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Islet Transplantation for Brittle Type 1 Diabetes: The UIC Protocol

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This prospective phase 1/2 trial investigated the safety
and reproducibility of allogeneic islet transplantation
(Tx) in type I diabetic (T1DM) patients and tested a
strategy to achieve insulin-independence with lower
islet mass. Ten C-peptide negative T1DM subjects with
hypoglycemic unawareness received 1–3 intraportal
allogeneic islet Tx and were followed for 15 months.
Four subjects (Group 1) received the Edmonton
immunosuppression regimen (daclizumab, sirolimus,
tacrolimus). Six subjects (Group 2) received the Uni-
versity of Illinois protocol (etanercept, exenatide and
the Edmonton regimen). All subjects became insulin-
independent. Group 1 received a mean total number
of islets (EIN) of 1460 080 ± 418 330 in 2 (n = 2) or
3 (n = 2) Tx, whereas Group 2 became insulin- in-
dependent after 1 Tx (537 495 ± 190 968 EIN, p =
0.028). All Group 1 subjects remained insulin free
through the follow-up. Two Group 2 subjects resumed
insulin: one after immunosuppression reduction dur-
ing an infectious complication, the other with exe-
natide intolerance. HbA1c reached normal range in
both groups (6.5 ± 0.6 at baseline to 5.6 ± 0.5 af-
ter 2–3 Tx in Group 1 vs. 7.8 ± 1.1 to 5.8 ± 0.3 after
1 Tx in Group 2). HYPO scores markedly decreased in
both groups. Combined treatment of etanercept and
exenatide improves islet graft function and facilitates
achievement of insulin-independence with less islets.

Key words: Islet transplantation, transplantation cell
therapy, transplantation research, type I diabetes

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A. Gangemi and P. Salehi contributed equally to this work.

Introduction

Although major advances in insulin treatment have im-
proved glycemic control, hypoglycemic unawareness re-
mains a major obstacle for optimal glycemic control in
many diabetic patients (1). Even under strict study condi-
tions, the incidence of severe hypoglycemia remains high;
this complication not only impairs quality of life but also is
life-threatening (2,3).

Islet transplantation can eliminate severe hypoglycemia
and restore almost normal glycemic control (4). However,
there are several limitations to the widespread application
of this procedure (4). These limitations include the need for
several donors for sufficient islet yield, lifelong immuno-
suppression and its attendant risks, and loss of islet func-
tion over time due to incompletely defined reasons.

At present, clinical trials in islet transplantation face strin-
gent federal regulations that define islets as a biological
drug and islet transplantation as an experimental proce-
dure. Limited resources make fully powered, larger-scale
trials prohibitive. Despite limitations, collaborative efforts
and single-center experiences are improving results and
expanding the body of knowledge as a whole. Herein, we
present a phase 1/2 trial investigating whether islet trans-
plantation utilizing a protocol designed at the University of
Illinois at Chicago is safe and reproducibly successful. The
study explored traditional therapy in the first arm, and then
tested a regimen of a TNFα receptor antagonist (etaner-
cept, Enbrel®) and a glucagon-like peptide-1 (GLP-1) ana-
logue (exenatide, Byetta®) to explore whether we could
improve islet engraftment with a marginal islet dose (5). Ex-
enatide is FDA-approved for treatment of type II diabetes
mellitus (6–8). Recently, exenatide was used to decrease
need for exogenous insulin and restore first-phase insulin
release in islet recipients who lost insulin-independence
after transplantation (9). Herein, we explored the de novo
use of a combination of etanercept and exenatide to im-
prove allogeneic islet transplant outcomes in brittle type l
diabetic patients.

Materials and Methods

Study design

This prospective Phase 1/2 trial was conducted under FDA IND 11 807 and
approved by the Institutional Review Board. In this single-center, open label,

uncontrolled trial we performed 1–3 allogeneic pancreatic islet transplants per subject and followed up for 64 weeks (~15 months) after transplant. This follow-up period was determined to avoid exceeding the maximum acceptable number of blood draws per individual at any time point and to include all safety and metabolic parameters reported herein. Thereafter, subjects were enrolled in a 5-year follow-up study.

