arginine bolus. Results are used to calculate the maximal potentiated arginine induced insulin secretion (**AIRmax**), a sensitive marker of beta cell mass, and the maximal C-peptide secretion (**ACRmax**). AIRmax is the *primary endpoint for the study. This is selected as the primary endpoint because it is the measure that most directly relates to islet mass in diabetes and transplant studies in humans and animal models.

4. Continuous glucose monitoring (CGM): 6 days of CGM monitoring (minimum of 72 hours for endpoint inclusion) at day 90, 365, and 730 will be obtained for mean glucose, standard deviation, % of time in hypo- and hyperglycemia (80).
5. Hemoglobin A1c level is measured as a marker of average glycemic control.
6. Insulin use is assessed from glucose and insulin diaries maintained by patients for 14 consecutive days, overlapping with day 90. Calculated as units/kg/day.
7. Hypoglycemic episodes: All severe hypoglycemic events will be recorded. This is defined based on American Diabetes Association criteria as a hypoglycemic episode ≤54 mg/dL or (if not measured) associated with prompt recovery upon administration of glucose in which a patient is mentally or physically incapacitated and requires assistance from another person in order to treat hypoglycemia.
8. Health related quality of life: Assessed by the SF-36, from which 8 subscale scores can be generated for physical and mental/emotional HRQOL, as well as standardized physical composite summary and mental composite summary scores. In addition specific diabetes burden will be assessed by the Problem Areas in Diabetes (Diabetes Distress Scale). These instruments are administered as part of our standard follow up protocol in all TPIAT recipients at 3 months, 6 months, and yearly after surgery.

In addition, at 90 days post-transplant, patients will have a fasting C-peptide and fasting glucose drawn (as part of MMTT), to calculate basic measures of early function, including: 1) Fasting C-peptide to glucose ratio; and 2) SUITO index (calculated as fasting C-peptide [ng/ml] / (fasting blood glucose - 63 [mg/dl]) × 1500) (78). In addition, a random glucose and C-peptide level are obtained at day 7,14, and day 28 after surgery.

In between visits at the study center, patients will be asked to continually maintain a log of self-monitored blood glucoses and insulin doses. Patients will be instructed to check fingerstick blood glucose at least 4 times per day (pre-meal and bedtime). Patients will be instructed to send logs to the study PI and coordinator at least once monthly for adjustment of insulin dosing. Insulin doses are adjusted by the PI based on site standard protocol, to maintain the following goals: fasting glucose <126 mg/dL, 2 hours after a meal <180 mg/dL, and HbA1c ≤6.5%. Patients have HbA1c levels drawn quarterly for clinical monitoring (standard of care). Any patient not meeting these goals off insulin is not considered insulin independent per protocol.

Safety monitoring: The PI and study coordinators will monitor for adverse events throughout the study period, as described in the human subjects monitoring plan. The coordinator or investigator will collect a history for safety assessment at day 60 (may be done by telephone visit if patient is not able to return to study center). Additional monitoring for safety will include history at each scheduled visit day 0- 30 for symptoms of DVT or PE (using Wells criteria) with lower extremity ultrasound or spiral CT performed if response moderately to highly suspicious for deep vein thrombosis (DVT) in the lower extremities (Well's>2) or pulmonary embolism (PE) (Well's >4) respectively. Note that in this population all patients are expected to have a Wells score of 3 (meets 'moderate' suspicion threshold) as 100% are immobilized and nearly 100% have intermittent tachycardia from pain and thus a score of 4 or greater will be achieved only in the context of symptoms to suggest PE. Likewise, the Well's score of >2 accounts for the surgery, immobilization and bilateral lower edema swelling (normal post-TPIAT) that will occur in all patients. At day 60, the telephone encounter will include assessment of symptoms for PE or DVT. An EKG will be performed at baseline, day 28 and day 90.

Design and Methods to Evaluate Specific Aim #2

SPECIFIC AIM #2: To gather pilot data on the long-term impact of etanercept or alpha-1 antitrypsin administered during the peri-transplant period by evaluating islet function at 1 and 2 years post-transplant, as assessed by area under the curve for C-peptide on mixed meal tolerance testing (oral stimuli), acute insulin response to glucose-potentiated arginine stimulation (IV stimuli), and insulin requirements (units/kg/day) at 1 and 2 years post-TPIAT.

Hypothesis 1: *AIRmax, AIRglu, and AUC C-pep from metabolic testing performed at yearly intervals post-transplant will be greater in the A1AT-treated patients and etanercept-treated patients, compared to controls.*

Hypothesis 2: *Mean daily insulin requirements (units/kg/day) will be lower in the A1AT-treated group vs control and in the etanercept treated vs control.*

Hypothesis 3: *AIRmax and AIRglu at 3 months will predict insulin independence at 1 year and 2 years post-transplant.*

For specific aim #2, patients will return to the study center at day 365 ± 28 days and 730 ± 56 days post-transplant for repeat metabolic testing. Testing performed will be identical to that described under approach for specific aim 1. Both insulin use and the proportion of patients insulin independent will be recorded.

