

1.1 Metabolic Testing

1.1.1 Study Endpoints

Because the assessment of islet graft function is dependent on complex physiologic relationships between the graft and its recipient, no single test adequately addresses the viability of the transplant. The primary endpoint of HbA1c < 6.5% and absence of hypoglycemic episodes addresses the clinically important outcome. Insulin-independence will be used as a clinically relevant measure of islet graft function for the secondary endpoint, and additional stimulatory tests of islet graft function utilizing meal (MTT) and glucose (FSIGT) challenges will be performed to assess additional secondary endpoints. Also, the effect of islet graft function on glycemic control (HbA1c), glycemic lability (MAGE and LI), hypoglycemia (Clarke and HYPO scores), glucose variability (CGMS), and quality of life (QOL) will be assessed as additional secondary endpoints.

1.1.2 Metabolic Assessments

All subjects will use a study provided One Touch® Ultra glucometer for measuring capillary glucose levels.

1.1.2.1 INSULIN REQUIREMENTS

Subjects will record their total daily insulin dose on self-monitoring diaries. Subject should be given exogenous insulin as needed to maintain fasting capillary glucose levels ≤ 140 mg/dL (7.8 mmol/L) at a minimum of 4 out of 7 days a week; 2-hour post-prandial capillary glucose levels should not exceed 180 mg/dL (10.0 mmol/L) more than 3 times per week.

1.1.2.2 GLYCEMIC CONTROL

Glycemic control will be assessed by HbA1c (%) measured centrally, per the University of Washington SOP, every 3 months following transplant.

1.1.2.3 GLYCEMIC LABILITY

Glycemic lability will be assessed by both the mean amplitude of glycemic excursions (MAGE)⁴⁹ and the lability index (LI)⁵³ measured every 3 months following transplant, including 365 days post-initial transplant..

The MAGE requires 14 - 16 capillary blood glucose measurements over two consecutive days taken before and 2-hours after breakfast, lunch, and dinner, and at bedtime with an optional measurement at 3 AM. A glycemic excursion is calculated as the absolute

difference in peak and subsequent nadir (or vice versa) glucose values, with the direction (peak to nadir versus nadir to peak) determined by the first quantifiable excursion in the two day period. All excursions > 1 S.D. of the 7 – 8 glucose readings for the day in which they occurred qualify for the analysis, where they are summed and divided by the number of qualified excursions to give the MAGE in mmol/L (or mg/dl) glucose. A MAGE > 11.1 mmol/L (200 mg/dl) is indicative of marked glycemic lability.

The LI requires 4 or more daily capillary blood glucose measurements over a 4 week period and is calculated as the sum of all the squared differences in consecutive glucose readings divided by the hours apart the readings were determined (range 1 to 12 hours) in mmol/l²/h wk⁻¹. A LI greater than or equal to the 90th percentile (433 mmol/l²/h wk⁻¹) of values derived from an unselected group of type 1 diabetes patients is evidence for severe glycemic lability.

1.1.2.4 HYPOGLYCEMIA

Episodes of severe hypoglycemia will be documented every month as defined by an event with symptoms compatible with hypoglycemia in which the subject required the assistance of another person and which was associated with either a blood glucose level < 54 mg/dl (3 mmol/L) or prompt recovery after oral carbohydrate, intravenous glucose, or glucagon administration.¹⁹

In addition, composite indices of hypoglycemia frequency, severity, and symptom recognition will be assessed by both the Clarke survey¹⁴⁴ and the HYPO score⁵³. The Clarke survey will be completed every 6 months following transplant, including 365 days post initial transplant. The HYPO score will be measured every 3 months following transplant, including 365 days post initial transplant.

The Clarke survey involves subject completion of eight questions scored by the investigator according to an answer key that gives a total score between 0 and 7 (most severe), where scores of 4 or more indicate reduced awareness of hypoglycemia and increased risk for severe hypoglycemic events.

The HYPO score involves subject recording of blood glucose (BG) readings and hypoglycemic events (BG < 3.0 mmol/L [54 mg/dl]) over a 4-week period and recall of all severe hypoglycemic episodes in the previous 12-months. A HYPO score greater than or equal to the 90th percentile (1047) of values derived from an unselected group of type 1 diabetes patients indicates severe problems with hypoglycemia.

1.1.2.5 MIXED-MEAL TOLERANCE TEST (MTT)

Basal (fasting) and stimulated plasma glucose and C-peptide levels will be determined every 3 months following transplantation, including 365 days post-initial transplant, using the MTT. Subjects will be instructed not to eat or inject short-acting (or bolus) insulin after 8 PM the night before the test. Evening or bedtime administration of long-acting insulin will be permitted, as will consumption of water. Subjects receiving CSII (insulin “pump” therapy) may remain on the basal rate of insulin. Subjects will arrive fasting to the transplant or diabetes clinic where the capillary blood glucose will be

checked. If the BG is < 70 mg/dl (3.89 mmol/l) or > 180 mg/dl (10 mmol/l), the test will be rescheduled for the next possible day. If the BG is 70 – 180 mg/dl (3.89 – 10 mmol/l), basal plasma glucose and C-peptide levels will be drawn. Immediately after, the subject will receive 6 ml per kg body weight (to a maximum of 360 ml) of Boost® High Protein Drink (or a nutritionally equivalent substitute) to consume in 5 minutes starting at time = 0. Then, at time = 90 minutes, stimulated plasma glucose and C-peptide levels will again be drawn.