The first objective was to demonstrate the safety of allogeneic islet transplantation in type I diabetic patients performed at the University of Illinois at Chicago (UIC). Therefore, the first four patients (Group 1) were transplanted according to the Edmonton protocol. The second objective was to implement a strategy to achieve insulin-independence with a minimal quantity of islets. Therefore, the next six consecutive patients (Group 2) were transplanted using the UIC protocol detailed below.

Primary efficacy endpoint

The primary efficacy endpoint was independence from insulin injections with adequate control of blood glucose. Insulin-independence was defined as freedom of exogenous insulin injection while achieving fasting glucose levels not exceeding 140 mg/dL more than three times in a week, and not exceeding 2-h postprandial values of 180 mg/dL more than four times in a week. The proportion of insulin-independent subjects meeting the criteria for glucose control was determined at 2 weeks and 1, 3, 6, 12 and 15 months after their final transplants.

Subjects who had reduced insulin requirements but did not achieve insulin-independence and presented reduced HbA1c and number of hypoglycemic episodes were considered to have partial islet graft function.

Absence of measurable levels of C-peptide (<0.03 ng/mL) after transplantation was considered islet failure.

Secondary endpoints

- HbA1c was compared between the last value before transplantation and 3, 6, 12 and 15 months after islet transplant. HbA1c reaching normal values of less than 6.5% by 3, 6, 12 and 15 months after first transplant and continuing for 12 months after the final transplant was considered a success.
- Fructosamine was compared between the last value before transplantation and 3, 6, 12, and 15 months after islet transplant.
- Oral glucose tolerance tests (OGTT) were conducted 6 and 12 months postislet transplant. OGTT was judged normal if blood glucose was below 140 mg/dL after 2 h, impaired if blood glucose was between 140 and 199 mg/dL, and diabetic if blood glucose was above 199 mg/dL.
- Mixed meal tests (MMT) were conducted before transplantation and 6 and 12 months posttransplant. Acute C-peptide response and blood glucose levels to a standard mixed meal test were compared.
- Glucagon stimulation test (GST) values were measured before transplantation and 6 and 12 months posttransplant. The fold-increase in C-peptide levels 6 min after glucagon injection was evaluated.
- Intravenous glucose tolerance test (IVGTT) values were taken at 6 and 12 months post transplantation. We calculated acute insulin response to intravenous glucose challenge (AIR-IVGGT) as an indicator of islet mass (10).
- Hypoglycemia was evaluated by the Ryan HYPO score (11) before and 12 months after transplantation. To calculate the HYPO score, subjects performed blood sugar self-assessments for 4 weeks during the 6 months pretransplant and 12 months after transplantation. Points were awarded for each occurrence of documented hypoglycemia and for neuroglycopenic symptoms.
- Glycemia status: Subjects measured and recorded blood sugar values seven times per day before and after transplantation: fasting, morn-

ing, postprandial, noon, afternoon postprandial, evening and evening postprandial.

Inclusion criteria

Subjects were selected according to the Edmonton protocol criteria (12). Enrolled subjects had type I diabetes mellitus for more than 5 years, complicated by either hypoglycemic unawareness, metabolic lability with documented severe hypoglycemia, or ketoacidosis despite state-of-the-art-intensive insulin therapy (13).

Exclusion criteria

Subjects were excluded from the study if they had coexisting cardiac disease, history of nonadherence to prescribed regimens, BMI >26 kg/m^2 or body weight >70 kg at screening visit, creatinine clearance <80 mL/min/1.73 m^2 by 24-h urine collection, insulin requirement >0.7 IU/kg/day or HbA1c >12%.

Screening of the study subjects

From November 2004 to May 2006, 88 individuals completed phone screenings, of whom 60 presented with exclusion criteria and 15 withdrew after screening because of the complexity of the study or potential risks involved (Table 1). Thirteen subjects were listed to receive an islet transplant, but three were excluded with unremediated medical conditions when recruitment closed, and the remaining 10 subjects received islet transplants.