As an exploratory analysis, patients will continue to be followed yearly by a telephone or face-to-face visit, depending on whether they are returning to UMN for clinical care, at which time mean daily insulin use will be recorded, and records will be reviewed for home blood glucometer results, hemoglobin A1c level, and mixed meal fasting and stimulated glucose and C-peptide performed at the patient's local lab, per our usual routine for clinical follow up.

In addition to the metabolic data collected under this protocol, all TPIAT patients are enrolled in a prospective follow up study tracking diabetes, pain, and health related quality of life outcomes on an ongoing basis. Under this protocol, patients complete the SF-36 health related quality of life instrument and institutional derived questionnaires that include self-report of daily insulin use, pain symptoms, and pain medications. These instruments are administered at 3 months and 6 months after TPIAT and then once yearly after TPAIT. Copies of these instruments are included in the supplementary materials for reference. These assessments will permit long-term follow up of the study participants for both insulin requirements and health-related quality of life outcomes.

Design and Methods to Evaluate Specific Aim #3

SPECIFIC AIM #3: To evaluate the mechanistic effects of etanercept or alpha-1 antitrypsin on inflammation, islet cell viability/survival, and beta cell apoptosis. This will be assessed by repeated measurement of circulating cytokine and chemokine levels and circulating unmethylated DNA (a recently validated marker of beta cell death in T1DM) in patient serum during days 0 - 28.

Hypothesis 1*: Peak levels and area under the curve for inflammatory cytokines will be lower in recipients treated with alpha-1 antitrypsin and etanercept.*

Hypothesis 2: *Lower rates of beta cell death will be observed post-transplant in the A1AT-treated and etanercept-treated groups, compared to standard of care, as evidenced by lower levels of circulating unmethylated insulin DNA in the patient serum.*

To evaluate the potential mechanistic effects of the two drug therapies, we will obtain frequent serum measurements in recipients for critical markers including key cytokines/chemokines and markers of coagulation (the instant blood mediated inflammatory reaction), and markers of circulating exocrine tissue (trypsinogen). In addition, we will profile in each patient beta cell death (islet loss) using the novel marker for unmethylated insulin DNA. Unmethylated insulin DNA is specific to beta cells, and elevated levels in serum indicate beta cell death. Patient assessments are described in detail below.

1) ***Patient assessments:***
   a. Inflammatory Cytokines: Patient serum will be assayed for TNFα, IL-1, IL-6, IL-8, IL-10, MCP-1, IP-10, and CCL2—at pre-surgical (x2 draws) and pre-infusion baseline, and at times 1, 3, 6, 12, 24,

and 72 hours and 7 days after islet infusion. Assays will be performed using a Luminex ® multiplex bead cytokine kit. These measures were selected based on the literature suggesting relative importance in islet transplantation. These assessments are run in a CLIA certified cytokine laboratory (University of Minnesota cytokine laboratory).

In addition, thrombin-antithrombin complexes, C3a, and C-peptide will be obtained pre-infusion and at times 1, 3, and 24 hours post-transplant, as a marker of the instant blood mediated inflammatory response.

b. Unmethylated insulin DNA: This novel marker has recently been validated as a measure of beta cell loss in new onset type 1 diabetes. Dying beta cells release unmethylated insulin DNA into the serum (43). The assay technique is performed as described by Herold et al (43). DNA is purified from 200 microliters of serum, using QIAamp DNA blood kits, and bilsufite treated using EZ DNA methylation kit. Unmethylated insulin DNA is measured by real-time PCR. Levels are elevated preoperatively and in new onset T1DM, but lowered when T1DM subjects are treated with teplizumab to preserve beta cell mass. Thus, we propose that this novel marker will provide critical mechanistic data on post-transplant loss of beta cell mass. This marker will be drawn prior to surgery and infusion (x 2 samples) and at 15 min, 30 min, 1, 3, 6, 12, 24, 72 hours, and 7, 14, and 28 days post-transplant, and repeated at each follow up visit (90 day, 1 year, 2 year).

c. Alpha-1 antitrypsin levels: Measured at pre-treatment and prior to each infusion in the A1AT-treated group. In the control and etanercept groups, these levels will be measured at baseline and weekly through day 28 for comparison.

