Clinical Islet Transplantation Consortium
Islet Transplantation in Type 1 Diabetes
Statistical Analysis Plan for CIT-06

Contents

Glossary of Abbreviations

Abbreviation	Definition
ACE	American College of Endocrinology
AE	Adverse Event
AIR_{glu}	Acute Insulin Response to glucose
ATG	Rabbit Anti-thymocyte Globulin
BMI	Body Mass Index
BG	Blood Glucose
BW	Body Weight
CGMS	Continuous Glucose Monitoring System®
CIT	Clinical Islet Transplantation
DDS	Diabetes Distress Scale
DI	Disposition Index
DSMB	Data Safety Monitoring Board
EQ-5D	European Quality of Life
FSIGT	Frequently-sampled Intravenous Glucose Tolerance
GFR	Glomerular Filtration Rate
HbA1c	Glycosylated Hemoglobin
HFS	Hypoglycemia Fear Scale
HLA	Histocompatability Antigen
HSA	Human Serum Albumin

Abbreviation	Definition
IEQ	Islet Equivalents
ITT	Intent To Treat
LI	Lability Index
MAGE	Mean Amplitude of Glycemic Excursions
MCMC	Monte Carlo Markov Chain
MMTT	Mixed-meal Tolerance Test
NCI	National Cancer Institute
NIH	National Institutes of Health
OHS	Overall Health Status
PAID	Problem Areas in Diabetes
QOL	Quality of Life
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SI	Insulin Sensitivity
SMC	Summary Mental Component
SPC	Summary Physical Component
T1D	Type 1 Diabetes
TCAE	Terminology Criteria for Adverse Events
ULN	Upper Limit of Normal

1. Introduction

Type 1 diabetes (T1D) afflicts nearly 2 million people in the United States, most of them children or young adults. Exogenous insulin, administered by multiple injections or by a continuous subcutaneous infusion from a wearable pump, allows long term survival in those who develop the disease, and most who are treated in this way will have a very good health-related quality of life (QOL). However, insulin therapy does not provide normal glycemic control, and long-term survivors commonly develop vascular complications such as diabetic retinopathy (the most common cause of adult blindness) and diabetic nephropathy (the most common indication for adult kidney transplantation). A small minority of individuals with T1D develop hypoglycemia unawareness, a condition that is life threatening, is associated with severe deterioration in QOL and activity restriction, and is not amenable to medical therapy.

The hope of achieving near-normal glucose control without hypoglycemia in T1D has provided the impetus for developing effective strategies for β-cell replacement via pancreas or isolated islet transplantation. Islet transplantation is accomplished by a procedure in which the islets are infused into the portal vein. While this procedure is not without risk, the procedural morbidity is much less than that of whole pancreas transplant.

While about 80% of whole pancreas transplant recipients will be insulin independent at one year after their transplant, less than 10% of 447 islet recipients transplanted between 1990 and 1999 achieved one year insulin independence. This was attributed to low engrafted islet mass combined with high metabolic demand imposed by glucocorticoids used to prevent rejection. In the year 2000, the group from Edmonton reported a series of 7 consecutive islet transplant recipients treated with islets from multiple donors and a glucocorticoid-free immunosuppressive regimen (Markmann, S, Huang, & al., 2003). These islet recipients were insulin free at follow-up ranging from 4.5 to 15 months. All of the recipients had experienced severe hypoglycemic episodes prior to transplant, and afterwards, none did. The efficacy of the Edmonton approach has now been confirmed by several other centers, and represents a major breakthrough in the field.

A Clinical Islet Transplantation (CIT) consortium has been formed to conduct a multicenter trial with the goal of providing strong scientific evidence that the rate of favorable outcome in transplanted subjects is high enough to justify the risks of the islet transplant procedure and the required immunosuppression.

This document briefly describes the study design and provides a detailed statistical analysis plan (SAP) for treatment efficacy and safety assessments. Details of all study aspects are given in the formal study protocol (CIT-06).

2. Study Design and Objectives

2.1 *Study Design*

This is a prospective, single-arm, multi-center study assessing the benefit of islet transplantation in type 1 diabetic (T1D) kidney transplant recipients. The participating centers will treat a total of 24 subjects.

2.2 *Study Objective*

The primary objective is to test the hypothesis that islet transplantation in patients with established kidney transplants leads to improved metabolic control as measured by serial HbA1c levels and a reduced occurrence of hypoglycemic events.

Secondary objectives of this study will assess whether successful islet transplantation leads to improved Quality of Life (QOL), improved metabolic control as determined by a wide variety of measurements and reduced risk of cardiovascular and renal complications from diabetes.

2.2.1 Selection of Subjects

Please see Section 3 of the CIT-06 protocol for subject inclusion and exclusion criteria.

2.2.2 Study Treatment Regimen

2.2.2.1 Investigational Agent: Allogeneic Islets

The investigational agent is the Purified Human Pancreatic Islet product. The final product is a sterile suspension of ≥70% viable, ≥30% pure, allogeneic human purified islets in 200 mL of transplant media containing 2.5% human serum albumin (HSA), 25 mM Hepes for administration by intraportal infusion. Each product lot may comprise up to 3 bags containing 200 mL each. The final product dose is ≥5,000 islet equivalents (IEQ)/kg recipient body weight (BW) for the first infusion, and ≥4,000 IEQ/kg recipient BW for subsequent infusions.

The transplant procedure involves infusion of the final product into a branch of the portal vein, which is accessed by percutaneous transhepatic cannulation using ultrasound guidance and fluoroscopic localization of the liver, or under direct visualization via a minilaparotomy.

2.2.2.2 Immunosuppression

For the first islet transplant, the study medication is administered concomitantly with a consensus regimen of immunosuppressive and anti-inflammatory medications that includes rabbit anti-

thymocyte globulin (Thymoglobulin®), Methylpredinisolone (Solumedrol®)), Basiliximab (Simulect®) (for subsequent transplants only), and Etanercept (Enbrel®).

2.3 *Study Endpoints*

2.3.1 Primary Endpoint

The primary endpoint for this study is the proportion of subjects with both an HbA1c ≤ 6.5% and an absence of severe hypoglycemic events at 1 year after the *first* islet transplant or a reduction in HbA1c of at least 1 point and an absence of severe hypoglycemia at 1 year after the *first* islet transplant.

2.3.2 Secondary Endpoints

Because there are a large number of secondary endpoints, it is impractical to account for all multiple comparisons. However, a few secondary endpoints have been identified as **key** secondary endpoints. Analysis of these key secondary endpoints will account for the multiplicity of tests.

2.3.2.1 Key secondary endpoints

The target level for HbA1c chosen for this study is 6.5%. This value was chosen because it is the level recommended by the American College of Endocrinology. We have included achieving a HbA1c level of 7.0%, alone and as a composite with freedom from **severe hypoglycemic events** at 1 year after the first and last islet transplants, as secondary endpoints because they correspond to the American Diabetes Association recommendations and will be of interest to the medical community. In addition, the primary endpoint for the Islet Alone study (CIT-07) uses 7.0% for the HbA1c cut-off. Inclusion of this endpoint will allow direct comparison with the Islet Alone study.

The key secondary endpoints are the following:

At 365 ± 14 days after the *last* islet transplant (the primary at 365 days after the last transplant):

> The proportion of subjects with both an HbA1c ≤ 6.5% and an absence of severe hypoglycemic events from Day 28 to Day 365, or a reduction in HbA1c of 1 point and an absence of severe hypoglycemia from Day 28 to Day 365.

At 365 ± 14 days after the *first* islet transplant:

- The proportion of subjects with HbA1c < 7.0% and free of severe hypoglycemic events from Day 28 to Day 365.
- The proportion of subjects free of severe hypoglycemic events from Day 28 to Day 365.
- The proportion of subjects with HbA1c < 7.0%.

- The proportion of subjects with HbA1c ≤ 6.5%.
- The proportion of subjects with a reduction in HbA1c of at least 1 point.
- The proportion of insulin-independent subjects.
- The change in Clarke score from baseline to Day 365.

2.3.2.2 Additional efficacy endpoints

Other secondary efficacy endpoints include measures at one year, two years, and three years after the first and/or after the last islet transplant.

One year (365 ± 14 days) after the last islet transplant:

- The proportion of subjects with HbA1c < 7.0% and free of severe hypoglycemic events from Day 28 to Day 365.
- The proportion of subjects free of severe hypoglycemic events from Day 28 to Day 365.
- The proportion of subjects with HbA1c < 7.0%.
- The proportion of subjects with HbA1c ≤ 6.5%.
- The proportion of subjects with a reduction in HbA1c of at least 1 point.
- The proportion of insulin-independent subjects.
- The change in Clarke score from baseline to Day 365.

Two years (730 ± 14 days) after the first and after the last islet transplant:

- The proportion of subjects with an HbA1c ≤6.5% AND free of severe hypoglycemic events from Day 28 to Day 730, or a reduction in HbA1c of 1 point and an absence of severe hypoglycemia from Day 28 to Day 730, inclusive.
- The proportion of subjects with HbA1c < 7.0% AND free of severe hypoglycemic events from Day 28 to Day 730.
- The proportion of subjects free of severe hypoglycemic events from Day 28 to Day 730.
- The proportion of subjects with HbA1c <7.0%.
- The proportion of subjects with HbA1c ≤6.5%.
- The proportion of subjects with a reduction in HbA1c of at least 1 point.
- The proportion of insulin-independent subjects.
- The change in Clarke score from baseline to Day 730.

Three years (1095±14 days) after the first and after the last islet transplant:

- The proportion of subjects with an HbA1c <7.0% AND free of severe hypoglycemic events from Day 28 to Day 1095, inclusive.
- The proportion of subjects with HbA1c ≤ 6.5% AND free of severe hypoglycemic events from Day 28 to Day 1095 or a reduction in HbA1c of 1 point and an absence of severe hypoglycemia from Day 28 to Day 1095.
- The proportion of subjects free of severe hypoglycemic events from Day 28 to Day 1095 after the last islet transplant.
- The proportion of subjects with HbA1c <7.0% at 1095 day after the last islet transplant.
- The proportion of subjects with HbA1c ≤6.5% at 1095 years after the last islet transplant.
- The proportion of subjects with a reduction in HbA1c of at least 1 point.
- The proportion of insulin-independent subjects at 1095 days.
- The change in Clarke score from baseline to Day 1095.

2.4 *Metabolic Measures*

Specific metabolic measures include the following:

At 75 ± 5 days following each islet transplant:

- Insulin independence

At one year (365 ± 14 days) following the first and the last islet transplant(s):

- Cardiovascular events [death, cerebrovascular accident (CVA), myocardial infarction (MI)], changes in carotid intima-medial thickness, atherogenic profile, and ratio of Apolipoprotein A1 and B
- Renal impact measures including renal allograft survival and function measured by serum creatinine (SCr) and urinary albumin creatinine ratio
- Number of severe hypoglycemic events
- The percent change from baseline insulin requirements
- HbA1c
- MAGE
- LI
- Clarke score [14]
- HYPO score

- Basal (fasting) and 90-min glucose and C-peptide (MMTT)
- β-score
- C-peptide/(glucose·creatinine) ratio
- AIR_{glu}, SI, and DI derived from the FSIGT test [5]
- Glucose variability and hypoglycemia duration derived from the CGMS®
- QOL, as defined in SAP section 3.6.5.
- The proportion of subjects receiving a second islet transplant
- The proportion of subjects receiving a third islet transplant
- Rate of favorable outcome at each center preparing islets (rate of subjects with an HbA1c ≤6.5% and absence of severe hypoglycemic events from Day 28 to Day 1095, or reduction in HbA1c of 1 point and absence of severe hypoglycemia from Day 28 to Day 1095)

At two years (730 ± 14 days) and three years (1095 ± 14 days) after the last islet transplant:

- Cardiovascular events [death, cerebrovascular accident (CVA), myocardial infarction (MI)], changes in carotid intima-medial thickness, atherogenic profile, and ratio of Apolipoprotein A1 and B
- Renal impact measures including renal allograft survival and function measured by serum creatinine (SCr) and urinary albumin creatinine ratio
- The percent change from baseline insulin requirements.
- The number of severe hypoglycemic events
- HbA1c
- Clarke score
- Basal (fasting) and 90-min glucose and c-peptide (MMTT)
- β-score
- C-peptide: (glucose• creatinine) ratio
- AIR_{glu}, SI, and DI derived from the FSIGT test [5]
- CGMS
- QOL

2.5 *Quality of Life Variables*

In this study, five QOL measures are considered. Two scales are obtained from the SF36 questionnaire (version 2). These scales are the Summary Physical Component score (SPC) and the Summary Mental Component score (SMC). Additional QOL assessments include the Overall Health Status (OHS) measure based on the European Quality of Life (EQ-5D) instrument, the Diabetes Distress Scale (DDS), and the Hypoglycemia Fear Scale (HFS) scales.