Donor selection

Potential donors were screened at the time of organ offer. Donors with positive serology results (except cytomegalovirus), diabetes or suspected diabetes, sepsis, cancer or methanol toxicity were ruled out. Safety screening

Table 1: Screening outcomes and subject exclusions*

Candidate/Subject status	Phone screen n = 88	Pre-screen	Phase I Screen	Phase II Screen	Total
Excluded by screening	44	12	4	0	60
Withdrew from screening	8	5	0	2	15
Subjects excluded after listing for transplant	NA	NA	NA	NA	3
Subjects active	36	19	15	13	10

*Reasons for exclusion (60)

Age > 65 years	1
Diabetes manageable with intensive insulin therapy	6
Daily insulin requirement >0.7 IU/kg	7
Diabetes <5 years	2
Kidney disease	7
Previous organ transplant	2
Weight >70 kg or BMI >26	31
Smoking	2
Reported psychiatry disease/nonadherence to therapy	1
Referral to a center closer to home	1

Reasons for withdrawal after phone screening (15)

Desire for children	1
Potential risk of procedure/immunosuppression	7
Family illness	1
Unknown	6

Reasons for exclusion after listing for transplant (3)

Unable to control weight	1
New onset kidney	1
New onset heart disease	1

included criteria defined in the suitability determination for donors of human cellular and tissue-based products final rule and the Centers for Disease Control high-risk criteria for blood-borne pathogens. Quality screening exclusion criteria included donor age < 25 or > 75 years, warm ischemia, cold ischemia > 12 h, non-heart beating donors, and BMI < 19. Negative crossmatch with the donor was required for transplant. We routinely performed flow cytometeric T- and B-cell cross-match with a cut-off at 20 channel shifts for T and 40 channel shifts for B cells.

Islet isolation

The pancreata were trimmed, distended with cold Liberase solution (Liberase-HI, Roche, Indianapolis, IN), and digested using a modified Ricordi automated method (14). After collection and wash, the tissue was incubated in UW solution (DuPont Pharma, Bad Homburg, Germany) for 30 min. Islets were purified by a continuous UIC-UB gradient (15) on a cell separator (Cobe 2991, Cobe, Lakewood, CO) and then cultured in Culture Media (Mediatech, Herndon, VA) at 37°C for up to 12 h.

Islet evaluation

Islet yield: We assessed quantity and purity of the preparations by Dithizone staining (16).

Viability: The percentage of dead and live cells was estimated by fluorescent staining with Syto-Green/Ethidium Bromide (17,18).

In vitro *function*: Islet function was expressed as a stimulation index (SI) after static incubation with low and high glucose conditions (19).

Islet transplantation

After the portal vein was accessed percutaneously under fluoroscopic and ultrasound guidance, the islets were resuspended into 60 mL syringes and slowly injected into the intraportal catheter. During infusion, syringes were turned constantly to avoid sedimentation or clumping of islets. Heparin was administered throughout the procedure for a total dose of 5000 units per transplant followed by enoxaparin (Lovenox®) 30 mg subcutaneously twice daily for 1-week posttransplant.

Study medication

Edmonton protocol group 1: Based on the Edmonton protocol, subjects received an immunosuppressive regimen of daclizumab, sirolimus and tacrolimus (12). Subjects received daclizumab 1 mg/kg intravenously immediately before transplant and 75 mg at 2, 4, 6 and 8 weeks after transplant. Sirolimus was administered at a loading dose of 0.2 mg/kg immediately pretransplant, and continued at 0.1 mg/kg/day each morning. Tacrolimus 1 mg was administered immediately before transplantation, and then adjusted to maintain target trough levels of 3–6 ng/mL throughout the study (Figure 1).

UIC protocol group 2: In addition to the Edmonton protocol immunosuppression regimen, the UIC protocol included the TNF-alpha receptor antagonist, etanercept and the glucagon-like peptide-1 (GLP-1) analogue, exenatide. Patients received etanercept 50 mg intravenously before islet transplantation and 25 mg subcutaneously at 3, 7 and 10 days after transplant. Exenatide 5 µg was administered subcutaneously twice daily for 1 week within 60 min before or after the morning and evening meals. If tolerated well, the dose was increased to 10 µg twice daily for a total of 6 months after the last islet transplant.

Autoantibody measurement

Antibodies were measured before and after transplant. Alloantibodies were assessed by flow cytometry measurement of class I and II panel reactive antibodies (One Lambda Inc, Los Angeles, CA). Anti-islet antibodies were

assessed by indirect immunofluorescence (normal titers <1:4), and anti-GAD antibodies by radioimmunoassay (normal values 0–70 mGAD-U/mL).