d. Trypsin and Trypsinogen activated peptide (TAP): To determine if dying acinar cells release activated proteases which degrade the insulin molecules in systemic circulation, trypsin levels will be measured in the patient's serum pre- and at 12 and 24 hours post-islet infusion and by detection of TAP in the urine at 24 hours post-transplant by enzyme immunoassay (81). TAP is a small, measureable peptide that is released when trypsinogen is activated to trypsin.

e. Serum markers of hepatocyte, Kupffer, and sinusoidal endothelial cell injury: Serum obtained at pre-infusion baseline and at 3, 12, 24, and 72 hours, and 7 days after islet infusion will be used for assessment of markers of liver cell injury, which may occur in islet transplant recipients, especially when exocrine acinar tissue is co-transplanted with the islets. We hypothesize that A1AT and etanercept may reduce occurrence of acute liver injury that is associated with islet infusion, and may thereby promote a more hospitable hepatic environment for islet engraftment.

   i. CK-19 and CK-18 fragments, and additional serum markers such as F-protein, and Glutathione-S transferase can serve as markers of hepatocyte damage or death (82-84).
   ii. Hyaluronic acid, vWF, thrombomodulin, -- markers of sinusoidal endothelial injury (85).

2) Islet graft and pancreas assessments:

a. The islet isolation team will provide key descriptive data for the islet product. Because chronic pancreatitis may theoretically inflict damage on the isolated islets, these assessments will provide information on baseline comparability of the islet grafts between the three treatment groups. The following islet assessments will be performed using a small islet aliquot (≤2,000 IEQ total required combined for all assays) for all study participants:

   i. Islet count, expressed as total islet equivalents (IEQ) and IEQ/kg body weight, which is a routine measure for all clinical transplants.
   ii. Oxygen consumption ratio (OCR)/ viability: Assay method is refined from that described by Papas et al (86) and/or assessed by newer Seahorse technology; this assay measures the viable islet tissue to be transplanted. This has been shown to be a superior measure of islet viability over FDA/PI.
   iii. An aliquot of 500 IEQ islets and a pancreatic biopsy will be embedded into paraffin and available for analysis of insulin content, glucagon content, replication markers (Ki67), and apoptosis (TUNL).

iv. Nuclei counts: A nuclei count is performed using the digest counting sample.
v. Beta cell count: Derived from proportion of insulin-positive staining islet cells in the histopathology sample (i.e. the fractional representation of beta cells in the graft) multiplied by the nuclei count obtained from the digest sample.

SAMPLE SIZE AND PLAN FOR DATA ANALYSIS

This is a pilot, parallel-arm study to compare etanercept, A1AT, and control groups; the primary outcome is maximal potentiated arginine induced insulin secretion (AIRmax) measured at 90 days posttransplant. We propose to enroll 15 patients in each group which will give 80% power to detect a difference between any two groups of at least 1.06*(within-group standard deviation of the outcome). From preliminary data for AIRmax measured more than one year post-transplant, the study will have 80% power to detect a difference of at least 33 mU/mL in pairwise comparisons of AIRmax between each of the 3 groups, corresponding to a 24% increase over standard treatment (control group).

The table below shows estimated minimum detectable differences for main outcomes, based on means and standard deviations from preliminary testing performed at 1 year post-transplant (for IVGTT and MMTT) or beyond 1 year post transplant (GPIAS) in this population of TPIAT recipients.

Test	Derived Measure	minimum detectable difference	n	Mean	SD
GPAIS	AIRmax (mU/mL)	33	10	137	31
IVGTT	AIRglu (mU/mL*min)	162	33	169	153
	ACRglu (ng/mL *min)	8.8	36	9.8	8.3
MMTT	AUC C-peptide (ng/mL *min)	147	39	295	139
	AUC glucose (mg/dL *min)	3790	39	16,037	3,579

For specific aim 1, the primary outcome, AIRmax, and other measurements at day 90 will be compared between treatment groups with a linear model to adjust for transplanted islet mass and the randomization stratum, BMI (< or ≥27 kg/m2). For aim 2, the same analysis will be performed for measurements made at 1 year and 2 years post-transplant. In addition, we will assess whether day 90 AIRmax and AIRglu measurements predict insulin independence at 1 and 2 years post-transplant using a logistic regression model. For aim 3, repeated measurements during the weeks immediately post-transplant will be summarized by peak and area under the curve for comparison by the same linear model used for aim 1, i.e., adjusting for transplanted islet mass and the randomization stratum. We will also compare the groups based on the longitudinal series of measurements using a mixed-effects linear model with fixed effects of treatment group, transplanted islet mass and randomization stratum, and a random intercept for each participant to model the correlation between repeated measurements from the same person.

POTENTIAL RISKS AND BENEFITS

Forseeable Risks

Medical risks arise from two primary components of the study: the study medications, and the islet functional (metabolic testing). Non-medical risks include risk of breech of privacy.