The SF36 scales will be standardized to the US population. The EQ-5D OHS questionnaire provides two sub-scales: the Behavior subscale that is based on 10 questions and ranges from 0 to 40 and the Worry subscale that is based on 13 questions. The scale is computed as the sum of the responses to the 13 questions and ranges from 0 to 52. The DDS is based on 17 questions rated on a 6 point Likert scales and ranges from 17 to 102. The HFS has two subscales: the Behavior scale which ranges from 0 to 40 and the Worry scale that ranges from 0 to 52.

2.6 *Time to Event Outcomes*

Multiple time-to-event outcomes will also be studied. All events will be defined for the period from the first transplant to 1095 days after the last islet transplant.

- Time to death (all cause and also cardiac related) and time to cardiovascular events (death, CVA, MI)
- Time to HbA1c $\leq$ 6.5%
- Time to first severe hypoglycemia episode
- Time to HbA1c decreasing by at least 1%
- Time to global treatment failure composite outcome measure defined as the time to the first occurrence of any of the following:
    - Death
    - Stroke
    - MI
    - Above the ankle amputation

All of the above will be ascertained from the SAE reports and will be adjudicated if an adjudication committee is available.

    - Renal Failure (Any one of the following):
        - Initiation of dialysis; or
        - Renal transplantation
    - Blindness: Visual acuity of 20/200 in both eyes.
    - Poor diabetes control (if any of the following occur):
        - HbA1c > 9% and reduced less than 1% below the entry level, found 6 months or more following the first transplant and confirmed on repeat examination at least

30 days and no more than 60 days later. If confirmed, failure is the time of the initial draw;

- Three consecutive monthly HbA1c values greater than the baseline value, with the first one being 6 months or more following the first transplant; or
- Occurrence of two severe hypoglycemic events (requiring assistance from another person) within a three month period. Failure will occur at the time of the second hypoglycemic event.

2.7 *Safety endpoints*

Safety endpoints include the following:

At 75 ± 5 days following each transplant, at 365 ± 14 days following the first and last islet transplant(s), at 730± 14 days following the last islet transplant, and at 1095 ± 14 days following the last islet transplant:

- The incidence and severity of adverse events (AEs) related to the islet transplant procedure, including: bleeding (>2 g/dL decrease in hemoglobin concentration); segmental portal vein thrombosis; biliary puncture; wound complication (infection or subsequent hernia); and increased transaminase levels >5 times upper limit of normal (ULN)
- The incidence and severity of AEs related to the immunosuppression including: allergy; reduction in glomerular filtration rate (GFR); increase in urinary albumin excretion; addition or intensification of anti-hypertensive therapy; addition or intensification of anti-hyperlipidemic therapy; oral ulcers; lower extremity edema; gastrointestinal toxicity; neutropenia, anemia, or thrombocytopenia; viral, bacterial, or fungal infections; and benign or malignant neoplasms
- The incidence of change in the immunosuppression drug regimen
- The incidence of immune sensitization defined by presence of anti-HLA (histocompatibility antigen) antibodies absent prior to transplantation
- The proportion of subjects who withdraw from study treatment due to an organ transplant

At 365 ± 14 days following the first and last islet transplant and at 1095 days following the last islet transplant:

- The incidence of worsening retinopathy as assessed by change in retinal photography

3. Subgroup Analysis

A Subject may enter the study because she/he is having severe hypoglycemic events that require assistance or because she/he is unable to reduce HbA1c level to below 7.5% on Intensive Insulin

Therapy (see inclusion criterion 7 in the protocol). The latter group may be very small. However, if there are at least 6 subjects in each group, we will perform subgroup analyses that examine these two subgroups separately. Analyses will include all primary and secondary outcome measures and use the same analysis strategies that were used for those measures. Please note that power for these analyses will be very small but the results may lead to a better understanding of the mechanisms of outcome.

4. Sample Size and Power Calculations

Sample sizes were calculated for the primary endpoint as a composite dichotomous variable as planned in the study: "HbA1c $\leq$ 6.5% and an absence of severe hypoglycemic events at 1 year after the first islet transplant or a reduction in HbA1c of 1 point and an absence of severe hypoglycemia at 1 year after the first islet transplant ".

The DCCT trial (The_Diabetes_Control_and_Complications_Trial_Research_Group, 1993) provided the consortium longitudinal data on 711 diabetic subjects who had been under intensive monitoring of HbA1c for at least 16 months. For our purposes, we assumed that enrolled patients for this trial will undergo similar intensive insulin therapy for their diabetes (IIT) as was done in the DCCT and that a subject would be eligible for participation in CIT-06 if her/his average value of HbA1c at months 3 and 4 was greater than 7.0% Using this criterion, 390 of the 711 DCCT subjects would have qualified. Among those DCCT patients who would be eligible for CIT-06 the observed favorable outcome rate based on 12 months follow-up was 27% (101/373, se=2.2). Since data on hypoglycemic events was not available in the DCCT data, this favorable outcome rate overestimates the true rate in the IIT group. The projected sample size will therefore be adequate. Because our patients have long standing type I diabetes and have already experienced kidney failure, it is likely that they will have more severe diabetes than those enrolled in the DCCT and that their experience with IIT will be less favorable. In addition, our information from the DCCT calculations does not include information on hypoglycemic events which was common in the DCCT patients (ref). For both of these reasons, we believe that the sample size and power calculations that are reported below are likely to be adequate.

Table 3.1 provides sample size calculations that use 27% as an estimate of the true favorable outcome rate in the IIT group. Sample sizes for alternative rates are also displayed. These were selected by using 23% (27%-2 times standard error) and 31% (27% + 2 times standard error) where the standard error is the standard error of the estimate from the DCCT study. Sample size calculations assume that an exact one sided test will be used and were calculated using the PASS (Hintze, 2011) sample size program. This table displays the sample sizes required for a one-sided 0.05 binomial test to achieve 80% and 90% power to detect true favorable outcome rates of 40%, 45%, 50%, 55%, and 60% in transplanted subjects compared to the selected rates in the IIT group. If the true favorable outcome rate at one year in IIT subjects is 27% then we would need to transplant 27 subjects to achieve 90% power to detect an improvement to 55% in transplanted subjects. We would need 19 subjects to achieve 80% power to detect 55% versus 27%. We

would need to transplant 40 subjects to ensure 90% power and 27 subjects to ensure 80% power if the true IIT rate is 31% and the true rate in islet transplant subjects is 55%.

Table 4.1: Sample sizes required to achieve 80% and 90% power

Rate in Transplanted Subjects	Power	Historic Rate - IIT		
		23%	27%	31%
40%	90%	64	113	246
	80%	46	84	175
45%	90%	39	64	105
	80%	31	46	76
50%	90%	28	39	59
	80%	21	30	43
55%	90%	21	27	40
	80%	15	19	27
60%	90%	15	19	27
	80%	12	16	17

We have chosen to transplant 24 subjects. This will provide approximately 90% power to detect a difference of 57% favorable outcome rate in islet transplanted subjects versus the estimated 27% favorable outcome rate in the DCCT population. Table 3.2 displays minimal detectable differences for sample sizes of 18, 24 and 30 subjects. With 24 subjects the smallest difference that can be detected with 90% power by a one-sided 5% level test is 57% compared to 27%. The minimal detectable difference is 52% compared to 27% with 80% power.

Table 4.2: Minimal detectable differences if the true IIT rate is 27%

	Power	
	90%	80%
18	62%	57%
24	57%	52%
30	53%	49%

Table 3.3 summarizes data provided by clinician investigators in Europe and North America. GRAGIL, CITR, and the Nordic Group reported data for HbA1c levels at one year after first transplant but did not report data on hypoglycemic events. Rates for HbA1c $\leq$ 6.5% varied from 50% to 83.3%. Overall, 70% (33 of 47) of islet transplanted patients from the three studies reporting 12 month HbA1c values would have satisfied the HbA1c requirement for a favorable outcome one year after their first islet transplant (95% confidence interval 57% to 83%). These rates do not include those whose HbA1c levels decreases by 1% or more at one year but did not reach 6.5%. While these reports do not give the incidence of hypoglycemia in these patients, the Edmonton group has reported that the incidence of hypoglycemic events in those with a successful islet transplant is very low. The lower portion of Table 3.3 provides HbA1c data on potentially eligible subjects identified during preliminary screening at several CIT clinics. Note that the baseline HbA1c levels are similar to those from the studies that present outcome data. These data argue that we can expect to observe favorable outcome rates that are not lower than those observed by the three groups.

We expect to observe a success rate greater than 55%. The proposed IAK study has approximately 90% power to detect this difference.

Table 4.3: HbA1c levels and favorable outcomes reported by investigators in Europe and North America

Reporting Group	Occasion	Number of transplanted subjects	HbA1c % (mean±sd)	HbA1c ≤ 6.5 one year after first transplant
GRAGIL	Pre transplant	8	7.7±0.6	50% (3/6)[1]
CITR[2]	Pre transplant	12	NR[3]	83.3% (10/12)
Nordic Group[2]	Pre transplant	29	9.0±1.7	69.0% (20/29)
	Post transplant	29	7.4±1.5	
Maffi	Pre transplant	54	8.1±1.6	NR
	Post transplant	46	7.5±1.2	
Giessen[4]	Pre transplant	21	8.7±0.4	NR
	Post transplant	21	7.1±0.3	
HbA1c levels reported by CIT Investigators				
Edmonton	Pre transplant	8	9.3±2.0	
Pennsylvania	Pre transplant	38	8.6±1.7	
Northwestern	Pre transplant	9	6.9±1.6	
Miami, Pennsylvania, and Northwestern	Change pre transplant to 12-months post transplant	16	-1.5±1.8	

[1] HbA1c < 6.5% but hypoglycemia events not reported

[2] The CITR has data on 17 IAK patients who are at least one year post first transplant.

[3] NR=not reported

[4] Both IAK and SIK reported

4.1.1 Precision of Estimates of Secondary Endpoints

There is no longitudinal data on our secondary endpoints so it is not possible to provide precision estimates for those outcomes for which change from baseline will be analyzed. However, data are available for cross-sectional means and standard deviations for our secondary endpoints. Table 3.4 provides estimates of the precision of estimates of the means for each secondary endpoint based on half the width of a 95% confidence interval (approximately 0.4 standard deviations). That is, we can say with 95% confidence that the true value of the parameter will be no further from the observed estimate than the value reported as the precision estimate.