Challenge test with or without exenatide

In Group 2 subjects, we measured frequently sampled intravenous glucose tolerance tests (FSIVGTT), C-peptide, proinsulin, amylin and glucagon levels with and without exenatide. Four subjects underwent FSIVGTT at least 12 h after withdrawal of exenatide. Subject 5 was not tested because of illness from infection subsequent to myonecrosis, and subject 9 did not tolerate exenatide. In a second session, the same subjects injected exenatide before testing. Group 1 subjects underwent only IVGTT without exenatide. We calculated acute insulin response to glucose as the mean of the 3-, 4-, and 5-min insulin values following the glucose injection subtracting the basal value (20). Insulin sensitivity index was calculated using the computer MINMOD mathematical model for FSIVGTT (21).

Statistical analysis

Data were expressed as mean ± SD. Comparisons were performed using two-tailed *t*-test, Paired *t*-test or Fischer's exact test where applicable. SPSS 13.0 (Chicago, IL) was used to run analyses. A p-value < 0.05 was considered statistically significance. Unless otherwise indicated, all data represent 15 months follow-up after the first islet transplant.

Results

Demographics

Recipients: Ten C-peptide negative diabetic subjects (nine females, one male) received 18 islet allo- transplantations over 2 years (Table 2). Subjects in Groups 1 and 2 had similar age distribution (49.2 ± 11.3 and 44.8 ± 10.0 years, p = 0.6), diabetes duration (30.2 ± 9.2 and 27 ± 10.8 years, p = 0.6), body weight (61.03 ± 4.27 and 63.8 ± 5.30 kg, p = 0.2), BMI (22.4 ± 1.08 and 22.35 ± 1.01, p = 0.4) and pretransplant insulin requirements (39.3 ± 2.9 and 32.1 ± 8.6 U/day, p = 0.5). HbA1c was significantly higher in Group 2 than Group 1 (6.5 ± 0.7 and 7.8 ± 1.1 p = 0.046). GAD autoantibody levels were also more elevated in Group 2 than Group 1 (41.8 ± 60.3 vs. 2.3 ± 2.1, p = 0.24). It should be noted that 2/6 patients in Group 2 had GAD levels greater than 100.

Donors and islet graft characteristics

Donor and graft characteristics for both groups are outlined in Table 3.