Risks of metabolic testing: IV placement is required for study testing, and can be associated with bruising or discomfort. The IV placement is done only by trained personnel using standard hospital technique. Because these patients have difficult IV access, the IV is often placed by the hospital vascular access team using ultrasound

guidance, to make the process as tolerable as possible for the patient. The mixed meal test requires Boost HP which can cause nausea or discomfort. There is modest hyperglycemia with the IVGTT and GPAIS which may cause increased urination or thirst, but this is transient (≤1 hours) and not associated with any long-term sequelae for the patient or islet graft. Blood draws for both days of metabolic testing will total up to 118 mL over the 2 day study visit.

The total amount of blood drawn over a 2 year period is 620 mL. This includes the screening, all early post-transplant labs, and the three follow up visits for metabolic testing. Subsequent to 2 years, only routine clinical yearly labs are obtained at the patient's local laboratory (no additional study labs).

Risks of study drugs: The known potential risks of alpha-1 antitrypsin (Aralast NP) and etanercept are detailed in the table below, as described by package insert and Micromedex/DRUGDEX. Patients with the highest risk of an infusion reaction from A1AT—those with IgA deficiency (IgA level≤5 mg/dL)—are excluded from this trial.

Both products are overall well tolerated. Aralast NP has been associated with elevation of liver enzymes, which were generally transient and normalized within 3 months despite continued administration of Aralast NP, in 11% of patients. Other patient symptoms include headache, cough, muscle aches, and rash or pruritus. Infusion reactions are rare. Although other A1AT products have been associated with cough, sinus, or respiratory symptoms, this may be a manifestation of the underlying disease in the clinically treated population (A1AT deficient) and were not seen with Aralast NP.

Etanercept (Enbrel) is generally administered long-term for management of autoimmune disease, particularly arthritis and psoriasis. Treated patients often are pre-disposed to autoimmunity, are treated long-term with this agent, and often receive other immunomodulating drugs. Major differences in this trial as compared to the conventional use of this medication include the short-term duration of treatment and lack of other immunosuppressive treatments; this is expected to overall reduce the risk of infection and other serious adverse events. Enbrel administration (long-term) for management of arthritis or psoriasis has been associated with increased risk of infection (including, rarely, tuberculosis and opportunistic infections) and sepsis. Serious infectious AEs most often occurred in patients who were treated with other concomitant immunosuppressive therapy; such drugs are prohibited in the current trial. The drug is halted or interrupted in case of sepsis or other serious infection.

The most common AE associated with Enbrel is injection site reaction (occurs in about 1/3rd of patients). Most cases are mild and do not necessitate drug discontinuation. Mild infections --most frequently URI, and less often UTI, bronchitis, sinusitis-- are also commonly reported in treated patients. Such infections were also present in a high frequency of adult patients with rheumatoid arthritis who were on placebo treatment (infection in 32% of placebo patients vs 35% of Enbrel-treated). Post-marketing surveillance suggests a statistically higher incidence of tuberculosis (in 0.006- 0.02% of treated patients), and therefore patients are screened for latent TB at screening. There are case reports of more serious or unusual pathogens that are of unclear relationship to Enbrel given the confines of few patient events and frequent co-administration of other immuosuppressives in these patients; these have included reports of septic arthritis, herpes zoster, legionella, pneumocystis, histoplasmosis, apergillosis, coccidiomycosis, and listeriosis. DRUGDEX ® notes that according to the FDA adverse event reporting system (AERS) the majority of patients affected by unusual or serious infections were receiving other concomitant immunosuppressive therapy.

Other potential serious adverse events associated with Enbrel include exacerbation of CNS demyelinating disease, hematologic cytopenias (anemia, neutropenia, thromobocytopenia), and malignancy of unclear causal relationship. To reduce risk in this trial, we are excluding patients with prior history of neurologic demyelinating disease, and patients with significant anemia (hgb<10), thrombocytopenia (plt<150), or neutropenia (<1.0). Given the short duration of treatment, we do not expect malignancy to be a significant concern in this population. Other case reports of rare congestive heart failure, one case of membranous glomerulonephritis, are of unclear

relationship to Enbrel. There is one post-marketing report of new onset diabetes, one of new onset hyperthyroidism, and one of new hypothyroidism, which are likely related to underlying autoimmune predisposition and not the drug. Because there is at least the theoretical risk of developing immunogenicity against etanercept with its use, we will exclude from the study patients known to have conditions for which Enbrel may be later indicated.