Table 4.4 Estimated Precision for QOL *Outcomes*

Outcome Category	Outcome	Mean	Standard Deviation	Precision
Metabolic	HbA1c	6	0.7	0.28
	c-Peptide	2	0.7	0.28
	MMTT	133	55	22
	Hypo Score	850	750	300
	MAGE	8	4	1.6
	LI	223	200	80
SF-36 Health Survey	Physical Functioning	96	6	2.4
	Role-Limitations Physical	83	22	8.8
	Role-Limitations Emotional	82	18	7.2
	Bodily Pain	74	14	5.6
	Social Functioning	84	15	6.0
	Vitality	54	17	6.8
	Mental Health	60	13	5.2
	General Health	75	16	6.4

Outcome Category	Outcome	Mean	Standard Deviation	Precision
	Physical Component Summary	56	5	2.0
	Mental Component Summary	43	8	3.2
EQ-5D(VAS)		83	13	5.2
Hypoglycemia Fear Survey	Behavior	6	11	4.4
	Worry	5	12	4.8
Diabetes Distress Scale	Emotional Burden	24	25	10
	Physician-Related Distress	0	0	
	Regimen-Related Distress	14	22	8.8

5. General Analysis Definitions

5.1 *Study Period and Visit Window Definition*

5.1.1 Study Period

The trial consists of three periods: (1) the pre-transplant period, which includes screening, enrollment, and wait list time; (2) the period that includes the islet transplant procedure(s); and (3) follow-up visits through 24 months following the last transplant. The study period of this trial is a 36-month follow-up after the final islet transplant. Subjects may undergo up to 3 transplants in the course of this study; the last transplant can occur not later than 8 months following the first transplant.

In the first period, individuals who meet the general inclusion criteria will be approached regarding participation in the study. After informed consent has been obtained, they will be formally enrolled into the study. Eligibility will be confirmed based on the results of the screening visit procedure detailed in Appendix 1 of the CIT-06 protocol. Enrolled subjects who meet the eligibility screening for the studies will be put on the waiting list for an islet transplant.

After completion of the screening assessments confirming eligibility for the study, a subject will undergo 12 months of intensive insulin therapy (IIT) by an experienced diabetologist (see Appendix 3). If a subject received at least 12 months of **intensive diabetes management** prior to enrollment and experienced at least on episode of **severe hypoglycemia** during that time, the subject would not be required to receive IIT while on study as long as they have a Clarke score of 4 or more. A subject is required to return to the study site for clinic visits every 3 months while undergoing IIT and while on the waitlist. Monthly HbA1c sampling between the required 3 month interval visits may be drawn locally, sent from the local lab to the study site and then shipped to the central lab for testing. Eligibility will be reconfirmed after 4 months of IIT and after 12 months of IIT. All eligibility assessments must be within the windows required for initial study enrollment. Once eligibility has been reconfirmed, a subject will be placed on the **wait list**. The subject will continue IIT while on the **wait list**.

Once a compatible islet preparation becomes available, a subject's eligibility will be re-confirmed and eligible subjects will begin immunosuppression therapy on Day -2 (Day 0 is defined as the day of transplant).

During the post-transplant follow-up period, subjects may receive up to two additional transplants. After receiving his/her first islet transplant, if a subject does not meet the criteria for insulin independence described in Study Definitions in the CIT-06 protocol, but has either a basal or stimulated C-peptide level ≥ 0.3 ng/mL (0.1 nmol/L), s/he will be considered for a second islet transplant. A subject who does not meet this criterion will be considered a transplant failure. A second islet transplant will be considered at 30 days but before 8 months after the first islet transplant and when all required metabolic assessments are complete. If, after the second islet transplant, both basal and stimulated C-peptide levels remain <0.3 ng/mL (0.1 nmol/L), the

recipient will be considered to have failed the endpoint, and immunosuppression will be managed as described in the protocol. A third islet transplant will be considered only if all the criteria described in Section 7.6 of the CIT-06 protocol are met. Islet transplant recipients who have completed 8 months of follow up after their first infusion will no longer be eligible for additional islet transplants under the CIT-06 protocol.

5.1.2 Visit Windows

The number of visits that occur before the first islet transplant will be determined by time on the waiting list and cannot be determined in advance. Screening tests and baseline measurements that are obtained during this period must be obtained within specified windows relative to the day induction immunosuppression is initiated, as described in Appendix 1 of the CIT-06 protocol (section 7). Following each transplant, up to 23 visits may be scheduled. Subsequent transplants restart the clock at day 0. Table 4.1 describes all visits, their scheduled times relative to the islet transplant, and the allowable visit windows.

Table 4.1 - Study assessment time points and visit windows

Time Points (days relative to transplant)	Visit Number	Visit Window (days around target)	Equivalent (weeks or months)
Screening	01	N/A	N/A
IIT ≥ 12 months	02		
Waitlist/Baseline	03	N/A	N/A
Baseline	04		
0	05	N/A	N/A
3	06	N/A	N/A
7	07	±3	W1
14	08	±3	W2
21	09	±3	W3
28	10	±3	W4
56	11	±7	M2

Time Points (days relative to transplant)	Visit Number	Visit Window (days around target)	Equivalent (weeks or months)
75	12	±5	M2.5
180	13	±7	M6
270	14	±14	M9
365 after most recent transplant	15	±14	M12
Y1 365 days after first transplant	Y1	±14	M9
M 15	16	±14	M15
M 18	17	±14	M18
M 21	18	±14	M21
M24 after most recent transplant	19	±14	M24
Y2 730 days after first transplant	Y 2	±90	Y2 After first transplant
M27	20	±14	M27
M30	21	±14	M30
M33	22	±14	M33
M36 after most recent transplant	23	±14	M36
Y3 1095 days after first transplant	Y 3	±90	Y3 After first transplant

This follow-up schedule will restart with visit 05 on the day that a subsequent islet transplant is performed. Yearly visits occur after the first transplant. The detailed activities for each scheduled follow-up visit are described in Appendix 1 of the CIT-06 protocol.

5.2 *Study Populations*

The study population consists of individuals with T1D who have had a kidney transplant and who meet the eligibility criteria for the trial described in Section 3 of the CIT-06 protocol. This section of the SAP describes three study populations. All efficacy analyses will be done on the ITT population. Parallel analyses will be done on the per-protocol population. Safety analyses will focus on the safety population.

5.2.1 Intent-to-Treat Population

All efficacy and safety analyses will be based on a modified ITT principle: any subject in whom protocol-directed therapy (*e.g.*, induction immunosuppression) is initiated will be included in the ITT population. Subjects who are transplant eligible but for whom a protocol directed therapy is not initiated will be listed in the final study report but will not be included in the ITT population.

A subject who is transplant eligible but never receives protocol-directed therapy will not be included in the analysis.

5.2.2 Per-Protocol Population

A per-protocol analysis will include all subjects in whom the islet transplant procedure is initiated. The procedure will be considered initiated when the operator (*e.g.*, surgeon or interventional radiologist) has started the process of obtaining access to the portal vein (*i.e.*, entered the body with a needle or scalpel).

5.2.3 Safety Population

The safety population consists of any subject in whom protocol-directed therapy (*e.g.*, induction immunosuppression) is initiated. Subjects in this population might not receive an islet transplant.

5.3 *Treatment Assignment and Treatment Groups*

5.3.1 Treatment Assignment

This is a single-arm study. All eligible subjects for whom an acceptable isolation is identified will receive at least one islet transplant. Enrolled subjects who meet the eligibility criteria will be placed on a waiting list for a transplant. Once a compatible pancreas becomes available, the subject will be reevaluated to ensure that s/he satisfies all inclusion/exclusion criteria and therefore is still eligible for CIT-06.

5.3.2 Treatment Groups

The treatment in CIT-06 is the islet transplant and its associated immunosuppression. This is a single arm, open label trial and everyone receives the same study treatment.

5.3.3 Center Pooling Method

The primary analysis assumes no between-center variability with regard to the favorable outcome resulting from the treatment. The data from all centers will be pooled without any adjustment for centers.

6. Subject Disposition

The number of subjects enrolled and treated will be summarized and reported in the following categories:

- The numbers of subjects who are screened - total and grouped by center.
- The numbers of subjects who are excluded from study participation - total and grouped by center. The numbers will also be tabulated by the reason for exclusion.
- The numbers of subjects who are enrolled (sign informed consent for screening) - total and grouped by center.
- The numbers of subjects who are eligible for the transplant both after the enrollment and completion of IIT and before the transplant, grouped by center.
- The numbers of subjects who are lost to follow-up, grouped by center and reason. (The rules for premature termination of study treatment are fully described in Section 5.7.3 of the CIT-06 protocol).

A list of all enrolled subjects (grouped by center) who are prematurely terminated from the study (withdraw consent or are lost to follow-up) will be provided. The list will give subject identification, the specific reason for termination, immunosuppression regimen and the duration of treatment before the termination.

7. Protocol Deviations

Major protocol deviations will be summarized by center and for the total study and grouped into the following categories:

- Impacts the inclusion and/or exclusion criteria (PD1)
- Involves consent violations (PD2)
- Alters protocol-specified study therapy (PD3)
- Impacts the ability of the Sponsor to evaluate the endpoints of the study (PD4)
- Involves administration of prohibited medications (PD5)

The template of summary tables for the protocol deviations is provided in Appendix 6. Individual subjects with these protocol deviations will be listed with specifics on the deviation.

8. General Analyses Methods

In general, CIT-06 analyses will fall into two categories: analyses for continuous outcome variables and analysis for binary outcome variables.

8.1 *Analysis of continuous outcome variables*

Continuous scale variables will be displayed as mean, standard deviation, median, minimum and maximum. The Shapiro-Wilk test (Shapiro & Wilk, 1965) will be used to test the normality of this continuous variable. If the hypothesis of normally distributed data is not rejected at the 0.05 significance level, the usual normal 95% confidence interval $\bar{x} \pm t_{0.025}^{n-1} \times \frac{s_x}{\sqrt{n}}$ will be constructed for the true mean μ. If the data are not normally distributed, the transformations $y = f(x) = \log x, \sqrt{x}$ and $1/x$ of these variables will be examined sequentially until we fail to reject the hypothesis of normality using the Shapiro-Wilk test at the 0.05 significance level. If a transformation is identified such that normality holds then the sample mean $\bar{y}$ and sample standard deviation s_y will be calculated for the transformed variable. The 95% confidence interval will be constructed as

$$\left[f^{-1}\left(\bar{y} - t_{0.025}^{(n-2)} \frac{s_y}{\sqrt{n}} \right), f^{-1}\left(\bar{y} + t_{0.025}^{(n-2)} \frac{s_y}{\sqrt{n}} \right) \right].$$

If these transformations fail to achieve normality, the bootstrap method will be adopted. One thousand bootstrap samples from the original data set with replacement are obtained. For each bootstrap sample, the sample mean is calculated. The 2.5 and 97.5 percentiles, $p_{0.025}$ and $p_{0.975}$, will be identified from the 1000 bootstrap sample means. The interval $\left[p_{0.025}, p_{0.975} \right]$ will reported as the bootstrap 95% confidence interval for the true mean.

8.2 *Analysis of binary outcome variables*

Binary outcome variables will be displayed as percents with 95% exact confidence intervals (Rosner, 1995). The exact confidence interval (P_L, P_U) will be as the solutions to the following equations. If x is the observed outcome (that is, there are x successes in the 24 subjects) then the lower bound will be the solution P_L *to* the equation

$$0.025 = \Pr\left\{ \text{at least x successes} \middle| \text{probability of success} = p_L \right\} = \sum_{k=x}^{24} \binom{24}{k} p_L^k \left(1 - p_L\right)^{24-k}.$$

The upper bound will be the solution to the equation

$$0.025 = \Pr\left\{\text{no more than x successes} \middle| \text{probability of success} = p_U\right\} = \sum_{k=0}^{x}\binom{24}{x} p_U^k \left(1-p_U\right)^{24-k}.$$

Standard search methods will be used to solve these equations.

8.3 *Analysis of time to event measures*

All time to event measures will be summarized using Kaplan-Meier (product limit) life table methods. Results will be displayed as survival plots. Tables with estimates and 95% confidence intervals for the rates at 1-year, 2-years, and 3-years after the first and last islet transplants will be provided. Separate analyses will be performed for time from first transplant and time from last transplant.