Safety of procedure and medications

Procedure-related events: Two bleeds occurred in 18 islet infusions (11% of total islet infusions, 20% of total subjects). One subject experienced an intraperitoneal bleed during withdrawal of the intraportal catheter, which was self-limiting and did not require transfusions or surgical intervention. A second subject with situs viscerum inversus, left isomerism, variant portal venous anatomy and several small hemangiomas developed an intrahepatic hematoma probably resulting from inadvertent puncture of a small hemangioma coupled with peri-procedural heparin. This subject received two units of packed red blood cells for low posttransplant hemoglobin, and received an additional
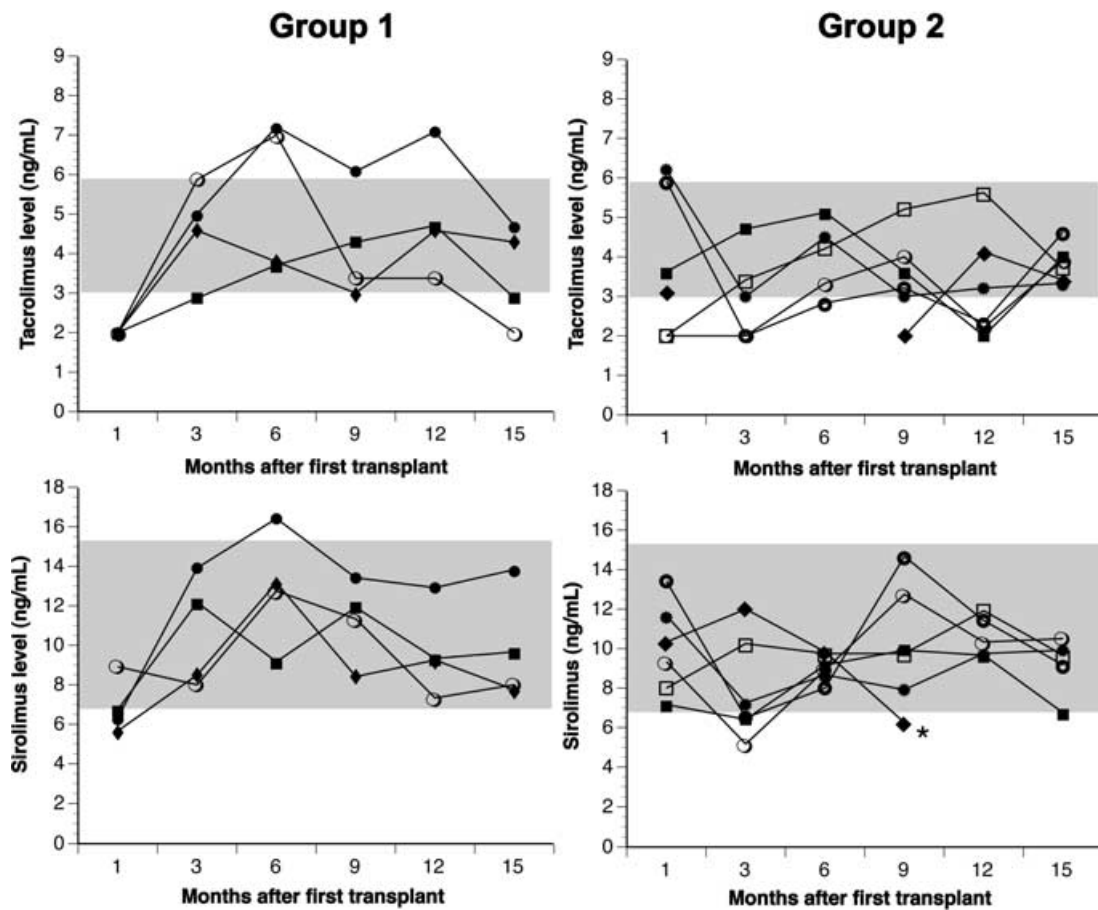
Figure 1: Immunosuppression drug levels for both groups during 15 months follow-up. Sirolimus dose was adjusted to maintain target trough serum blood levels of 12–15 ng/mL for the 3 months following the most recent islet infusion and lowered to 7–10 ng/mL subsequently. Target trough level for tacrolimus was 3–6 ng/mL throughout the study. Average AUC for tacrolimus level was 62.4 ± 14.1 in Group 1 versus 48.4 ± 8.1 in Group 2 (p = 0.14). *This patient developed sirolimus complications and switched to mycophenolate mofetil.

Table 2: Pretransplant characteristics for the 10 C-peptide-negative islet transplant recipients with hypoglycemic awareness

| | Recipient No. | | | | | | | | | | | |
| | Group 1 | | | | | Group 2 | | | | | | |
Characteristic	1	2	3	4	Mean(SD)	5	6	7	8	9	10	Mean(SD)
Age, years	61	56	43	35	48.8 (11.9)	39	46	37	62	36	47	44.5 (9.7)
Sex, F or M	F	M	F	F	–	F	F	F	F	F	F	–
Body weight, kg	54.9	66	60.7	61.7	60.8 (3.9)	71.4	61.8	60	62	66.3	58.6	63.4 (4.3)
Body mass index	22.9	21.0	20.8	23.0	21.9 (1.0)	22.3	22.5	18.6	23.1	24.1	21.0	21.9 (1.8)
Diabetes duration, years	36	37	32	17	30.5 (9.3)	29	34	21	10	14	24	22.0 (9.1)
Daily insulin, U/Kg	35	43	41	44	40.8 (4.1)	44	26	46	26	38	42	37.0 (8.9)
HbA1c	5.9	6.2	7.4	6.4	6.5(0.6)	8.1	6.9	8.2	9.5	6.7	6.7	7.8 (1.0)
Hyposcore	2,668	207	218	274	842 (1217.9)	189	2,576	228	71	793	1,020	812.8 (941.7)
Alloantibodies (class I & II PRA)	0	0	0	0	–	0	0	37	0	0	0	–
Autoantibodies:												
• Anti-GAD65	5.44	1.1	1.0	3.34	2.7 (1.8)	199.4	1.3	2.4	7.9	99.7	4.86	52.6 (74.4)
• Anti-ICA512	–	–	–	–	/	+	–	–	–	+	–	/

BMI = body mass index; PRA = panel reactive antibodies.