	Mild to moderate	Serious
Alpha-1 antitrypsin (Aralast NP ®)	Elevated liver enzymes (ALT or AST, 11%) Headache (0.3- 7%) Musculoskeletal discomfort (0-7%) Pharyngitis (1.6%) Cough (0.6%) Somnolence (0.3%) Rash (1.5%) Pruritus (1%) Altered taste (1.5%) Decreased platelet count (1.5%) Joint swelling (1.5%) Infusion reactions: fever, chills, chest pain, shortness of breath, dizziness, visual change (0.1%) Transient leukocytosis	Anaphylaxis
Etanercept (Enbrel ®)	Injection site reaction (37%) Mild infection (most often URI, 35%) Headache (17%) Rhinitis (12%) Pharyngitis (9%) Cough (6%) New autoantibodies (3-15%) Present in ≤5%: Dyspepsia/GI upset or pain, rash, edema, sinusitis, and elevated liver enzymes Other: Hematologic Cytopenia Skin cancer (non-melinoma) Opportunistic infection including legionella, listeria, fungal pneumonia	Anaphylaxis Serious infection Sepsis Opportunistic infection, including TB Demyelinating disease Blood dyscrasia/ blood malignancy

Risk of loss of privacy: There is a risk that a subject's study medical records will be viewed by those outside the study. Precautions taken to avoid breach of privacy include using study ID numbers rather than names on labs/documents that are not a component of the clinical record, maintaining electronic records on password protected and secure computers serviced by the University of Minnesota Academic Health Center, and storing study binders in a locked storage area within the PI's locked office.

Only the primary investigator, co-investigators, and study staff (coordinators) will have access to the patient's private study records. As indicated above, some labs obtained as a part of the study will be posted to the medical

record and may be viewed by other providers' caring for the patient in a clinical setting. These will be limited to test results that are important component of the patient's routine clinical care.

Risk management and emergency response

The primary investigator or one of the co-investigators will be on call for any medical concerns during routine study visits or infusion of the study medication. Patients will receive emergency or first aid care if an injury or illness occurs during the study visit, or during medication infusion. All doses of medication are administered in the hospital or in the adjacent clinical research center, with a hospital rapid response and code team available on site in case of an emergency.

If patients experience an illness or symptom between during the study that could be related to the study drug, they will be directed to receive routine clinical care from their primary physician.

Potential Benefits:

Direct benefits: If the study treatment is successful, then those participants treated with study drug (A1AT or etanercept) may require less insulin or be more likely to come off insulin than those patients receiving standard care.

If the treatment is successful, the potential benefit of the study treatment is significant, including potential for reduced incidence of diabetes, or better controlled diabetes after surgery. The total pancreatectomy and islet autotransplant is a major surgical procedure. Minor complications (including nausea/ vomiting, delayed gastric emptying, pain) are not uncommon and recovery is gradual. There is a high risk of partial or total diabetes. A1AT or etanercept used only temporarily, and have relatively few safety risks over this short duration of treatment. Thus, we expect the additional study treatment to contribute only minor risks compared to the overall risks of the total pancreatectomy and islet autotransplant.

Other benefits: The knowledge gained from the extensive metabolic and islet graft assessments performed in this trial will advance the understanding of islet autotransplantation.

ADVERSE EVENT DOCUMENTATION AND TERMINATION CRITERIA

Adverse events: All adverse events will be recorded for all subjects, and graded according to the CTCAE version 4.0. All AEs, regardless of severity will be collected from day 0 to the day 90 visit (drug exposure window). Subsequent to day 90, only severe AE's (as subsequently defined) are collected, from day 90 to the final face to face visit at day 730. AE's include new or worsening symptoms reported by the subject, signs on physical exam (performed at each study visit), and/or abnormalities on study labs. AE's will be reviewed by the DSMB as described in the section below. Serious adverse events will be reported to the IRB per institutional guidelines (within 10 days for an event that meets rapid reporting requirements), and to the DSMB and the FDA within 72 hours in accordance with IND regulations.

Each event will be analyzed to determine is relatedness to study medication (definitely, probably, possibly, unlikely, or unrelated) and the severity will be graded as follows, modified from the Common Toxicity Criteria:

- MILD (Grade 1) – transient or mild discomfort; no limitation in activity; no medical intervention require.
- MODERATE (Grade 2) – mild to moderate limitation in activity, some assistance may be needed; no or minimal medical intervention/therapy required.
- SEVERE (Grade 3) – marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalization is possible.
- LIFE-THREATENING or DISABLING (Grade 4) – extreme limitation in activity, significant assistance required; significant medical intervention/therapy required, hospitalization or hospice care probable.
- DEATH (Grade 5) – event is a direct cause of death.

All adverse events will be graded as mild, moderate, or severe, according to the guidelines established in the current Common Terminology Criteria for Adverse Events (CTCAE).