9. Analysis of Demographic and Baseline Measures

9.1 *Baseline Data*

Baseline data collected for the ITT analysis consists of demographic information and medical/physical assessments during the waiting period for islet transplant. These data will be grouped into the following categories:

- Demographic variables
  - Age
  - Sex
  - Race (White, Black, Hispanic, Asian and Other)
- Body Habitus and Quality of Life
  - Body Weight
  - Height
  - Body Mass Index (BMI)
- Diabetes Control
  - Insulin requirement (units/day)
  - HbA1c
  - Fasting and post prandial plasma glucose
  - Fasting and post prandial C-peptide
  - 90-minute C-peptide following consumption of Boost® or equivalent
  - 90-minute glucose following consumption of Boost® or equivalent
  - Number of severe hypoglycemic events in the last year
  - MAGE score
  - LI
  - Clarke Score
  - HYPO score
  - β-score
  - C-peptide/(glucose·creatinine) ratio

- Measures of Cardiovascular function
    - Carotid intimal thickness
    - Atherogenic profile (fasting lipids, c-reactive protein, serum amyloid A, Apolipoprotein A1, Apolipoprotein B, and the ratio of Apolipoprotein A1 and Apolipoprotein B).
- Measures of Renal function
    - Serum Creatinine
    - Spot urine albumin creatinine ratio, and protein excretion.

- QOL Measures (defined in Protocol Section 9.1.10)
    - SF-36
    - EQ-D5
    - Diabetes Distress Scale

9.2 *Analysis of Baseline Data*

The number of subjects who do not meet the eligibility criteria will be reported, grouped by center. The numbers will be further broken down by the reasons for exclusion. These numbers and the corresponding rates will be reported in the summary table provided in SAP Appendix 7.

Descriptive statistics of baseline data, grouped by center, will be presented in a summary table. Continuous data will be summarized by mean, standard deviation, median, minimum, and maximum. Categorical data will be presented as numbers and percentages. The template of this table is described in SAP Appendix 1. Since the number of subjects transplanted in each center is small, there will be no significance tests for the differences of the baseline data among the seven centers.

9.2.1 Analysis of demographic variables

9.2.1.1 Age

The distribution of age at baseline will be displayed as mean, standard deviation, median minimum and maximum.

9.2.1.2 Sex and race

Sex and race will be displayed as counts and percents.

9.2.2 Analysis of Baseline Diabetes Control Variables

Diabetic control variables include insulin requirement, fasting and post-prandial plasma glucose and C-peptide, glucose and C-peptide 90 minutes post Boost® (or equivalent) glucose challenge, number of hypoglycemic events in the last year, MAGE score, LI, Clarke score, HYPO score, β-score, and C-peptide/(glucose·creatinine) ratio (the ratio of C-peptide to the product of glucose and creatinine).

The distribution of number of hypoglycemic events in the last year will be presented in tabular form. The remaining variables are ordinal scale and will be analyzed as described for continuous variables.

9.2.3 Analysis of Baseline Body Habitus Variables

Body habitus variables include height, weight, and body mass index (BMI). BMI is computed as the ratio of weight to the square of height (kg/m^2). All three variables are continuous scale and will be analyzed in the same manner as described for continuous variables.

All QOL scales are continuous and will be analyzed in the manner described for the continuous scale variables. Each scale will be tabulated as mean, standard deviation, median, minimum and maximum. 95% confidence intervals will be computed in the manner described for continuous variables.

10. Analysis of Efficacy Measures

10.1 *Analysis of Primary Endpoint*

HbA1c is the standard measure of glucose control and is used in all major studies as an endpoint for glycemic control. It has been valuable as a risk predictor of diabetes complications. However, since HbA1c is an integrated average, it does not provide information about the range of glucose values a subject experiences. This limitation is a rationale for also including hypoglycemic event occurrence as part of the primary endpoint and various glycemic excursion measures as secondary endpoints.

The primary endpoint (as defined in Section 2.3.1) is the proportion of subjects with both an HbA1c $\leq$ 6.5% and an absence of severe hypoglycemic events at 1 year or a reduction in HbA1c of 1 point and an absence of severe hypoglycemia at 1 year after their first transplant. For brevity in the following discussion, we will denote this outcome as "favorable outcome at one year".

The primary analyses will be an intention to treat analysis. It is expected that some subjects will be screened and found eligible but will never receive an islet transplant either because a compatible isolation never becomes available, because the subject decides to no longer participate in the study, or because some other event precludes them from participation. The intention to treat population will be defined as all subjects for whom induction antibody therapy for an islet transplant is begun. In addition, a per-protocol analysis will include all subjects in whom the islet infusion procedure is initiated. The procedure will be considered initiated when the operator has started the process of obtaining access to the portal vein (*i.e.*, entered the body with a needle or scalpel).

The primary aim of the analysis is to estimate the true rate of favorable outcome at one year after their first transplant in all eligible subjects for whom induction for an islet transplant is begun. The observed favorable outcome rate and a one-sided 95% exact binomial confidence interval will be used to estimate the true favorable outcome rate. The primary analysis will compute a one-sided exact binomial test of the null hypothesis that the true rate is less than or equal to 27% versus the alternative that the true rate exceeds 27% (see sample size justification). The analysis will be considered to support efficacy if the null hypothesis is rejected at the 5% level of significance. The lower bound for the confidence interval p_L will be determined as the value that satisfies the following equation:

$$0.05 = \sum_{x \geq r} \binom{24}{x} p_L^x \left(1 - p_L\right)^{24-x}$$

The rates of favorable outcome and exact one-sided 95% confidence intervals will be computed for each contributing center.

This analysis will be conducted for the ITT population. The primary endpoint should be available for all treated subjects. An exception will be if a death occurs, if the subject withdraws consent to be followed, or if immunosuppression is begun but the subject never receives a transplant. In these cases the endpoint will be classified as failure to achieve a favorable outcome. Should the endpoint not be evaluated for a particular individual for other reasons, a failure will be imputed unless an evaluation is done at a time longer than one year after transplant, in which case, that later value will be imputed. All imputations will be reported with the primary analysis. The rates and the exact one-sided 95% confidence intervals for complete data and imputed data will be compared to ascertain the sensitivity of the imputation.

10.2 *Analyses of Secondary Endpoints*

Except for the primary analyses there are no explicit hypotheses in the protocol. Changes in the secondary outcomes are of interest as they will relate to efficacy as measured by the primary outcome variable. All analyses are descriptive and are intended to document the changes in these important variables. Secondary outcomes will be used to support the decision for efficacy of islet transplantation in this population but are not intended to be used explicitly for making a decision for the efficacy of islet transplantation in this population.

10.2.1 Analysis of Key Secondary Endpoints

Because this is a single intervention study, the tests will be one sided tests for whether the true rates are greater than the endpoint's' predetermined "minimum rate for efficacy". These minimum rates were determined by the investigators to be large enough to have credibility for the islet transplant community. The minimum rate for efficacy is provided in Table 10.1 for each of the key secondary outcomes.

Table 10.1: Key Secondary Outcomes and Minimum Rates for Efficacy

	Key Secondary Outcome	Minimum Rate for Efficacy
1	At 365 ± 14 days after the *last* islet transplant (the primary at 365 days after the last transplant): The proportion of subjects with both an HbA1c ≤ 6.5% and an absence of severe hypoglycemic events from Day 28 to Day 365, or a reduction in HbA1c of 1 point and an absence of severe hypoglycemia from Day 28 to Day 365.	50%
2	At 365 ± 14 days after the *first* islet transplant: The proportion of subjects with HbA1c < 7.0% and free of severe hypoglycemic events from Day 28 to Day 365.	50%
3	At 365 ± 14 days after the *first* islet transplant: The proportion of subjects free of severe hypoglycemic events from Day 28 to Day 365.	50%
4	At 365 ± 14 days after the *first* islet transplant: The proportion of subjects with HbA1c < 7.0%.	50%
5	At 365 ± 14 days after the *first* islet transplant: The proportion of subjects with HbA1c ≤ 6.5%.	50%
6	The proportion of subjects with a reduction in HbA1c of at least 1 point	50%
7	At 365 ± 14 days after the *first* islet transplant: The proportion of insulin-independent subjects.	20%
8	At 365 ± 14 days after the *first* and *last* islet transplant: The change from baseline in the Clarke score.	Favorable increase of 1 point

As with the primary endpoint, the key secondary endpoints should be available for all transplanted subjects and the analysis will be conducted for the ITT population. If an endpoint is

not available for a transplanted subject then it will be imputed using the same rules that were used for the primary endpoint. That is the measure will be imputed as a failure unless later data would make the outcome a success.

For each of the binary outcomes (1-7), the observed rate for each key secondary outcome will be used as the point estimate. The analysis will also compute an exact binomial one-sided test for the null hypothesis that the true rate of the outcome is less than or equal to the predetermined minimum rate for efficacy against the alternative that the true rate exceeds the minimum rate for efficacy. Change from baseline in the Clarke score will be analyzed as a continuous variable.

We will use the Benjamini and Hochberg (Benjamini & Hochberg, 1995) method to account for the multiplicity of the key secondary tests. This method controls the false discovery rate (FDR) rather than the more familiar family-wise error rate (FWER). It provides a powerful approach for identifying those positive tests that are not likely to be true while controlling a reasonable measure of the expected number of false positive tests (the FDR).

The multiple testing procedure considers testing m hypotheses $H_1, H_2, \ldots, H_m$. Each test yields the corresponding p-values $P_1, P_2, \ldots, P_m$. Let $P_{(1)} \leq P_{(2)}, \ldots, \leq P_{(m)}$ be the ordered p-values, and let $H_{(i)}$ denote the null hypothesis corresponding $P_{(i)}$. The Bonferroni-type multiple testing procedure is defined by the following:

Let q^* the maximum false discovery rate and let k be the largest i for which $P_{(i)} \leq \frac{i}{m} q^*$; then

reject all $H_{(i)}$ $i=1,2,\ldots,k$.

For this study there are 8 key secondary endpoints so m=8 and we will fix q^* at 0.1.

For each key secondary outcome, the observed rate will be used as the point estimate. The rate and a 95% exact one-sided confidence interval will be reported along with the p-value from an exact one-sided test of the corresponding null hypothesis.

10.2.2 Additional Efficacy Endpoints

Excluding the first key secondary endpoint, the additional efficacy endpoints are just the key secondary endpoints measured at one year, two years, and three years after the first and after the last islet transplant. They will be analyzed in exactly the same way as the key secondary endpoints but no adjustment will be made for multiple comparisons. The secondary endpoints that are not directly related to the primary and key secondary efficacy endpoints are discussed in detail in the following sections.

10.2.3 Additional Post-Hoc Analyses

The HbA1c component of the composite primary endpoint for CIT-06, aimed at controlling HbA1c levels at or below 6.5% or reducing HbA1c levels by 1 percentage point, is based on a minimum rate for efficacy of 27%. This is in contrast to the HbA1c component of the primary endpoint for the CIT-07 trial, aimed at controlling HbA1c levels at or below 7.0%, which is based

on a minimum rate for efficacy of 50%. There are several CIT-06 key secondary outcome measures that, while assessing HbA1c levels at the 6.5% threshold or the 1 percentage point decrease threshold, still use the CIT-07 minimum rate for efficacy of 50%. The study team found the SAP lacking in that it never stipulated that these key secondary outcomes also be evaluated at the minimum rate for efficacy for the CIT-06 primary outcome, 27%. Therefore, we propose to add the three corresponding analyses (from Table 10.1) to the analysis plan in a post-hoc manner. That is, they will not be considered key secondary outcomes, which would otherwise impact the pre-specified plan for adjusting for multiple key secondary comparisons, and they will not replace the current key secondary outcomes, but rather they will be considered additional post-hoc comparisons.