Table 3: Donor graft characteristics for the 10 C-peptide-negative islet transplant recipients with hypoglycemic awareness

	Group 1											Group 2						
	1			2		3		4			5	6	7		8	9		10
Transplant	1st	2nd	3rd	1st	2nd	1st	2nd	1st	2nd	3rd			1st	2nd		1st	2nd	
-Donor																		
Age, years	59	37	36	30	47	37	43	46	57	51	30	64	30	35	43	41	49	60
Sex, F or M	M	M	F	M	F	M	M	F	M	M	M	F	F	M	F	F	M	F
Body weight, kg	100	100	74	91	86	76	110	143.4	130	82.1	105	57.5	93.1	124	94	108	118	98
Body mass index	29	31	28	30.4	30.5	24	32	45.3	37	28	39	24.7	37.5	34.5	39.1	38	35	34.8
Cause of death	CVA	MVA	CVA	MVA	CVA	CVA	MVA	CVA	MVA	CVA	CVA	CVA	CVA	CVA	CVA	MVA	MVA	CVA
Blood glucose																		
• Admission	150	161	146	257	195	210	157	152	221	210	131	121	122	180	97	138	150	148
• Peak	167	252	378	257	195	227	233	187	221	477	451	261	159	190	155	150	162	148
• Preharvest	167	203	142	227	107	227	181	124	176	158	451	261	138	179	125	150	95	126
HLA-A/B matches	0	1	0	0	1	1	2	0	1	2	1	1	1	0	0	0	0	1
HLA-DR matches	0	2	1	0	1	1	0	0	1	2	–	0	0	2	0	1	0	1
-Graft																		
Cold ischemia time, hr	8.5	8	7	6.2	12	9.25	4	9.21	11	10	12	6.34	9.5	8	7.11	10.11	5	6.2
Tissue volume, mL	7.9	8	6.1	6.8	4.2	5	4.2	8.8	4.7	4.1	7.8	6.1	9	1.5	2.0	5.5	1.8	1.3
Total islet equivalent	652 000	378 000	386 000	405 000	569 000	450 000	372 000	716 000	1 094 000	921 000	417 000	377 000	635 000	633 000	450 968	883 000	482 000	462 000
Islet equivalent/kg	11 241	6798	6942	6136	8621	7413	6128	11 604	17 731	14 927	5840	6180	10 583	10 550	7273	13 318	7269	7884
Islet purity,%	50	30	55	60	50	70	50	50	60	65	30	70	60	90	70	40	60	45
Islet viability,%	90	80	90	95	85	87	90	80	95	90	80	90	95	92	90	90	90	90
Stimulation index	8.6	3.9	1.5	2.67	0.4	4.85	–	2.26	2.4	9.5	5.4	4.96	3.1	1.5	5.05	10	2.1	3.45
Endotoxin, EU/kg	0.91	0.46	0.04	0.26	0.15	0.74	0.01	1.18	0.56	0.06	1.12	1.11	1.05	0.01	1.08	1.03	0.18	1.17

CVA = cerebrovascular accident; MVA = motor vehicle accident.

three units after an unlucky blunt abdominal trauma provoking an intrahepatic rebleeding 6 weeks posttransplant. No interventions were needed for any of the islet transplant recipients. No portal vein thrombosis was observed.

Medication-related events: All subjects lost weight with a mean decrease from 62.3 ± 4.5 kg to 59.3 ± 5.6 (p = 0.2) after islet transplantation. BMI was reduced significantly from 22.5 ± 1.2 to 21.6 ± 1.5 (p = 0.04). Group 2 lost insignificantly more weight than Group 1 (3.4 ± 2.2 vs. 2.5 ± 3.1 kg, p = 0.6).

Most subjects could tolerate the immunosuppressive regimen. One recipient in Group 2 developed viral stomatitis, severe anemia and elevated creatinine that resolved after switching from sirolimus to mycophenolate mofetil (22). As a cohort, there was no significant change in kidney function (0.78 ± 0.17 at base line to 0.93 ± 0.29 mg/dL after 15 months for Group 1, p = 0.11 and 0.97 ± 0.2 to 1.07 ± 0.41 for Group 2, p = 0.35). However, one subject in Group 1 and two subjects in Group 2 showed some increase in creatinine from the baseline (Figure 2). All recipients (10/10) developed transient anemia.