Serious Adverse Events (SAE)
Defined as any adverse event that suggests a significant hazard, contraindication, side effect, or precaution. This includes, but may not be limited to any of the following events:

1. Death: A death occurring during the study or which comes to the attention of the investigator during the protocol-defined follow-up after the completion of therapy, whether or not considered treatment-related, must be reported.
2. Life-threatening: Any adverse therapy experience that places the patient or subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred (i.e., it does not include a reaction that, had it occurred in a more serious form, might have caused death).
3. In-patient hospitalization or prolongation of existing hospitalization.
4. Persistent or significant disability or incapacity.
5. Congenital anomaly/birth defect.
6. Other serious (Important medical events).

Events of special interest: Any infectious event will be specifically recorded, in as much detail as possible, for patients in all three study arms. While the lack of concomitant immunosuppressive therapy and short duration of treatment with Enbrel is expected to result in a lower risk of infection risk compared to that reported in post-marketing studies (in patients with long-term treatment and other immunomodulators), we recognize that this patient population has special risks associated with the major surgical procedure. As detailed below, drug therapy will be suspended in the cases of sepsis or serious infection that requires procedural intervention for treatment; more than one such infection in a single treatment arm will prompt DSMB review.

Regulatory oversight: The study will be reviewed by the human subjects committee of the Institutional Review Board as per standard research practice. Because the drugs being studied are currently FDA-approved, we have requested the FDA to review our protocol to determine if it meets criteria for a new IND. If the FDA determines that this protocol is non-exempt due to the unique patient population, we will request an investigator-initiated IND from the FDA for etanercept and alpha-1 antitrypsin. The study will be reviewed for safety at every 6 month intervals by the islet transplant data safety and monitoring board at UMN. In addition the DSMB will be contacted regarding any SAE felt possibly related to study drug, within 72 hours of the event. This is further described below.

Clinical trial monitor: A clinical trial monitor external to the study and not paid by the study will be provided by the Clinical Translational Sciences Institute (CTSI). The study monitor will review study conduct every 6 months. The purpose of the CTSI clinical monitor is to verify that: (1) the rights and well-being of human subjects are protected; (2) trial data are accurate, complete, and verifiable; and (3) conduct of the trial is in compliance with GCP guidelines and with applicable regulatory requirements. Activities of the CTSI monitor include (but are not limited to) review of facilities, storage and dispensing of investigational products, protocol compliance, informed consent, appropriate recruitment and screening of subjects, case report forms, accurate data recording, and adverse event reporting.

Drug or Study Termination
Study drug termination in an individual patient: This is an intent-to-treat study. Any patient treated with study drug will be followed for endpoint assessment (unless the patient withdraws from the study).

Study drug will be discontinued and the patient will continue to be followed for any of these events:

- Serious allergic reaction
- patient refusal to continue study drug (but willingness to continue with endpoint assessment)
- ALT or AST >5x ULN **and** bilirubin >1.5x ULN. Note that elevation in ALT and AST is expected to occur as a result of islet infusion itself. However, bilirubin elevation is uncommon. If ALT and/or AST subsequently fall to <5x ULN and bilirubin to <1.5x ULN, the study drug will be resumed, if in the opinion of the investigator, enzyme elevations were suspected secondary to the surgical procedure.
- Serious infection including sepsis (positive blood culture AND suspicion of sepsis due to associated hypotension, need for vasopressors, or organ failure; or significant physician concern for sepsis due to latter symptoms in the absence of a positive blood culture) or intra-abdominal abscess requiring an operative intervention. Treatment will be temporarily held until the infection is adequately treated, and then resumed if, in the opinion of the patient's physician, it is safe to do so. Hypotension and tachycardia are common on POD 0- POD 1, due to the surgery, and alone, would not necessitate halting therapy. Antibiotic prophylaxis is routinely given in this population.
- Wound infection or minor infection – Superficial wound infections are relatively common in this population. In the etanercept arm only, etanercept will be held until infection is treated with antimicrobial therapy, per standard care with this agent.
- Any diagnosis of malignancy
- Any new diagnosis of CNS demyelinating disease, including multiple sclerosis, optic neuritis, transverse myelitis, or a new diagnosis of Guillan Barre.
- Pregnancy (not expected in first few months after major abdominal surgery)
- Other new medical condition which in the opinion of the investigators, or the patient's surgical and medical team, present a contra-indication to continuing treatment.
- Occurrence of diabetic ketoacidosis
- Occurrence of DVT or PE

Study Termination/ Study arm termination: If two or more patients in one arm experience any of the following safety events: (1) documented sepsis, (2) an intra-abdominal infection that requires drainage and/or operative intervention (excludes superficial wound infection), (3) PE or DVT, or (4) diabetic ketoacidosis, all within the first 90 days of surgery, the study will be temporarily halted, and the external DSMB will be asked to review the data. The study arm in question (alpha-1 antitrypsin or etanercept) will be resumed only with permission from the DSMB. If the DSMB deems that the serious infections were likely related to the study drug, no additional patients will be treated with this agent. Any patient death, regardless of relation to study drug will prompt halting of study pending DSMB review.