The following table describes the additional post-hoc analyses in terms of Table 10.1, where the additional analyses are broken out from the key secondary analyses table.

Table 10.2 Additional Post-Hoc Analyses

	Additional Post-Hoc Analyses	Minimum Rate for Efficacy
1	At 365 ± 14 days after the *last* islet transplant (the primary at 365 days after the last transplant): The proportion of subjects with both an HbA1c ≤ 6.5% and an absence of severe hypoglycemic events from Day 28 to Day 365, or a reduction in HbA1c of 1 point and an absence of severe hypoglycemia from Day 28 to Day 365.	27%
5	At 365 ± 14 days after the *first* islet transplant: The proportion of subjects with HbA1c ≤ 6.5%.	27%
6	The proportion of subjects with a reduction in HbA1c of at least 1 point	27%

11. Analysis of metabolic outcomes

Separate identical analyses will be reported for outcomes measured at one year after the first and after the last islet transplant, at two years after the first and after the last islet transplant, and at three years after the first and after the last islet transplant.

11.1 *The percent reduction in insulin requirement*

Subjects will record their total daily insulin dose on self-monitoring diaries. The percent reduction from the baseline insulin requirement ((follow-up minus baseline) divided by baseline) will be computed for each subject for each time point. The analysis of the reduction percentage will use the method described above for a continuous variable.

11.2 *HbA1c*

The primary endpoint requires that either HbA1c at one year after the first transplant is less than or equal to 6.5% or has dropped at least one percent. A secondary endpoint requires that HbA1c is less than 7.0%. The analyses will present the observed percent and 95% confidence intervals for the true percent for the three outcomes: (1) the proportion of subjects with HbA1c $\leq$ 6.5%, (2) the proportion of subjects with HbA1c <7.0%, and (3) the proportion of subjects with a drop of at least 1.0%.

Additional analyses of HbA1c will adopt the method for a continuous scale variable. Data will be displayed as mean, standard deviation, minimum and maximum. In addition, 95% confidence intervals will be computed as described in the methods section. Change from baseline will be computed and analyzed using the same methods.

11.3 *Mean amplitude of glycemic excursions (MAGE)*

The MAGE requires capillary glucose readings over two consecutive days (a minimum of four readings a day), and is defined as the arithmetic mean of blood sugar increases (or decreases) when both increases and decreases (or vice-verse) at subsequent points in time are greater than 1 standard deviation of the blood sugar for the same two day period (Service, Molnar, Rosevear, Ackerman, Gatewood, & Taylor, 1970). If the MAGE is $\geq$11.1 mmol/L, the subject is considered to have labile diabetes.

The first analysis for this variable directly uses MAGE and adopts the method for a continuous scale variable. In addition, subjects will also be categorized into two groups depending on whether or not the MAGE $\geq$ 11.1 mmol/L. The analysis of the rate of labile diabetes will be displayed using the methods described for a binary outcome variable.

11.4 *Glycemic lability index*

LI is a measure of blood glucose lability which is based on the change in glucose over time. The LI requires 4 or more daily capillary blood glucose (BG) measurements over a 4 week period.

For each week, the sum of the squared differences in consecutive glucose readings is divided by the hours apart the readings are determined. Only differences in time that are greater than or equal to 1 hour and less than or equal to 12 hours are used in calculations. This sum is calculated for each of the four weeks and the LI is the mean of the four weekly values (Ryan, Shandro, K, & al., 2004), that is:

$$\text{LI}=\frac{1}{4}\times\sum_{i=1}^{4}\sum_{j=1}^{N_i}\frac{(\Delta Gluc_{i,j})^2}{\Delta h_{i,j}}$$

where $\Delta Gluc_{i,j}$ is the j^{th} eligible difference of glucose readings in the i^{th} week, $\Delta h_{i,j}$ is the time interval in hours for the j^{th} eligible difference of glucose readings in the i^{th} week. N_i is the total number of the eligible differences of glucose readings in the i^{th} week.

Most subjects have scores under 300 mmol/L^2/h·wk^{-1} with a median of 223 (25 – 75$^{\text{th}}$ percentiles 130 – 329 mmol/ L^2/h·wk^{-1}). An LI ≥433 mmol/ L^2/h·wk^{-1} (90$^{\text{th}}$ percentile) indicates serious problems with glycemic lability. The first analysis for this variable directly uses LI and adopts the method described for continuous scale variables. Change from baseline will also be computed and analyzed using the same methods.

Subjects will also be categorized into two groups depending on whether or not the LI ≥433 mmol/ L^2/h·wk^{-1}. The analysis of the rate of serious problems with glycemic lability will also be analyzed using the method for binary outcomes.

11.5 *Ryan hypoglycemia severity (HYPO) score*

The HYPO score involves subject recording of blood glucose readings and hypoglycemic events (BG <3.0 mmol/L [54 mg/dL]) over a 4-week period and recall of all severe hypoglycemic episodes in the previous 12 months. The HYPO score is a scalar quantity based on the severity of hypoglycemic events over a four week period. A hypoglycemic event occurs when a blood sugar reading is less than 54 mg/dL and a series of self-reported questionnaire items determine the severity. The HYPO score is the sum of points awarded to each hypoglycemic event, where a large HYPO score indicates more severity (Ryan, Shandro, K, & al., 2004).

A HYPO score greater than or equal to the 90$^{\text{th}}$ percentile (1047) of values derived from an unselected group of type 1 diabetic subjects indicates severe problems with hypoglycemia

The analysis of the raw HYPO score adopts the method for continuous scale variable. Change from baseline will also be calculated and analyzed using the same method.

Subjects with HYPO scores greater than or equal to 1047 will be classified as having severe problems with hypoglycemia, and the analysis of the true rate of this event adopts the method for binary outcome variables.

11.6 *Clarke Score*

The Clarke survey (Clarke, Cox, Julian, Schlundt, & Polonsky, 1995) provides a total score between 0 and 7 (most severe), where scores of 4 or more indicate reduced awareness of hypoglycemia and increased risk of severe hypoglycemic events.

The analysis will treat this variable as continuous and adopts the method for a continuous variable. Change from baseline will also be calculated and analyzed using the same method. The subject will be considered to have reduced awareness if the score is greater than or equal to 4. The rate of reduced awareness will be analyzed using the method for a binary outcome variable.

11.7 *Basal (fasting) and 90-min glucose and C-peptide derived from the mixed-meal tolerance test*

The partial graft function of islet transplantation is indicated by continued C-peptide production. Basal and 90-min glucose and C-peptide derived from the MMTT will be measured at each time point. The glucose levels (fasting and 90 minute) are continuous variables and will be analyzed using the method for continuous scale variables. Change from basal to 90 minutes will be calculated and analyzed using the same methods. Change from baseline for basal, 90 minutes and the difference between 90 minutes and basal will also be analyzed using the same methods.

11.8 *β-score*

The β-score (Ryan, Paty, Senior, Lakey, Bigam, & Shariro, 2005) is an assessment of β-cell graft function after islet transplantation and is treated as a continuous variable and ranges from 0 (no graft function) to 8.

The β-score is generated from a composite scoring system based on fasting plasma glucose values (mmol/L), HbA1c(%), daily insulin consumption (units/kg) or oral hypoglycemic agents use, and stimulated C-peptide levels (nmol/L). For each of these measures, scores of 0, 1, and 2 are assigned to abnormal, intermediate, and normal values, respectively. The β-score is then calculated as the sum of the four scores.

The β-score will be analyzed using the method for a continuous variable.

11.9 *C-peptide/(glucose·creatinine) ratio*

This measure accounts for both the dependence of C-peptide secretion on the ambient glucose concentration and the dependence of C-peptide clearance on kidney function (Zavaroni, Deferrari, Lugari, & al, 1987) (Zerboni, Mangili, & Luzi, 1999). This ratio will be treated as a continuous variable. The analysis of this variable adopts the method for a continuous variable.

11.10 *Acute insulin response to glucose (AIRglu), insulin sensitivity, and disposition index derived from the insulin-modified frequently-sampled intravenous glucose tolerance (FSIGT) test*

The insulin modified FSIGT test will be performed at 75±5 days following the first and final infusion (if applicable). AIR_{glu}, SI and DI, derived from the test, provide a composite measure of β-cell function. AIR_{glu} is calculated as the incremental area-under-the-curve for insulin between 0 and 10 minutes post injection. SI, a measure of insulin-dependent glucose disposal, is derived from Bergman's minimal model using MinMod Millenium software (Pacini & Bergman, 1986) (Saad, Anderson, Laws, & al, 2004). The DI is calculated by AIR_{glu}*SI. The three variables are all continuous and will be analyzed using the method for a continuous variable.

11.11 *Glucose variability and hypoglycemia duration derived from the continuous glucose monitoring system (CGMS)*

CGMS involves the subcutaneous placement of a glucose sensor connected by tubing to a pager-sized monitoring device that stores glucose data obtained every 5 minutes over a 72-hour period (Steja, 2005). Data from a 72-hour period near the target time point will be used to derive the glucose variability and hypoglycemia duration.

The glucose variability is the standard deviation of all measured glucose values and is a continuous variable. This variable will be analyzed using the method for a continuous variable. The data from the 72-hour period are used to derive the number and duration of all hypoglycemic episodes (measured glucose <3.0 mmol/L [54 mg/dL]). Then the total duration of hypoglycemia can be calculated. The hypoglycemia duration is a continuous variable and is analyzed using the method for a continuous variable. The distribution of the number of hypoglycemic events will be tabled.

11.12 *The percent reduction in insulin requirement*

The percent reduction from the baseline insulin requirement at each time point will be calculated. The analysis of the percent reduction adopts the method for a continuous variable.

12. Analysis of Quality of Life Measures

The analysis of QOL will be conducted for the ITT population. QOL measures are obtained every three months while the patient is on the wait list. Observed values from the last of these prior to transplant will be used as the baseline value. After transplant, QOL measures are obtained at 75±5 days after each transplant (unless a subsequent transplant has occurred), and at one year, 2 years and 3 years after the first and last transplants. Separate analyses will be calculated for each measure at each time point.

12.1 *Generic Measures*

Generic and disease-specific measures will be used to assess QOL. Questionnaires will be completed at enrollment and every 3 months during the screening period and at one year, 2 years, and 3 years after the first and last transplants.

12.1.1 Version 2 SF-36 survey

The version 2 SF-36 health survey, standard (4-week) recall form will be adopted for general QOL measure in this study. This widely used, generic instrument derives eight scales (physical functioning, role-physical, bodily pain, general health, vitality, social functioning, role-emotional, mental health) and two summary components (physical and mental). For these analyses we will use only the physical and mental composite scales.

12.1.2 European Quality of Life Questionnaire

The EQ-5D is a public domain instrument. This instrument is a utility measure that generates a descriptive profile and single index value for health status. The descriptive portion addresses five health dimensions (mobility, self-care, usual activities, pain/discomfort, and anxiety/depression). The second portion of the EQ-5D is a (0-100) visual analogue scale that is used to report overall health status.

12.2 *Disease-Target Measures*

12.2.1 Diabetes Distress Scale

The DDS represents the latest iteration of the Problem Areas in Diabetes (PAID) scale. This is a 17-item self-administered questionnaire selected from a longer battery of 28-items.

12.2.2 Hypoglycemia Fear Scale

The HFS is a 23-item self-administered survey for measuring the fear experienced with respect to hypoglycemia. The HFS has two subscales. The first measures hypoglycemia avoidance behavior, and the second measures worry about hypoglycemia.

12.3 *Statistical Analyses of Quality of Life Measures*

We will analyze QOL data at each time point independently. Separate univariate analyses will be performed for each scale and for the change from baseline for each scale. Since all QOL measures are at least ordinal scale, we will use the methods described for continuous outcomes. We will also perform a longitudinal analyses for the composite scores for QOL measure.

