Study Protocol: Hydroxychloroquine and Metabolic Outcomes in Patients Undergoing Total Pancreatectomy and Autologous Islet Transplantation: A Clinical, Molecular, and Genomic Study

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1. Introduction:

Total pancreatectomy (TP) has been used as the therapy of last resort in patients with chronic pancreatitis (CP). The procedure is often coupled with transplantation of islets of Langerhans recovered from the resected pancreas (autologous islet transplantation, AIT) intended to prevent or attenuate the effects of surgically-induced diabetes in select patients based on pre-surgical metabolic assessments [1-3]. AIT is not significantly affected by the stress of post-transplant cellular rejection that one encounters in allotransplantation. However, islet loss and altered beta cell functionality have been demonstrated to be inevitable [4-6]. A compelling level of evidence exists on the effects of the innate immunity-driven inflammation on the decline of functional beta cell mass in the autologous transplant setting [5]. Rationally-selected anti-inflammatory approaches for patients undergoing total pancreatectomy and autologous islet transplantation (TPAIT) may be a potential approach for the protection of islets from the initial inflammatory challenge resulting in preservation of beta cell function and leading to sustained long-term insulin independence.

2. Background and Significance:

An extensive amount of preclinical and clinical data support the viability of islet transplantation as therapy for treating insulin-dependent diabetes. Islet transplantation has become an increasingly acceptable approach to reverse diabetes and has been largely tested in the context of autologous as well as allogeneic donor-recipient pairs [2, 7-9]. The outcomes of AIT in diabetes remission differ across patients that undergo TPAIT. Recent literature has shown at a median follow-up of 28-months post transplantation that about a third of patients was insulin independent, one third was fully insulin-dependent, and one third showed some islet graft function but still required exogenous insulin [1, 2]. In the latter case, even a partial graft function confers benefit to patient's health and quality-of-life.

A critical factor in predicting successful islet transplantation which translates into insulin-independence, is the islet equivalent (IEQ) count [1, 2]. Recent data from the largest TPIAT cohort reported a 3-year insulin independence rates that range from 12% in those receiving <2,500 IEQ/kg to 72% in those receiving >5,000 IEQ/kg of body weight [2]. Despite compelling evidence of the latter, about a third of patients receiving more than double IEQ/kg remain insulin dependent post-TPAIT. More interestingly, a recent report on a subject receiving as few as 954 IEQ/kg achieved and maintained insulin independence

up to 4 years after transplantation [10]. Therefore, additional factors besides the IEQ count, contribute to and limit islet survival and function post-AIT.

Islet cell survival and function, both short- and long-term are governed by the degree of cellular damage: both pre-existing from the underlying inflammation seen in chronic pancreatitis, and inflicted damage by the isolation and transplantation processes. Reports on beta cell mass among CP patients estimate 29% loss of the fractional beta cell area as a result of chronic inflammation and fibrosis, coupled with an additional 21% reduction in overall pancreatic mass resulting in more pronounced deficit in total islet mass [11]. As for inflicted damage, the islet isolation procedure is a key pre-transplant factor in islet mass loss [12, 13]. Harmful events also occur in the early post-AIT period and are induced by stressors such as hypoxia, hyperglycemia, and the infiltrating innate immune cell-derived cytokines and other pro-inflammatory factors [5, 14, 15]. A pro-coagulatory and pro-inflammatory cascade is activated within minutes after islet infusion back into the host and actively accelerate the injury to transplanted islets [16]. The levels of inflammatory cytokines such as IL-1Ra, IL-6, IL-8 and IL-10 have been reported to peak within the first 6 hours of islet infusion and gradually subside to pre-transplant levels days from islet transplantation [5, 16].

Stemming from experimental and clinical transplantation data, there is general agreement that providing anti-inflammatory cover during the early post-islet infusion period can protect the graft [15, 16]. Protection of the islet graft from the initial inflammatory challenge should prevent cell loss and preserve beta cell function leading to sustained long-term insulin independence and delay of complications associated with uncontrolled hyperglycemia [17, 18]. Recent studies have proposed the use of TNF-α blockers/inhibitors to short-circuit the innate inflammation known to be detrimental to beta cell function and survival [19-25]. Etanercept, a TNF-a blocker, has been incorporated into a number of immunosuppressive protocols for islet allotransplantation which have proven successful [19, 20, 23-26]. In the multicenter Clinical Islet Transplantation (CIT) study, the use of etanercept resulted in improvement in beta cell secretory capacity of more than 40% of normal and a three-fold gain in islet engraftment efficiency [20]. When these patients were evaluated at 1 year post-transplant, they remained free of insulin use with a trend towards further improvement in beta cell secretory capacity to more than 50% of normal [20]. Even though one cannot conclude that etanercept alone was an important factor in improving the outcomes, its inclusion in the immunosuppressive regimens is believed to have made some contribution that could be relevant in preserving beta cell mass and function consequent to TNF-alpha blockade. This same mechanism of islet loss is also relevant in the autologous islet transplantation settings.