The data safety and monitoring board for islet transplantation at the University of Minnesota is headed by Dr. Bruce Redmon. The DSMB meets every 6 months to review all active islet transplant clinical trials. The DSMB will review the study semi-annually, to ensure safety of the study participants.

STUDY PERSONNEL AND ORGANIZATION

Study Investigators/ Collaborators:

Dr. Melena Bellin is the primary investigator on the project. Dr. Bellin is an Assistant Professor of Pediatric Endocrinology at the University of Minnesota and the Director of Research for the Islet Autotransplant Program. She will be responsible for the overall planning and conduct of the trial. Dr. Bellin will directly supervise the nurse and CMA research coordinators, and will direct the efforts of the collaborators. Dr. Bellin will assume the primary role for subject recruitment, retention, and follow up. She has previously successfully conducted trials within the IAT population.

The surgery team includes co-investigators *Drs. Gregory Beilman, Ty Dunn, Timothy Pruett, and Srinath Chinnakotla*. They will assist with subject recruitment and subject visits, and help supervise subject care while patients are in the hospital setting post-operatively, and will also provide academic input. While Dr. Chinnakotla

is the pediatric transplant surgeon, his patients include young adults (18- early 20s) and he assists with coverage for the adult service and thus remains an important contributor. Dr. Gregory Beilman is a COL in the armed forces reserve and will have a significant academic role in the project. Dr. Ty Dunn will also assume a larger role in providing back up coverage for PI Dr. Bellin when Dr. Bellin is not available on site.

Dr. Bernhard Hering is the Scientific Director of the Schulze Diabetes Institute. He has extensive experience in islet transplantation research, including auto-transplant, allotransplant, and xenotransplants. He will assist in project planning and will provide academic support to Dr. Bellin.

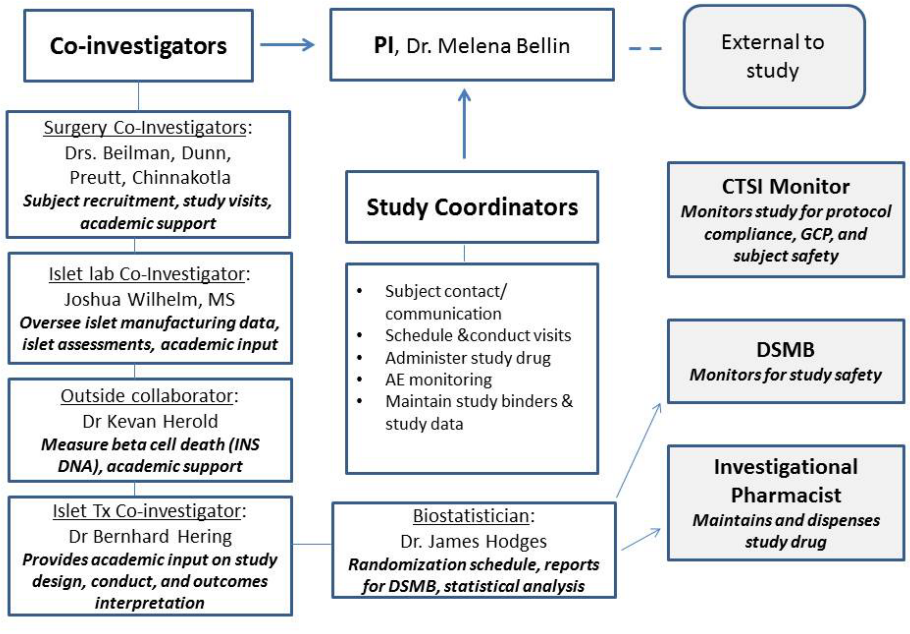
Dr. James Hodges will provide biostatistical support for randomization and data analysis, as well as assist in sample size planning for future studies based on preliminary data gathered in this trial.

Josh Wilhelm MS oversees the islet isolation lab, coordinates islet graft assessments, and maintains the islet isolation database. He will also provide academic input, particularly on assessing the comparability of the islet product between study groups.

Outside collaborators: *Dr. Kevan Herold* is a Professor of Immunobiology and Medicine (Endocrinology) at Yale, and will provide academic expertise on a novel assay to measure loss of beta cells in the immediate post-operative period. Dr. Herold additionally has expertise in alpha-1 antitrypsin through his role in the RETAIN trial of alpha-1 antitrypsin in type 1 diabetes.