Each analysis will display mean, standard deviation, median, minimum, and maximum for the outcome. A 95% confidence for the scale and for the change from baseline for that scale will be computed using the method described for continuous outcome measures.

12.3.1 Analysis for SF-36 survey

Separate analyses for the SPC and SMC scales for each time point. Each composite score and their change from baseline values will be analyzed using the methods described for continuous variables.

In addition, we will perform the following longitudinal analyses. For each subject, we will include his/her score at baseline, 75±5, 365±14 days, 730±14 days, and 1095 ±14 days following the last islet transplant. We will use the linear mixed models with a term for time (baseline, 75 days, 365, 730, and 1095 days following the last islet transplant) and repeated measures on subjects.

The Shapiro-Wilk test is applied to test the hypothesis that the QOL scores are normally distributed. If the normality hypothesis is not rejected at significance level 0.05, a linear mixed model analysis will be performed using the observed test scores to test whether the mean QOL score is the same among the five time points. The results would be treated as follows:

- If the p-value for time is greater than 0.05, we conclude that there is no evidence to claim that QOL is changing over time.
- If the p-value is less than 0.05, we would compute the overall 95% confidence intervals for the pair-wise differences in the QOL score.

If the normality hypothesis is rejected at significance level 0.05, we will use the bootstrap procedure (sampling subjects with replacement) to construct the overall 95% confidence intervals for the pair-wise difference in the QOL score between any two time points. We will usethe Benjamini and Hochberg (Benjamini & Hochberg, 1995) adjustment for multiple tests.

12.3.2 Analysis for European Quality of Life Questionnaire

Separate analyses of Overall Health Status (OHS) score from the EQ-5D will be computed for 75±5 days after each transplant (unless a subsequent transplant has occurred), at one year, 2 years, and 3 years after the first and last transplants. We will calculate the sample mean and construct the 95% confidence interval of the mean OHS using the method for a continuous variable described above.

The longitudinal analysis will be repeated for the OHS.

12.3.3 Analysis for Diabetes Distress Scale

Separate analyses of DDS will be computed for 75±5 days after each transplant, and at one year, 2 years, and 3 years after the first and after the last islet transplants. We will calculate the sample mean and construct the 95% confidence interval of the mean DDS using the method for a continuous variable.

The longitudinal analyses will be repeated for the DDS.

12.3.4 Analysis for Hypoglycemia Fear Scale

Separate analyses of HFS will be computed for 75±5 days after each transplant, and at one year, 2 years, and 3 years after the first and last transplants. We will calculate the sample mean and construct the 95% confidence interval of the mean DDS using the method for a continuous variable described in SAP section 9.2.2.

The longitudinal analyses will be repeated for the HFS.

12.4 *Analysis of Secondary Time to Event Outcomes*

Time to event outcomes include the following:

- Time to death (all cause and also cardiac related) and time to cardiovascular events (death, CVA, MI)
- Time to HbA1c $\leq$ 6.5%
- Time to first severe hypoglycemia episode
- Time to HbA1c decreasing by at least 1%
- global treatment failure composite outcome measure defined as the time to the first occurrence of any of the following:
  - Death
  - Stroke
  - MI
  - Above the ankle amputation

All of the above will be ascertained from the SAE reports and will be adjudicated if an adjudication committee is available.

  - Renal Failure (Any one of the following):
    - Initiation of dialysis; or
    - Renal transplantation
  - Blindness: Visual acuity of 20/200 in both eyes.

- Poor diabetes control (if any of the following occur):
  - HbA1c > 9% and reduced less than 1% below the entry level, found 6 months or more following the first transplant and confirmed on repeat examination at least 30 days and no more than 60 days later. If confirmed, failure is the time of the initial draw;
  - Three consecutive monthly HbA1c values greater than the baseline value, with the first one being 6 months or more following the first transplant; or
  - Occurrence of two severe hypoglycemic events (requiring assistance from another person) within a three month period. Failure will occur at the time of the second hypoglycemic event.

The endpoints and the analyses in this protocol have adopted two principal time points for each transplanted subject: (1) the time of the first transplant and (2) the time of the last transplant. It is of interest to examine the time to events following each of these time points. The following analyses will be repeated using each of these milestones as time zero in the analysis. In the unlikely event that a subject has already reached an event at the time of the second transplant he/she will not be included in the analyses for that event for time from last transplant.

All time to event outcomes will be summarized using Kaplan-Meier life table methods and survival plots. Estimates and 95% confidence intervals will be provided for rates at 1-year, 2-years, and three years after the first and after the last islet transplant. Confidence intervals will be computed as the estimate ± 1.96 standard errors of the product-limit estimates.

12.5 *Sensitivity Analysis*

Measurements of secondary endpoints may be missing at any time point. In our analysis report for the secondary endpoints, we will not impute missing values in the analysis but will report results for the observed data only. With close monitoring and data validation, the chance of having missing values will be minimized except that some serious adverse events (SAEs) may force subjects to withdraw from the study treatment or withdraw consent without a particular reason during the study. The interpretation of our analysis is strictly applied to the efficacy measurable population. However, a sensitivity analysis will be performed to determine the potential effect that missing values have on the analyses of the secondary endpoints.

Missing values due to SAE will be excluded from this analysis. The analysis on these subjects will be provided in SAP section 14.

We will conduct a sensitivity analysis assuming a missing at random mechanism: *i.e.*, we assume that a subject will withdraw consent at random with probability *r*. This probability will be estimated at each time point.

We will use the method of multiple imputation (Schafer, 1999) to develop estimates and standard errors that account for the missing data. We will compare the magnitude of the differences between multiple imputation estimates and observed data estimates to assess the potential affect

of the missing data. The assumption of missing at random cannot be verified and these results must be viewed with caution.

13. Other Exploratory Analyses

13.1 *Change from baseline*

For each secondary outcome and time point where it is appropriate, we will explore the change from baseline over time. The endpoints and time points are as follows:

- insulin requirements (measured quarterly years 1 and 2 post-first and post-last transplant then every 6 months until year 3 post-last transplant)
- HbA1c (measured at day 28 and then quarterly years 1, 2, and 3 post-first and post-last transplant)
- Clarke Score (measured at 6 months and year 1 post-first and post-last transplant then every 6 months until year 3 post-last transplant)
- β score (measured quarterly year 1 post-first transplant, quarterly years 1 and 2 post-last transplant then every 6 months until year 3 post-last transplant)
- Basal glucose (measured quarterly year 1 post-first transplant, quarterly years 1, 2, and 3 post-last transplant)
- 90-minute MMTT glucose (measured quarterly year 1 post-first transplant, quarterly years 1, 2, and 3 post-last transplant)
- Basal c-peptide (measured quarterly year 1 post-first transplant, quarterly years 1, 2, and 3 post-last transplant)
- 90-minute MMTT c-peptide (measured quarterly year 1 post-first transplant, quarterly years 1, 2, and 3 post-last transplant)
- C-peptide/(glucose*creatinine) ratio (measured monthly year 1 post-first transplant and post-last transplant, then quarterly post-last transplant)
- Serum creatinine (measured monthly year 1 post-first transplant and post-last transplant, then quarterly post-last transplant)
- Atherogenic profile (measured year 1 post-first transplant and yearly post-last transplant)
- Quality of Life measures (measured day 75, day 180, yearly post-first and post-last transplant)
- Carotid IMT (measured year 1 post-first transplant and yearly post-last transplant)

Data analyses will be based on change from baseline for each variable. Data analysis will use linear mixed models to describe the profiles of change across time while accounting for correlation within subjects. We will use two strategies. The first strategy will select specific time points where data are routinely measured (e.g. 1 year after first transplant, and 2 and 3 years after last transplant). Estimates of mean change from baseline at these specific time points and 95% confidence intervals will be reported. Contrasts will be used to compare mean changes among time points. We will use the method of Benjamini and Hochberg (Benjamini & Hochberg, 1995)

to account for the multiplicity of comparisons. Next, we will fit polynomial regression models to describe the profile of mean change with time. Data will be displayed graphically and parameter estimates and confidence intervals for regression parameters will be reported. We will use the multiple imputations method to determine the potential effect of missing data.

13.2 *Comparison of time profiles of eligible subjects who are transplanted with eligible subjects who are not transplanted*

For each secondary outcome and time point where it is appropriate, we will compare transplanted subjects to those subjects who remain on intensive insulin therapy (IIT) but do not receive a transplant. Subjects can remain on IIT for up to one year so the time points and comparisons will be determined by the measures that are obtained while subjects are on wait list. Prior to transplantation, measures are obtained when subjects satisfy the IIT entry criteria (baseline) and either quarterly or every six months while subjects are on the waitlist. The proposed endpoints and time points are as follows:

- insulin requirements (measured every six months)
- HbA1c (measured months 3, 6, 9, 11, 12 encompassing the IIT and WL periods)
- MAGE (measured every six months)
- LI (measured every six months)
- Clarke Score (measured every six months)
- Hypo Score (measured every six months)
- Quality of Life measures (measured quarterly while on wait list)

Analysis will use the linear mixed model approach to describe and test for differences across the time points between transplanted subjects post-transplant and non-transplanted subjects while they are on wait list. Time zero will be defined as the day that the IIT criteria was satisfied for those who do not get a transplant and as the day of transplant for those that are transplanted. Because the two populations will be different at baseline, the analyses will need to adjust for the baseline value of the same outcome as well as age, gender, and baseline BMI. We will use the linear mixed models approach for these analyses. We will use models which treat time as a categorical variable. We will use a standard "factorial" design with terms for time, treatment (transplant or no transplant), and the interaction between time and treatment. If the interaction is significant (which we expect) then we will test for the difference between treatments at each time point. We will report means and 95% confidence intervals for the difference at each time point. We will use the method of Benjamini and Hochberg (Benjamini & Hochberg, 1995) to account for the multiplicity of tests.

13.3 *Comparison of pre-transplant and post-transplant time profiles of subjects who are transplanted.*

For those who have been transplanted, we will compare the profiles of change over time while the subject was on waitlist to change over time after the subject has received a transplant. We begin collecting data pre-transplant when the subject becomes eligible for transplant. Time zero for the pre-transplant values will be when the subject becomes eligible for transplant. Time zero for post-transplant observations will be the day of transplant. The endpoints and times will be the same as those described in the previous section. Analysis will use the linear mixed model approach. The models will have terms for time, transplant status (pre-transplant or post-transplant), and the interaction between time and transplant status. If the interaction is significant (which we expect) then we will test for the pre-post difference at each time point. We will report means and 95% confidence intervals for the difference at each time point. We will use the method of Benjamini and Hochberg (Benjamini & Hochberg, 1995) to account for the multiplicity of tests.

14. Safety Analyses

Safety analyses will be conducted for the safety population. Summaries will be prepared for the targeted safety endpoints listed in the secondary endpoints and for all observed AEs organized by body system. The CIT consortium modified the National Cancer Institute (NCI) toxicity table to create a document relevant for trials of adult pancreatic islet transplantation. The resulting reference manual, "Terminology Criteria for Adverse Events (TCAE) In Trials of Adult Pancreatic Islet Transplantation," provides descriptive terminology and a grading (severity) scale which will be utilized for adverse event (AE) reporting.

Regular safety analyses will be prepared for the Data Safety Monitoring Board (DSMB). These summaries will be used to monitor the overall safety profile of the study. These analyses will summarize all AE data that are available at the time of the DSMB meeting. Analyses will summarize AEs by MedDRA term and body system. Separate incidence summaries will be prepared for serious AEs, for nonserious AEs and for all AEs combined. Separate tables will summarize severity and attribution. Each AE can be attributed to the investigational agent (allogeneic islets), the immunosuppression, both or neither. Identical safety summaries will be included in the final statistical report.