Hydroxychloroquine (HCQ) is a quinolone anti-malarial agent, FDA-approved for the treatment of autoimmune diseases. HCQ is considered the first-line agent for the treatment of rheumatoid arthritis,

systemic lupus erythematosus and sjogren syndrome [27]. The immune effects of HCQ are modulated though several mechanisms, particularly through decrease in pro-inflammatory cytokine production such as IL-1 and IL-6 and reduction of proteolysis and antigen presentation. Compared to TNF-a blockers, HCQ is administered orally, is relatively inexpensive, and has excellent tolerability with minimal side effect profile.

Previous studies have demonstrated that patients treated with HCQ have a lower incidence of diabetes [28-32]. Hypoglycemia and/or reduction in HbA1c levels have been encountered in HCQ-treated patients, both in those with or without diabetes [33-37]. In three randomized, controlled trials conducted with type 2 DM patients, the use of HCQ resulted in reduced insulin need and improvement in HbA1c levels and beta cell function in all three trials [38-40]. A few mechanisms have been proposed regarding the effects of HCQ in improving glycemic profiles, most notably through the inhibition of inflammatory cytokines resulting in decreased beta cell loss as demonstrated in a rat model of pancreatic endocrine failure [41]. Another proposed mechanism is through improvement of insulin metabolism at the level of peripheral tissues [42].

We **hypothesize** that HCQ administration during the peri-transplant period will preserve islet mass and improve beta cell function in TPAIT by attenuating inflammation. The aim of our proposed study is to demonstrate a higher stimulated C-peptide level as well as better glucose control in response to a mixed meal tolerance test (MMTT) at 6 and 12 months following TPAIT in patients treated with HCQ compared to controls. A better response in the HCQ arm would suggest improved islet survival and metabolic performance, potentially facilitating higher rates of insulin independence.

We foresee that that the use of HCQ will help facilitate engraftment and preservation of the transplanted islet mass, both increasing the likelihood of graft survival initially and fostering long-term engraftment and improved outcomes in functional beta cell mass for patients who undergo TPAIT. If the study goals are achieved, and more controlled clinical studies confirm a potential positive outcome, anti-inflammatory treatments may become part of the standard protocol in patients undergoing TPAIT, and long-term complications of surgical diabetes may be attenuated. It is also anticipated that if the proposed treatment demonstrates improved metabolic function, higher rates and longer duration of insulin independence, this may encourage patients suffering from CP and qualifying for TP, to undergo surgery at a time in which a significant islet mass is still retrievable.

Data obtained from this pilot study will be used to inform a future phase I/II clinical trial to determine the optimum dosage and timing of this anti-inflammatory treatment to maximize improvement in beta cell function. In particular, the mechanisms of inflammation in the early phase after transplant may differ

from those associated with long-term islet loss, which we believe is an important variable to better understand.

2.1 Innovation:

2.1.1 Part I: Mitochondrial Function and Metabolic Outcomes in TPAIT

From a molecular standpoint, mitochondrial function of beta cells is directly related to the ability of beta cells to secret insulin [43]. Physiologically, as the insulin release is regulated by cytoplasmic adenosine triphosphate (ATP)/adenosine diphosphate (ADP) ratio, a mitochondrial defect will lead to defect in insulin release. Mitochondrial efficiency is important for the survival of transplanted islets due to increase in metabolic demand [44], and decrease in oxygenation [45].

In patients with chronic pancreatitis, as there are several factors influencing the islet health and function, such as inflammation, fibrosis, limited blood flow, compartment-like effect and loss of innervation [46], the mitochondrial function is probably compromised by this pathological microenvironment.