Each blood sample collected for c-peptide and glucose determination will be drawn according to University of Washington (Seattle, WA) SOP and will be shipped frozen to U of W for measurement in the core laboratory

1.1.2.6 B-SCORE: A COMPOSITE INDEX OF POST-TRANSPLANT GRAFT FUNCTION

The β -score will be determined every 3 months post-transplant, including 365 days post-initial transplant, from the HbA1c, insulin requirements, fasting (basal) plasma glucose, and basal or stimulated C-peptide as developed by Ryan et al.¹⁴⁰ The score may range from 0 (no graft function) to 8, with all subjects reported with a score of 8 also having 90-minute plasma glucose levels during a MTT that are \leq 10.0 mmol/L (180 mg/dl), indicative of excellent graft function.

1.1.2.7 THE C-PEPTIDE TO GLUCOSE, CREATININE RATIO

The C-peptide to glucose, creatinine ratio (CPGCR) will be determined every month post-transplant, including 365 days post-initial transplant, from the fasting (basal) plasma glucose and C-peptide, and a simultaneous serum creatinine. 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.^{145, 146} The CPGCR is calculated as $[\text{C-peptide (ng/ml)} * 100] / [\text{glucose (mg/dl)} * \text{creatinine (mg/dl)}]$. An index of islet graft function, this measure correlates well with both the 90-minute plasma glucose levels during a MTT and with the β -score (Faradji R et al., unpublished data).

1.1.2.8 INSULIN-MODIFIED FREQUENTLY-SAMPLED INTRAVENOUS GLUCOSE TOLERANCE (FSIGT) TEST

The acute insulin response to glucose (AIR_{glu}), insulin sensitivity (SI), and disposition index (DI) will be determined pre-transplant and at 75 days following each islet infusion and at 365 days following the first and last islet infusion using the FSIGT test. This assessment provides a composite measure of β -cell function, the disposition index (DI), which relates the effect of insulin sensitivity (S_i) on first-phase insulin secretion (AIR_{glu}). Understanding the effect of insulin sensitivity on insulin secretory dynamics post-transplant is important because insulin resistance imposes an increased demand on β -cell function to maintain the same level of glycemia. Whether insulin resistance,

possibly attributable to immunosuppressive drugs, is an important problem post-transplant is not known. Preliminary data indicate that insulin sensitivity may actually be improved post-transplant, despite immunosuppression, possibly due to the improved glycemia that occurs with transplantation (Rickels MR et al., unpublished data). These results require confirmation by longitudinal analysis.

The insulin-modified FSIGT test¹⁴¹ involves blood sampling at baseline (t = -10, -5, and -1 min) and at t = 1, 2, 3, 4, 5, 7, 10, 12, 14, 16, 18, 20, 22, 25, 30, 40, 50, 70, 100, 140, & 180 minutes post-injection of glucose at t = -30 seconds with an injection of insulin at t = 20 min. Each blood sample collected for insulin, c-peptide and glucose determination will be drawn according to University of Washington (Seattle, WA) SOP and will be shipped frozen to U of W for measurement in the core laboratory. The acute insulin response to glucose (AIRglu) is calculated as the incremental area-under-the-curve for insulin between 0 and 10 minutes post-injection (the same calculation can be performed for C-peptide). Glucose effectiveness (SG), a measure of insulin-independent glucose disposal, and insulin sensitivity (SI), a measure of insulin-dependent glucose disposal, are derived from Bergman's minimal model using MinMod Millennium® software, and further allow for determination of the disposition index (DI = AIRglu • SI). Total blood amount of blood sampled is 72 mL.

1.1.2.9 CONTINUOUS GLUCOSE MONITORING SYSTEM® (CGMS)

Glucose variability and hypoglycemia duration will be determined at 75 days following each islet infusion and at 365 days following the first and last islet infusion using CGMS (Medtronic Minimed, Northridge, CA). CGMS involves the subcutaneous placement of a glucose sensor connected by tubing to a pager-sized monitoring device that stores glucose data over a 72-hour period. Subjects will have the sensor placed in the diabetes clinic and wear it continuously for 72 – 84 hours. Then they will drop the monitoring device off or ship it to the clinic 4 days later for analysis. Subjects will need to calibrate the sensor to their capillary blood glucose readings 4 times daily with no interval between readings exceeding 12-hours. Data from each 72-hour period will be analyzed for mean glucose concentration, mean glucose variability (absolute value of measured glucose minus 5.5 mmol/L [100 mg/dl]), number and duration of hyper- (> 10.0 mmol/L [180 mg/dl]) and hypo- (< 3.0 mmol/L [54 mg/dl]) glycemic episodes, and total duration of hypoglycemia.^{109, 147}