The protocol also describes targeted safety endpoints. The planned analyses for general safety outcomes and for these targeted safety endpoints are described in the following few sections. While data summaries of variables describing renal failure and variables describing changes related to immunosuppression (immunosuppression and bone marrow suppression variables) are included in the SAP, no pre-planned analyses of these variables were described. Because of their extreme clinical importance, post-hoc analyses of these data tables will be reported in the clinical study report and labeled as post-hoc.

14.1 *Adverse Events*

An AE is any occurrence or worsening of an undesirable or unintended sign, symptom (including an abnormal laboratory finding), or disease that is temporally associated with the use of a medicinal product whether considered related to the medicinal product or not. An SAE is defined as any AE occurring at any dose that suggests a significant hazard, contraindication, side effect, or precaution. This includes but is not limited to any of the following events (21CFR§312.32):

- Death.
- A life-threatening event. A life-threatening event is any adverse therapy experience that, in the view of the investigator, places the patient or participant at immediate risk of death from the reaction as it occurred.
- Inpatient hospitalization or prolongation of existing hospitalization.
- Persistent or significant disability.
- Congenital anomaly or birth defect.

- An event that required intervention to prevent permanent impairment or damage. An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based on appropriate medical judgment, it may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.
- Other conditions specified in the protocol.

In addition, events that occur at a higher than expected frequency, as determined by appropriate medical judgment, may be considered SAEs.

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

Grade 1 = Mild adverse event.

Grade 2 = Moderate adverse event.

Grade 3 = Severe and undesirable adverse event.

Grade 4 = Life-threatening or disabling adverse event.

Grade 5 = Death.

AEs not included in the CIT-TCAE listing, will be recorded and graded 1 to 5 according to the General Grade Definition provided as in the table below:

Table 12.1-General Severity Definition of Adverse Event

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

All AEs will be reported and graded whether they are or are not related to disease progression or treatment. The relationship of an AE to islet transplantation, which includes the transplant procedure and/or the islet product, or to the immunosuppression and/or infection prophylaxis will be defined by using the descriptors provided in Table 12.2.

Table 12.2 -Attribution of Adverse Event

Code	Description	Definition
UNRELATED CATEGORY		
1	Unrelated	This adverse event is clearly not related to allogeneic islets; the islet transplant procedure; immunosuppression or infection prophylaxis.
RELATED CATEGORIES		
2	Unlikely	The adverse event is doubtfully related to allogeneic islets; the islet transplant procedure; immunosuppression or infection prophylaxis.
3	Possible	The adverse event may be related to allogeneic islets; the islet transplant procedure; immunosuppression or infection prophylaxis.
4	Probable	The adverse event is likely related to allogeneic islets; the islet transplant procedure; immunosuppression or infection prophylaxis.
5	Definite	The adverse event is clearly related to allogeneic islets; the islet transplant procedure; immunosuppression or infection prophylaxis.

The collecting and recording procedures for AEs are described in detail in Section 8.2 of CIT-07 protocol.

14.2 *Analysis of Safety Endpoints*

The safety secondary endpoints in this study target AEs related to islet transplantation (transplant procedure and/or islet product) and the immunosuppression and/or infection prophylaxis.

The targeted AEs related to the islet transplantation include:

AE-IIP-1: Bleeding (>2 g/dL decrease in hemoglobin concentration)

AE-IIP-2: Segmental portal vein thrombosis

AE-IIP-3: Biliary puncture

AE-IIP-4: Wound complication (infection or subsequent hernia)

AE-IIP-5: Increased transaminase levels (>5 times ULN)

The targeted AEs related to the immunosuppression and infection prophylaxis therapy include:

AE-IP-1: Allergy

AE-IP-2: Reduction in GFR

AE-IP-3: Increase in urinary albumin excretion

AE-IP-4: Addition or intensification of anti-hypertensive therapy

AE-IP-5: Addition or intensification of anti-hyperlipidemic therapy

AE-IP-6: Oral ulcers

AE-IP-7: Lower extremity edema

AE-IP-8: Gastrointestinal toxicity

AE-IP-9: Neutropenia, Anemia, or Thrombocytopenia

AE-IP-10: Viral, Bacterial, or Fungal Infections

AE-IP-11: Benign or Malignant Neoplasms

Results will be reported for the following time points: 75 days and one year after the first and last transplant and at 2 years, and 3 years after the first and after the last islet transplant.

We will report incidence of each AEs categorized by MedDRA term and body system. Tables with relation to the islet product and to the immunosuppression will be reported. For each category, the number of resolved events will be reported. The mean, median, standard deviation and range for the number of days until the AE is resolved will also be reported. Results will be summarized using the template provided in Appendix 2.

The incidence rate of immune sensitization, defined as detection of anti-HLA antibodies at 75 ± 5 days following the first and last islet transplant and at and one year after the first and last transplant and at 2 years, and 3 years after the first and after the last islet transplants. Exact two-sided 95% confidence interval of the incidence rate will also be reported.

14.2.1 Statistical Analyses of Adverse Events

AEs will be analyzed according to the body system described in the *CIT-TCAE* manual. Any event that appears in a body system will be categorized into one of the five severity grades according to the *CIT-TCAE* manual or the general definition of severity given in Table 7 and analyzed accordingly. For each event, we will analyze its incidence rate per 100 person-days. Suppose for each subject, the incidence of a particular event is a Poisson process with the homogeneous incidence rate λ. At the time of analysis, if a subject is only followed *r* days after the initiation of induction immunosuppression, the incidence of this event is distributed according to the Poisson distribution with mean $r\lambda/100$. The maximum likelihood estimate of the

incidence rate will be obtained based on data collected from the available subjects at the analysis time. The 95% confidence interval derived using the maximum likelihood estimator theory will be also reported. The results will be summarized in the table provided in SAP Appendix 4. Moreover, we will also list all the individuals who have ever had an incident of any of the AEs listed in Appendix 5 since their initiation of induction immunosuppression.

15. Interim Analyses and Safety Monitoring Analyses

The DSMB will be convened to review safety and efficacy data following National Institutes of Health (NIH) policy. When requested, formal interim analyses to assess safety and efficacy will be performed. Formal interim analyses will include distributions of endpoints, biomarkers and AEs. Additional analyses may be requested by the DSMB.

15.1 *Interim Analysis for Early Stopping*

We will use the method for interim analyses described by Emerson and Fleming (Emerson & Fleming, 1989) which is implemented in the SPlus module S+SeqTrial (TIBCO_Software, 2009) to provide cut-points for an interim analysis for futility. This module does not require specifying the timing of interim analyses and controls the overall type I and type II errors. In order to make what follows clearer we provide some notation. Let p represent the true probability of a favorable outcome in islet transplanted subjects.

The efficacy hypothesis is

$$H_{01} : p \leq 0.27 \text{ versus } \mathrm{H}_{a1} : p > 0.27.$$

Rejecting this null hypothesis would lead to concluding that the favorable outcome rate is better than 27% in patients who receive an islet transplant. In order to ensure that the study provides the maximum safety information, this study will not be stopped for efficacy.

The futility hypothesis is

$$H_{02} : p \geq 0.67 \text{ versus } H_{a2} : p < 0.67.$$

Rejecting this hypothesis would lead to concluding that the favorable outcome rate in patients who receive an islet transplant is not greater than 0.27. The value 0.67 guarantees that the probability of a type II error (accepting the null hypothesis when it is false) is less than or equal to 0.025. That is, the probabilities of type I and type II errors are equal.

In order to illustrate the method, Table 13.1 assumes two equally spaced analyses; an interim analysis when 12 subjects have completed their one-year post first transplant visit and a final analysis when all 24 subjects have completed their one-year post first transplant visit. The table provides cut-points for a recommendation for futility (Reject H_{o2}). Column 4 in this table (labeled number of favorable outcomes required) provides the largest number of observed favorable outcomes that would lead to recommending futility.

At the first interim analysis (when 12 subjects have completed the study) the monitoring plan would recommend for futility if no more than 2 of the first 12 islet transplant subjects experienced a favorable outcome at one year after their first transplant.

Table 13.1: Sequential Monitoring Plan

Patients Accrued		Fixed Sample p-value	Number of Favorable outcome Required*
12	Reject H_{02}	.5000	≤2
24	Accept H_{01}	.0243	<11

Source SPlus SeqTrial

15.2 *Safety Monitoring Analyses*

AEs and clinical outcomes are monitored closely in this study. To protect the safety of subjects, safety stopping rules for the protocol and individuals sites have been developed.

15.2.1 Protocol Suspension and Review

Criteria for protocol suspension and review are detailed in the CIT-06 protocol, section 6.2.1.

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

15.2.2 Site Suspension and Review

Criteria for suspension of study enrollment and first islet infusions at individual sites are detailed in the CIT-06 protocol, section 6.2.2.

After any site is placed on hold, no additional transplants will be performed at that site until the CIT Steering Committee and DSMB meet either in person or by conference call to review in depth the results and circumstances surrounding the islet functional failure or SAE to determine whether the trial enrollment of new subjects and conduct of additional transplants could be safely resumed at that site, or whether there could be implications for the continuation of the entire proposed pilot protocol also at other affiliated sites testing the same protocol.

In all cases of PNF, subjects will be asked to temporarily continue immunosuppression to decrease the risk of sensitization that could increase the risk of poor outcome should future transplants occur. A tapering schedule will be applied until immunosuppressants are completely discontinued.

16. Works Cited

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statisitcal Society. Series B (Methodological)*, 289-300.

Clarke, W., Cox, D. G.-F., Julian, D., Schlundt, D., & Polonsky, W. (1995). Reduced awareness of hypoglycemia in adults with IDDM. A prospective study of hypoglycemic frequency and associated symptoms. *Diabetes Care*, 517-522.

Emerson, S., & Fleming, T. (1989). Symmetric group sequential designs. *Biometrics*, 905-923.

Hintze, J. (2011). *Pass 11*. Kaysville, Utah.

Markmann, J., S, D., Huang, X., & al., e. (2003). Insulin independence following isolated islet transplantation and a single islet infusion. *Ann Surg.* , 741-749.

Pacini, & Bergman. (1986). MINMOD: a computer program to calculate insulin sensitivity and pancreatic responsivity from the FSIGT. *Comp Meth, and Prog in Biomed;*, 113-122.

Rosner, B. (1995). *Fundamentals of Biostatistics: Fourth Edition.* Belmont California: Wadsworth Publishing Company.

Ryan, E., Paty, B., Senior, P., Lakey, J., Bigam, D., & Shariro, A. (2005). Beta-score: an assessment of beta-cell function after islet transplantation. *Diabetes Care*, 343-347.

Ryan, E., Shandro, T., K, G., & al, e. (2004). Assessment of the severity of hypoglycemia and glycemic lability in type 1 diabetic subjects undergoing islet transplantation. *Diabetes*, 955-962.

Saad, M., Anderson, R., Laws, A., & al, e. (2004). A comparison between the minimal model and the glucose clamp in the assessment of insulin sensitivity across the spectrum of glucose monitoring. *Diabetes Care*, 722-726.

Schafer, J. (1999). Multiple imputation: a primer. *Stat Methods Med Res*, 3-15.

Service, F., Molnar, G., Rosevear, J., Ackerman, E., Gatewood, L., & Taylor, W. (1970). Mean amplitude of glycemic excursions, a measure of diabetic instability. *Diabetes*, 644-655.

Shapiro, S., & Wilk, M. (1965). An analysis of variance test for normality (complete samples). *Biometrika*, 591-611.

Steja, D. (2005). Can continuous glucose monitoring provide objective documentation of hypoglycemia unawareness. *Endocr Pract*, 83-90.

The_Diabetes_Control_and_Complications_Trial_Research_Group. (1993). The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependend diabetes mellitus. *NEJM*, 977-986.

TIBCO_Software. (2009). *TIBCO Spotfire S+ 8.1 for Windows User's Guide.* Seatle, Washington.

Zavaroni, I., Deferrari, G., Lugari, R., & al, e. (1987). Renal metabolism of C-peptide in man. *J Clin Endocrinol Metab*, 494-498.