Extracellular flux analyzer is a method to assess mitochondrial respiration and glycolysis through the measurement of oxygen consumption rate (OCR) and extracellular acidification rate (ECAR), respectively. With a microchamber system, the analyzer allows the measurement of the change in the concentration of dissolved oxygen and the free protons. As the analyzer is equipped with drug introducers, a physiological stress can be imposed to the cells to test their respiration capacity in such situations. A method to assess the human islet with this analyzer has been validated and used in research [47].

In this pilot study, we are investigating the degree mitochondrial dysfunction in islets of patients with chronic pancreatitis receiving TPIAT, reflected by measured OCR and whether the latter is associated with poorer metabolic outcome compared with control, normal islets. This information will further contribute to the knowledge of autoislet function, and will identify other factors that are related to metabolic outcome that can be targeted for islet therapy in the future.

We **hypothesize** that mitochondrial dysfunction in islets of chronic pancreatitis patients is associated with a poor metabolic outcome after TP-IAT compared with control, normal islets. We also **hypothesize** that a trend towards less mitochondrial oxidative stress would be seen in patients receiving the anti-inflammatory drug HCQ prior to transplantation.

2.1.2 Part II: Genome-wide Gene Expression in TPAIT Patients

Previous investigations have identified pathways that regulate human beta-cell proliferation, function and maturation [48-51]. The genes regulating beta-cell proliferation and function may change dramatically with various factors, such as age [52]. Little is known about the gene signatures for islets from an inflammatory environment, particularly long-term CP patients, whose pancreata have been altered by the disease over time. The gene expression may influence the islet engraftment, survival, function and proliferation and impact the metabolic outcomes in islet transplantation.

We **hypothesize** that the gene expression signature plays a significant role in the clinical outcomes in TPAIT in patients with CP. Specifically, this study would help to determine:

1. Whether there is heterogeneity in the autoislet gene expression profiles prepared for TP-AIT
2. How the heterogeneity in the gene expression profiles is related to clinical outcomes of AIT

3. Study Design

3.1 Methods

This will be a pilot, 12-month phase II, open label, randomized, two-arm, single-blinded, placebo-controlled, parallel clinical trial of individuals undergoing TPAIT. The two study arms consist of HCQ-treated and placebo-treated individuals. To take into consideration an expected more favorable outcome in beta cell function when higher IEQ/Kg body weight is infused, MMTT and C-peptide measurement prior to surgery will be used to assign potential subjects to either a predicted lower or higher IEQ/Kg group. This “distinguo” will be useful in the analysis of the results. Subjects from each group will be evenly distributed and randomized into the two treatment arms.

3.2 Sample

All patients undergoing TPIAT for CP in the Cleveland Clinic Foundation (CCF) will be under the auto islet registry (Institutional Review Board (IRB) number 08-340) and are potential candidates for the study.

A total of **10 patients** scheduled for TPAIT at CCF, who meet the inclusion/exclusion criteria for TPAIT, confirmed by a multidisciplinary team, consisting of a hepatobiliary surgeon, gastroenterologist, endocrinologist, diabetes educator, social worker, nutrition therapist, psychologist, and pain management team will be recruited into the study. The inclusion/exclusion criteria for entry into the trial, which consists of two parts (criteria for TPAIT and then criteria to be enrolled into this pilot trial), are summarized in Table 1. Historically, gender representation has been equal in the cohort of patients undergoing TPAIT at CCF [1]. Although a trend to better outcome in females was observed even though the mechanisms are unknown [1], the most significant predictor of metabolic function in patients

undergoing TPAIT is the islet mass infused, which will be factored into this trial in two ways: 1) by pre-testing and assigning patients to predicted islet yield groups as has been described previously and 2) by normalizing parameters of metabolic function per IEQ/Kg infused.

Arm 1 (n=5) subjects will receive a pre-transplant 200 mg daily dose of HCQ 30 days before TPAIT and will continue on the drug for 3 months after surgery. This dosage will not exceed a daily dose of 5 mg/kg/day using actual body weight due to potential risk of retinal toxicity; patients weighing less than 40 kg will be excluded from study.

Arm 2 (n=5) subjects will receive placebo treatment following the same schedule as in Arm 1.

3.3 Feasibility of study

Since the program of TPAIT at CCF has become fully operational, approximately 10-13 patients undergo TPAIT within a 12-month period. It is anticipated that the requisite number of subjects proposed for the study will be enrolled within the time period allowed for the study.

3.4 Pace of enrollment

We anticipate that 12 months will be needed to recruit study subjects and 1-year for trial, totaling a study period of up to 24 months.