Other key study staff

RN Coordinator and *CMA Coordinator* will have a vital role in the day to day operations of the study. The coordinators will be the primary contact between the study and the study participant. They will be responsible for scheduling subject visits, maintaining communication between study visits, adverse event monitoring, maintaining study documentation, and performing the metabolic testing. The RN coordinator will also be responsible for study drug administration. Thus, the RN coordinator will have a more prominent role in drug administration and monitoring drug AEs, while the CMA coordinator will focus on performing lab draws, metabolic assessments, scheduling, and maintaining contact with study participants. The coordinators operate under direct supervision from the study PI. The study coordinators will have previous experience in clinical trials research within this population, and are certified clinical research coordinators (CCRC, certification obtained through the Association of Clinical Research Professionals). In addition, our *regulatory coordinator* (Jean Witson), who has extensive experience with IND / FDA regulated studies in islet transplant, will work with the study coordinators to be sure that all regulatory requirements are met.



External monitoring

A clinical trial monitor will be provided by the University of Minnesota CTSI, and will monitor the study to ensure compliance with GCP and FDA regulations (21 CFR 312 and 812). This individual is external to the study.

A *data safety and monitoring board (DSMB)* for islet transplantation at the University of Minnesota will review the safety and efficacy data, with particular attention to subject safety, every 6 months.

Lab facilities

The majority of lab assays will be run in the Fairview Hospital lab facilities, affiliated with the University of Minnesota. However, the following are not available or are inadequate at the Fairview lab and will be run in the facilities described below:

- Insulin levels (GPAIS and IVGTT): To obtain accurate results in this population, we require an insulin assay that is resistant to interference from hemolysis. Fairview does not offer an adequate assay for this purpose. We will ship serum for insulin levels to the Northwest Lipid Research Laboratories at the University of Washington. Timed test glucose and C-peptide measures will also be run at NWLRL due to their ability to handle such timed, repeated measures specimens.
- Unmethylated insulin DNA: This specialized assay will be run by Dr. Kevan Herold's lab at Yale University
- Trypsinogen, TAP, and liver injury markers: ELISA kits for the respective assays will be purchased and the laboratory assessments run by the technician in Dr. Gregory Beilman's lab.

For labs that require shipping, all specimens are labeled with the study ID number and contain no identifying information. Specimens are shipped overnight by FedEx on dry ice. All study personnel performing specimen shipping are trained in shipping through a session offered to research staff at the University of Minnesota. Appropriate precautions and labeling for biohazard materials are undertaken.

Visit Day	Screening	Day -1 or 0	Transplant visit*	Day 3	Day 7	Day 10	Day 14	** Day 21	** Day 28	Day 60	Day 90	Day 365	Day 730
Visit window	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**	**± 1**	**± 1**	**± 7**	**± 15**	**± 28**	**± 56**
Req'd for Tx arms:	**ALL**	**Etan/ A1AT**	**ALL**	**ALL**	**ALL**	**Etan**	**ALL**	**ALL**	**ALL**	**ALL**	**ALL**	**ALL**	**ALL**
General Assessments													
Informed Consent	x												
History	x				x		x		x	phone	x	x	x
Physical Exam	x				x		x		x		x	x	x
Hypoglycemia History											x	x	x
Insulin use (patient records), u/kg/day											x	x	x
AE assessment					x		x	x	x		x	x	x
Basic Labs/Safety													
Complete metabolic panel	x				x		x	x	x		x	x	x
CBC with plt/diff	x				x		x	x	x		x	x	x
D-dimer (safety assessment)	x		x	x	x		x	x	x				
IgA level	x												
Hepatitis B sAg and Ab	x												
Hepatitis C Ab	x												
HIV ELISA	x												
Urine pregnancy test (Fm)	x												
Manitoux skin test or Quantiferon (TB)	x												
Hemoglobin A1c	x										Quarterly through study		
EKG	x								x		x		
Metabolic Assessments													
Mixed meal test	x										x	x	x
GPAIS	x										x	x	x
IV glucose tolerance test	x										x	x	x
Continous glucose monitor (iPro)											x	x	x
C-peptide: glucose (fasting)											x		
C-peptide and glucose (random)					x		x		x				
Mechanistic Assessments													
Inflammatory Cytokines*			x	x	x								
Unmethylated insulin DNA*			x	x	x		x	x	x		x	x	x
Serum trypsin and urine TAP*			x										
A1AT levels	x	x			x		x	x	x				
Liver injury markers*			x	x	x								
Study Drug Administration													
Etanercept (only for Etan. arm)		day 0		x	x	x	x	x					
A1AT (only in A1AT arm)		day -1		x	x		x	x	x				

* These mechanstic assessments during "transplant visit" include mulitple draws. Please refer to text in protocol for specific times of draw.

Patients will be inpatient per usual clinical care through day 7 at minimum.

** +/-1 day window for labs and assessments. Study drug should be given on day 21 and day 28 whenever possible for consistency.

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