Zerboni, G., Mangili, R., & Luzi, L. (1999). High post-absorptive C-peptide levels in Type I diabetic patients without renal complications. *Diabetes Med.*, 1048.

17. Appendices

17.1 *Appendix 1 - Descriptive Statistics of Baseline Data*

	Center									
	Univ of Alberta	Univ of Miami	Univ of Minnesota	Univ of Pennsylvania	Emory Univ	Northwestern Univ	UIC	UCSF	Massachusetts General Hospital	Total
Demographic Variables										
Age n mean s.d. median range										
Sex: n (%) male female total										
Race: n (%) White Black Hispanic Asian Other										
	Disease Factors									

	Center									
	Univ of Alberta	Univ of Miami	Univ of Minnesota	Univ of Pennsylvania	Emory Univ	Northwestern Univ	UIC	UCSF	Massachusetts General Hospital	Total
Insulin Req. n mean s.d. median range										
HbA1c n mean s.d. median range										
# of Severe Hypo. n mean s.d. median range										
MAGE n mean s.d. median range										
LI n										

	Center									
	Univ of Alberta	Univ of Miami	Univ of Minnesota	Univ of Pennsylvania	Emory Univ	Northwestern Univ	UIC	UCSF	Massachusetts General Hospital	Total
mean s.d. median range										
Clarke Score n mean s.d. median range										
HYPO score n mean s.d. median range										
β-score n mean s.d. median range										
C-peptide glucose Creatinine Ratio										

	Center									
	Univ of Alberta	Univ of Miami	Univ of Minnesota	Univ of Pennsylvania	Emory Univ	Northwestern Univ	UIC	UCSF	Massachusetts General Hospital	Total
n mean s.d. median range										
Physical Conditions										
Body Weight n mean s.d. median range										
Height n mean s.d. median range										
QOL										
SPC n mean s.d. median range										
SMC										

	Center									
	Univ of Alberta	Univ of Miami	Univ of Minnesota	Univ of Pennsylvania	Emory Univ	Northwestern Univ	UIC	UCSF	Massachusetts General Hospital	Total
n mean s.d. median range										
OHS n mean s.d. median range										
DDS n mean s.d. median range										
HFS n mean s.d. median range										

17.2 *Appendix 2 - Summary Table of Adverse Events Related to the Islet transplant and Immunosuppression Therapy*

	Degrees of Event Severity					
Events	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Total
AE related to the islet transplantation (islet product or islet transplant procedure):						
AE-IIP-1 Number (%) Resolved? Number (%) mean s.d. median min-max AE-IIP-2 Number (%) Resolved? Number (%) mean s.d. median min-max AE-IIP-3 Number (%) Resolved? Number (%) mean						

	Degrees of Event Severity					
Events	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Total
s.d. median min-max AE-IIP-4 Number (%) Resolved? Number (%) mean s.d. median min-max AE-IIP-5 Number (%) Resolved? Number (%) mean s.d. median min-max						
AE related to the immunosuppresion and/or infection prophylaxis:						
AE-IP-1 Number (%) Resolved? Number (%)						

	Degrees of Event Severity					
Events	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Total
mean						
s.d.						
median						
min-max						
AE-IP-2						
Number (%)						
Resolved?						
Number (%)						
mean						
s.d.						
median						
min-max						
AE-IP-3						
Number (%)						
Resolved?						
Number (%)						
mean						
s.d.						
median						
min-max						
AE-IP-4						
Number (%)						
Resolved?						
Number (%)						
mean						

	Degrees of Event Severity					
Events	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Total
s.d. median min-max AE-IP-5 Number (%) Resolved? Number (%) mean s.d. median min-max AE-IP-6 Number (%) Resolved? Number (%) mean s.d. median min-max AE-IP-7 Number (%) Resolved? Number (%) mean s.d.						

	Degrees of Event Severity					
Events	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Total
median						
min-max						
AE-IP-8						
Number (%)						
Resolved?						
Number (%)						
mean						
s.d.						
median						
min-max						
AE-IP-9						
Number (%)						
Resolved?						
Number (%)						
mean						
s.d.						
median						
min-max						
AE-IP-10						
Number (%)						
Resolved?						
Number (%)						
mean						
s.d.						
median						

	Degrees of Event Severity					
Events	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Total
min-max AE-IP-11 Number (%) Resolved? Number (%) mean s.d. median min-max						

17.3 *Appendix 3 - Adverse Events: Number Observed and Rate with Patient Identifications Grouped by Severity and Attribution*

	Degrees of Event Severity					
Events	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Total
AE related to the islet transplantation (islet product or islet transplant procedure):						
AE-IIP-1 incidences	6 (12.5%) C11+ C12 C22 C34 C63 C66					
AE-IIP-2 incidences						
AE-IIP-3 incidences						
AE-IIP-4 incidences						
AE-IIP-5 incidences						
AE related to the immunosuppression and/or infection prophylaxis:						

AE-IP-1 incidences AE-IP-2 incidences AE-IP-3 incidences AE-IP-4 incidences AE-IP-5 incidences AE-IP-6 incidences AE-IP-7 incidences AE-IP-8 incidences AE-IP-9 incidences AE-IP-10 incidences						

AE-IP-11 incidences						

+ : Patient's identification, for example C11 stands for the incidence occurring on patient number 1 at Center #1

17.4 *Appendix 4 - Analysis of Adverse Events at Month # Since Transplant Eligibility Confirmation*

	AE related to the islet transplantation (islet product or islet transplant procedure) or immunosuppression and/or infection prophylaxis			AE **not** related to the islet transplantation (islet product or islet transplant procedure) or immunosuppression and/or infection prophylaxis		
All Adverse Events	# of incidences	The incidence rate per 100 person-days (λ)	The 95 % confidence interval of λ based on MLE theory	# of incidences	The incidence rate per 100 person-days (λ)	The 95 % confidence interval of λ based on MLE theory
Body System A Grade 5 Grade 4 Event 1 Event 2 ⋮ Grade 3 Event 1 Event 2 ⋮ Grade 2 Event 1 Event 2 ⋮ Grade 1 Event 1 Event 2						

⋮						
Body System B Grade 5 Grade 4 Event 1 Event 2 ⋮ Grade 3 Event 1 Event 2 ⋮ Grade 2 Event 1 Event 2 ⋮ Grade 1 Event 1 Event 2 ⋮						
Body System C Grade 5 Grade 4 Event 1 Event 2 ⋮ Grade 3 Event 1 Event 2						

⋮ Grade 2 Event 1 Event 2 ⋮ Grade 1 Event 1 Event 2 ⋮						

17.5 *Appendix 5 - Identification of Adverse Events at Month # Since Transplant Eligibility Confirmation*

	AE related to the islet transplantation (islet product or islet transplant procedure) or immunosuppression and/or infection prophylaxis		AE **not** related to the islet transplantation (islet product or islet transplant procedure) or immunosuppression and/or infection prophylaxis	
All Adverse Events	Identification of incidences	Number and percentage of patients who have had the incidence	Identification of incidences	Number and percentage of patients who have had the incidence
Body System A Grade 5 Grade 4 Event 1 Event 2 ⋮ Grade 3 Event 1 Event 2 ⋮ Grade 2 Event 1 Event 2 ⋮ Grade 1	C111 C112 C113* C221 C231 C232 C331 C421 C461	6 (12.5%)		

Event 1 Event 2 ⋮				
Body System B Grade 5 Grade 4 Event 1 Event 2 ⋮ Grade 3 Event 1 Event 2 ⋮ Grade 2 Event 1 Event 2 ⋮ Grade 1 Event 1 Event 2 ⋮				
Body System C Grade 5 Grade 4 Event 1 Event 2 ⋮				

Grade 3 Event 1 Event 2 ⋮ Grade 2 Event 1 Event 2 ⋮ Grade 1 Event 1 Event 2 ⋮				

*: C113 stands for the third incidence of Event 1 of Grade 4 in body system A that occurs in patient #1 at Center 1.

17.6 *Appendix 6 - Summary of Protocol Deviations*

	Number of Protocol Deviations					
Centers	PD1	PD2	PD3	PD4	PD5	Total
University of Alberta						
University of Miami						
University of Minnesota						
University of Pennsylvania						
Emory University						
Northwestern University						
University of California San Francisco						
University of Illinois Chicago						
Massachusetts General Hospital						

17.7 *Appendix 7 - Summary of Excluded Subjects*

Centers	Number of excluded subjects	Reason for exclusion* (n)	# of excluded / (# of excluded + # of included
University of Alberta			
University of Miami			
University of Minnesota			
University of Pennsylvania			
Emory University			
Northwestern University			
University of California San Francisco			
University of Illinois Chicago			
Massachusetts General Hospital			

***** From list of 29 possible reasons.

17.8 *Appendix 8 – Analysis Templates for the Secondary Efficacy Endpoints Measured at 75±5 Days Following the First and Last Infusion*

	Center									
	Univ of Alberta	Univ of Miami	Univ of Minnesota	Univ of Pennsylvania	Emory Univ	Northwestern Univ	Univ of California San Francisco	Univ of Illinois Chicago	Massachusetts General Hospital	Total
Continuous Outcomes										
Insulin Req. n mean s.d. median range 95% CI of mean										
HbA1c n mean s.d. median range 95% CI of mean										
MAGE										

n mean s.d. median range 95% CI of mean										
LI n mean s.d. median range 95% CI of mean										
Hypo n mean s.d. median range 95% CI of mean										
Glucose Change n mean s.d. median										

range 95% CI of mean										
β-score n mean s.d. median range 95% CI of mean										
C-pep:glucose ratio n mean s.d. median range 95% CI of mean										
AIRglu n mean s.d. median range 95% CI of mean										

SI n mean s.d. median range 95% CI of mean										
DI n mean s.d. median range 95% CI of mean										
Glucose variability n mean s.d. median range 95% CI of mean										
# of Hypoglycemia n										

mean s.d. median range 95% CI of mean										
Duartion of Hypoglycemia n mean s.d. median range 95% CI of mean										
Binary Outcomes										
MAGE ≥ 11.1 mmol/l n proportion (p) 95% CI of p										
LI ≥ 433 mmol/l2/h·wk-1 n proportion (p) 95% CI of p										

Hypo ≥ 1047 n proportion (p) 95% CI of p										

17.9 *Appendix 9- Analysis Templates for the Secondary Efficacy Endpoints Measured at 365±14 Days Following the First and last Infusion*

	Center									
	Univ of Alberta	Univ of Miami	Univ of Minnesota	Univ Of Pennsylvania	Emory Univ	Northwestem Univ	Univ of California San Fransicso	Univ of Illinois Chicago	Massachusetts General Hospital	Total
Continuous Outcomes										
Insulin Req. n mean s.d. median range 95% CI of mean										
HbA1c n mean s.d. median range 95% CI of mean										
MAGE										

n mean s.d. median range 95% CI of mean										
LI n mean s.d. median range 95% CI of mean										
Clarke Score n mean s.d. median range 95% CI of mean										
Hypo n mean s.d. median range										

95% CI of mean										
Glucose Change n mean s.d. median range 95% CI of mean										
β-score n mean s.d. median range 95% CI of mean										
C-pep:glucose ratio n mean s.d. median range 95% CI of mean										
Glucose variability										

n mean s.d. median range 95% CI of mean										
Binary Outcomes										
MAGE ≥ 11.1 mmol/l n proportion (p) 95% CI of p										
LI ≥ 433 mmol/l2/h·wk-1 n proportion (p) 95% CI of p										
Hypo ≥ 1047 n proportion (p) 95% CI of p										
Insulin Indenp. n proportion (p) 95% CI of p										

Second infusion n proportion (p) 95% CI of p										
Third infusion n proportion (p) 95% CI of p										
HbA1c<6.5% and free of severe hypoglycemic events n proportion (p) 95% CI of p										