3.5 Benefits and risks

Hydroxychloroquine is considered to be among the safest antirheumatic medications with the potential for any serious adverse event being extremely rare [53]. Gastrointestinal symptoms are the most common side effects, nausea being the most common. The relative safety of HCQ has resulted in no regular laboratory testing required to monitor for toxicities. Monitoring for adverse events is clinical. Retinopathy is the most notable complication of HCQ, though any serious ophthalmologic toxicity is rarely seen with regular follow up. The risk of retinopathy increases with higher dosages and longer durations of therapy. In a study of 2,361 patients on HCQ for at least 5 years, the prevalence of retinal toxicity was less than 2 percent during the first 10 years of therapy in patients treated with 4.0 to 5.0 mg/kg of real body weight [54]. The current 2016 revised recommendations of the American Academy of Ophthalmology recommend that all patients undergo a baseline eye examination before or within a year of beginning the treatment with HCQ [55]. Given that that the likelihood of HCQ-induced retinopathy is rare with short-term use (i.e. 4 months in this pilot study), no baseline ophthalmological evaluation will be mandated. However, patients with preexisting retinopathy will be disqualified from enrollment.

Table 1: Inclusion/Exclusion Criteria

Inclusion criteria for TPAIT [1, 56]	Exclusion criteria for TPAIT [1, 56]

Clinically confirmed diagnosis of CP Intractable abdominal pain History of failed operation(s) for CP Recurrent acute pancreatitis HbA1c <8.0% Sustained alcohol remission Chronic narcotic use	Insulin dependence Pancreatic carcinoma Pancreatic mass suspicious for carcinoma Cirrhosis Portal hypertension Continued alcohol abuse

Inclusion criteria for HCQ phase	Exclusion criteria for HCQ phase
Acceptance for TPAIT at CC Age range 18-65	Manufacturer's product label-contraindicated use of HCQ History of retinopathy Actual weight at enrollment <40 Kg Inclusion/exclusion criteria for TPAIT unmet

4. Research Procedures

4.1 Total pancreatectomy and islet isolation and transplantation

Pylorus-preserving TP with duodenectomy will be performed in all cases usually associated with splenectomy to preserve perfusion to body and tail. Warm ischemia time will be minimized. Finally, the pancreas and duodenum will be removed intact and flushed/drained via the splenic artery and vein respectively with UW solution [56]. Islet isolation and purification will be performed as described [56-58]. The islet Isolation Laboratory located at the Institute of Cellular Therapeutics at Allegheny General Hospital (AGH) has performed more than 60 islet isolating procedures for the Cleveland Clinic and will perform all of the islet isolation for the study [58]. The number of islets will be quantified as islet equivalents (IEQ). The isolated islet through standard method will be transported back to the Cleveland Clinic and transplanted as previously described [56].

4.2 Randomization

Fasting blood glucose (FPG) level <100mg/dl with stimulated C-peptide >4 ng/ml during MMTT pre-surgery, will be the criteria to assign subjects to groups with predicted lower versus higher post-isolation islet yield. If either condition is not met, patients will be assigned to the lower yield group. With a representation from both groups, study subjects will be then randomized to HCQ treatment Arm 1 or placebo Arm 2 in a 1:1 allocation before the pancreatectomy procedure without any bias on age, gender, body weight, or IEQ isolated.

4.3 Blinding

The study will be single-blinded. The PI, biostatistician who will analyze the data, consultants, and technicians running assays will be blinded to the study arm into which the subjects have been randomized. An alphanumeric identifier that refers to the study subject without any indicators of study arm allocation will be used. Only the surgeons and the research coordinator, but not the personnel conducting the metabolic studies, will be un-blinded as to the study arm randomization.

4.4 HCQ administration

Arm 1 (n=5): Subjects will receive a pre-transplant HCQ 200 mg daily dose 30 days prior TPAIT followed by HCQ use for an additional 3 months post-surgery. Oral medications are continued peri-operatively. Patients are typically ordered for "NPO except for medications" starting from post-operative day 1 which will not preclude the administration of HCQ in the peri-transplant period.

Arm 2 (n=5) subjects will receive placebo treatment following the same schedule as in Arm 1.

4.5 Exploratory mechanistic studies

All subjects will undergo a MMTT to assess islet cell function at 6 and 12 months following TPAIT (in addition to MMTT pre-surgery performed as standard of care, and whose results will be used for pre-randomization in this pilot). Baseline metabolic tests obtained too early after surgery may not be indicative of islet function, due to insulin supporting therapy administered for several weeks after transplantation. Also, compelling data indicate that stabilization of islet function may require up to 1 year to occur. Blood glucose and C-peptide serum levels will be measured in peripheral blood samples immediately prior and subsequent to MMTT. Laboratory tests will be measured in CCF hospital laboratory.

The research coordinator will contact the subjects at 3, 6 and 12 months for interview on the course of follow up and will assist in scheduling the 6 and 12-month appointments for MMTT.

4.6 Innovation:

4.6.1 Part I: Mitochondrial Function and Metabolic Outcomes in TPAIT

Small amounts of digest left after islet isolation, that would normally be discarded, will be used for this study. The islets from the digest will be collected and will undergo extracellular efflux analysis through the Seahorse XF analyzer located in the Kirwan Laboratory of the Cleveland Clinic Lerner Research Institute for mitochondrial function assessment. Commercially available normal human islet cells for experiments will be used as control. Controls will be shipped within 1 week of planned TPAIT date and will be compared simultaneously with islets isolated from study subjects. A protocol developed by Seahorse Bioscience in collaboration with the Mitochondrial Advancing Research through Collaborations

at Boston University School of Medicine will be used for the Seahorse XF analyzer islet analysis [59].The OCR of isolated islets will be analyzed with metabolic outcomes from post-transplantation follow-up.

4.6.2 Part II: Genome-wide Gene Expression in TPAIT Patients

Small amounts of digest from the procedure used for isolating islets, and what remains in the circuit after the isolation process is complete, that would normally be discarded, will also be used for islet gene expression assessment. These collected islets will be shipped to Seung Kim Laboratory at Stanford University School of Medicine for gene expression assessment.

For the flow cytometry, RNA isolation and RNA-sequence library preparation, islets or acinar tissues will be dispersed into single cells by enzymatic digestion and incubated. Cells will be then stained with conjugated primary antibodies and sorted, followed by cytometry data collection. The cells then will be lysed, and total RNA will be isolated. RNA quality and quantity will be measured, and RNA-Sequence libraries will be built. Sequencing RNA reads will be aligned with default parameters. Transcript counts will be obtained using a collapsed Ref-Seq reference transcriptome list.

4.7 Potential problems and alternative strategies:

The TPAIT procedure carries with it a risk of bacterial contamination from the pancreatic organ itself along with islet infusion, however CCF has one of lowest incidences (38%) than reported [60, 61]. Patients undergoing TPAIT are fully immunocompetent, and will be receiving antibiotics treatment as part of the post-operative protocols. Routine microbiological analysis will allow germ identification and prompt target therapy, if required. Patients will remain in the hospital for post-operative care (median 10 days) [1], housed in ICU after surgery. Hospitalization will be followed by outpatient daily visits for additional 5-7 days, which will allow for control of any possible event associated with the administration of HCQ. Blood glucose levels will be monitored and eventually corrected if potential hypoglycemic events associated to HCQ use should occur. Post-operatively, patients will be under continuous glucose monitoring and insulin drips and on an IV line, which will allow for a quick response to changes in glycemic levels.

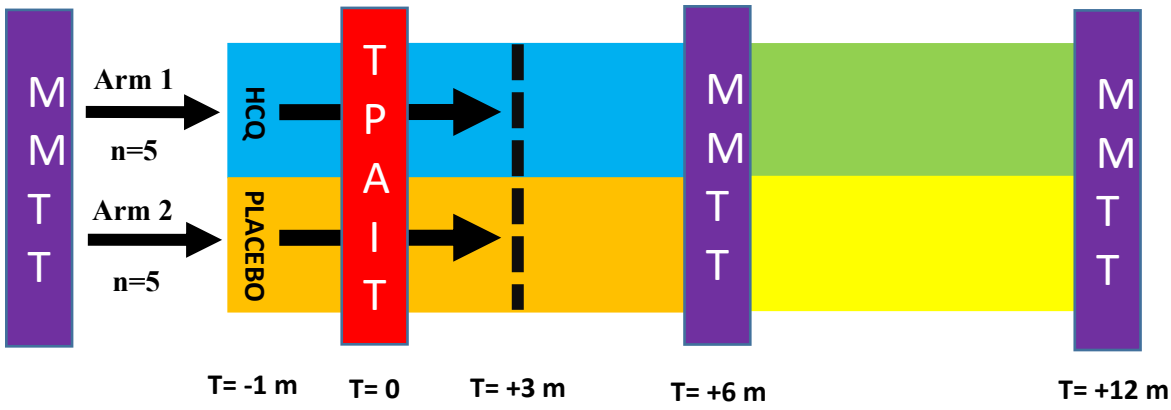
Metabolic test such as arginine stimulation [62] would have been more informative than the MMTT, allowing for better control of variables such as glucagon responses. If the data support the hypothesis, in the future, a larger and more controlled phase I/II study could consider the more cumbersome, but better informative arginine stimulation test.

Dr. Hatipoglu and her clinical staff will monitor the subjects for HCQ-emergent adverse events prior to discharge and the research coordinator will follow-up by phone contact throughout the duration of the trial.

Table 2: Study Timeline

	T= -1 months	T= 0 (TPAIT day)	T = +3 months	T = +6 months	T = +12 months
HCQ group (n=5)	- MMTT #1 - Start of HCQ	- Seahorse XF analyzer for isolated islets (Kirwan Lab) - Genomic analysis (Kim Lab)	- Discontinue HCQ	- MMTT #2	- MMTT #3
Placebo group (n=5)	- MMTT #1 - Start of placebo	- Seahorse XF analyzer for isolated islets (Kirwan Lab) - Genomic analysis (Kim Lab)	- Discontinue placebo	- MMTT #2	- MMTT #3

Figure 1: Study Timeline



5. Data Analysis

5.1 Study endpoints

5.1.1 Primary endpoints:

- Quotient of MMTT stimulated (90min) C-peptide/glucose/IEQ/Kg at 6 and 12 months after TPAIT.

Rationale for the proposed endpoint: MMTT is a common metabolic test used to assess the insulin and C-peptide response to a mixed meal challenge. It triggers physiologic pathways to induce maximal insulin and C-peptide secretion. It is simple to conduct, and the outcome is widely believed to represent beta cell insulin secretion capacity. In recipients of islet allografts, the ratio of MMTT C-peptide/glucose at 90min correlates with the islet mass infused and it proves a valuable indicator of islet function. Whereas IVGTT

is also broadly utilized to assess islet function, our preference has been given to a test that shows correlation with predicted islet yield (used in a pre-randomization step) and infused islet mass.

5.1.2 Secondary endpoints:

- Stimulated C-peptide area under the curve (AUC) in response to MMTT at 6 and 12 months after TPAIT.
- Stimulated glucose AUC in response to MMTT at 6 and 12 months after TPAIT.
- Stimulated C-peptide and glucose levels in response to MMTT at 6 and 12 month after TPAIT.

5.1.3 Innovative endpoints:

5.1.3.1 Part I: Mitochondrial Function and Metabolic Outcomes in TPAIT

Oxygen consumption rate (OCP) and extracellular acidification rate (ECAR) of isolated islets' mitochondria in subjects receiving HCQ and placebo.

5.1.3.2 Part II: Genome-wide Gene Expression in TPAIT Patients

Differential gene expression calls will be done on isolated islets. These will be compared to appropriate control gene expression sets [52].

5.2 Study outcomes:

5.2.1 Primary outcomes:

- Increased quotient of stimulated C-peptide/glucose level normalized for IEQ/Kg infused in response to MMTT at 12 months in the HCQ-treated compared to placebo arm.

5.2.2 Secondary outcomes:

- Higher C-peptide AUC in response to MMTT 12 months in the HCQ-treated compared to placebo arm.
- Higher ratio of C-peptide AUC to glucose AUC in response to MMTT at 12 months in the HCQ-treated compared to placebo arm.

5.2.3 Innovative outcomes:

- Lower rates of oxygen consumption within isolated islets from CP patients compared to commercial controls
- Higher rates of oxygen consumption within isolated islets of HCQ-treated subjects compared to placebo.
- Full RNA sequence library for isolated islets from CP patients

5.3 Data collection:

The clinical data will be collected under auto islet registry stored in REDCap. All data in the Islet Isolation Laboratory at the Institute of Cellular Therapeutics, Allegheny Health Network and Kirwan Laboratory of the Cleveland Clinic Lerner Research Institute will be de-identified and collected into Microsoft Excel as medium, then transferred to REDCap by the investigators. All investigators will have received proper HIPPA training.

5.4 Data access and confidentiality:

All data will be collected either directly into REDCap or indirectly through Microsoft Excel on CCF Desktop computer as medium. The data will contain no personal health identifier (PHI) when it is being shared with investigators outside of the CCF. A study ID will be assigned to each subject. The de-identified clinical data will be shared for the purpose of manuscript construction under the standard user data agreement from the CCF IRB Committee. All investigators will have received proper HIPPA training.

5.5 Statistical Analysis

Measurement of serum C-peptide levels during MMTT and, specifically, the quotient of C-peptide (CP)/glucose (G) (ng/ml x100/mg/dl) at 90min during the test, have shown to be indicators of islet function and islet mass after allogeneic islet transplantation. Increments in the quotient according to islet mass (IEQ)/kg have also been identified. We expect this quotient to be applicable to the TPAIT setting in line with several studies showing that MMTT is a valuable assay to assess islet function after TPAIT. We applied the proposed increments to estimate levels of the CP/G (0.35±0.07) based on our historical TPAIT data, indicating on average IEQ/Kg infused of 4500±1000. Our hypothesis is that higher survival of beta cell mass due to the treatment will result in higher CP/G quotient in the HCQ-treated arm, compared to the placebo group, after normalization for IEQ/Kg infused. A one-sided, 2 sample t-test was used for sample size calculation with the assumption of equal variance between the two study arms. Assuming a 20-30% difference in outcome (CP/G quotient) between study arms, which is equivalent to 0.08-0.12 CP/G difference and a 0.07 standard deviation, a sample size of 6 subjects (1:1 randomization) will detect a CP/G difference of at least 0.12, assuming 80% power, with a 0.05 significance level. We propose that a sample size of 10 subjects (5 for each arm) with the same assumptions but at 75% power, (Table 2) will be justified, given the expected feasibility of subject enrollment and the pilot nature of this trial.

Table 3: Statistical Analysis

	Sample size (per arm)		
	Δ=0.08	Δ=0.1	Δ=0.12
SD=0.07	n=9	n=7	n=5
SD=0.08	n=12	n=8	n=6
SD=0.09	n=15	n=10	n=7

6. Adverse Events and Data Monitoring Committee (DMC)

7. Consent

Patients will be consented to participate in this study. Those whom agree to participate will be enrolled. A nurse coordinator whom performs the consent for TPIAT will perform the consenting process for this study. A copy of the consent form is attached to this IRB application.

8. Budget

An outline of our budget is found in table 4. Data collection and entry will be performed by the study's investigators at no cost. MMTT testing at 6 and 12 months post-TPAIT is part of the standard-of-care testing in all centers performing TPAIT and thus no additional resources will be allocated for MMTT conduction. The cost of the RNA genomic analysis will be covered by the Seung Kim Laboratory at Stanford University School of Medicine at no additional cost to us.

An application will be submitted for the Research Program Committee (RPC) Grant to cover half of the study expenses (around $11,000). The planned date of submission of the application is February 6th, 2017. The remainder of the budget will be covered internally from EMI funding and the study's PI.

Table 4: Preliminary proposed budget

Item	Units needed	Estimated expense of items	Total expenses
HCQ (4 month supply) and Placebo (4 month supply)	600 HCQ pills, 600 matching placebo	- $1,200 for capsule manufacturing - $1,750 onetime pharmacy initiation fee - $60 monthly drug storage and inventory maintenance fee - $40 dispensing fee per occasion - $2,452 for generic HCQ (#600)	$1,200 $1,750 $240 $1,600 $2,452

Seahorse XF24 Islet Capture Microplates from Agilent Technologies	2	$369	$738
Islet controls from Lonza Pharma&Biotech	11	$945	$10,395
Genomic Analysis		$0 (to be covered by Kim's Lab)	$0
MMTT testing		$0 (to be covered by insurance providers)	$0
Expense of islet transport and care from CCF to AGH, from AGH to both Stanford and CCF		$0 (to be covered by Kim's Lab, AGH, and insurance providers)	$0
Statistical support		~$2,000	~$2,000
Data collection and entry		$0 (to be performed by study investigators)	$0
			~$20,375

9. Extramural Grant Application Plan

An application will be submitted for the Research Program Committee (RPC) Grant to cover the remainder cost of the study. The planned date of submission of the application is February 6th, 2017.

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