All subjects (6/6) in Group 2 experienced temporary nausea and/or vomiting and weight loss. Two subjects in Group 2 could not tolerate exenatide, one with preexisting diabetic autonomic gastroparesis, and the other with persistent vomiting and weight loss. Another subject in Group 2 developed myonecrosis of the right scalene muscle attributed to methicillin sensitive staph aureus (MSSA) vertebral osteomyelitis (C7-T1) that resolved with antibiotic therapy and reduced immunosuppression. This subject developed partial graft failure and resumed insulin therapy. Ultimately, she withdrew from the study because of medication side effects. One subject in Group 1 underwent an abdominal hysterectomy for irregular menstrual bleeding and ruptured ovarian cyst possibly related to immunosuppressive medications.

Primary efficacy endpoint
Insulin-independence: All subjects completed the 15 months posttransplant follow-up and became insulin-independent after one or more islet transplants (Figure 3). In Group 1, all subjects remained insulin-free for the 15-month posttransplant observation period. Group 1 subjects received a mean total number of islets (EIN) of 1460 080 ± 418 330 in 2 (n = 2) or 3 (n = 2) islet infusions to achieve primary insulin-independence (Figure 4). All patients in Group 2 became insulin-independent after the first transplant (537 495 ± 190 968 EIN, p = 0.028 compared to Group 1). Four of the six Group 2 subjects remained insulin-independent at the end of the 15 months follow-up. In Group 2 two subjects resumed insulin. One subject had partial graft failure at 19 weeks posttransplant when immunosuppression was reduced during an infectious complication (scalene myonecrosis and MSSA
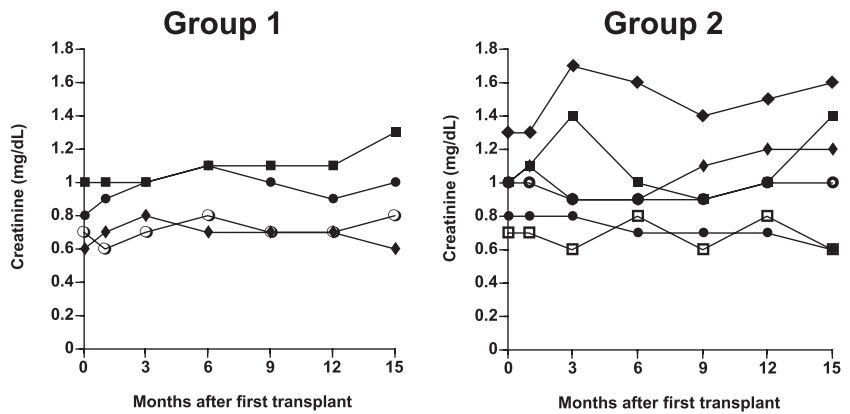
Figure 2: Creatinine levels for both groups during 15 months follow-up.

vertebral osteomyelitis). The second subject, who had severe preexisting gastroparesis and ongoing nausea, vomiting and weight loss, discontinued exenatide and resumed insulin 17 weeks post transplant. This subject received a second transplant and again became insulin-independent, but resumed insulin 5 months posttransplant. One subject experienced an increase in HbA1c to 6.5% and received a second islet preparation to avoid resuming insulin injections. Ultimately, subjects in Group 2 received a mean total EIN of 723 328 ± 461 446 in 1 (n = 4) or 2 (n = 2) islet infusions (Figure 4). During the 15 months observation period, none of the 10 patients in either group presented with complete graft failure.

Secondary endpoints

HbA1c: HbA1c reached normal range in both groups, decreasing from 7.2 ± 1.1% at baseline to 5.9 ± 0.4% (p = 0.001) after transplant. HbA1c decreased from 6.5 ± 0.6 at baseline to 5.6 ± 0.2 after at least two transplants in Group 1. HbA1c reached normal levels for subjects in Group 2 after one islet transplant, decreasing from 7.8 ± 1.0 at baseline to 6.1 ± 0.3 (Figure 3). It should be noted that the baseline HbA1c was significantly higher in Group 2 than Group 1 (p = 0.046).

Fructosamine: Fructosamine reached normal levels after transplant for subjects in both groups, decreasing from
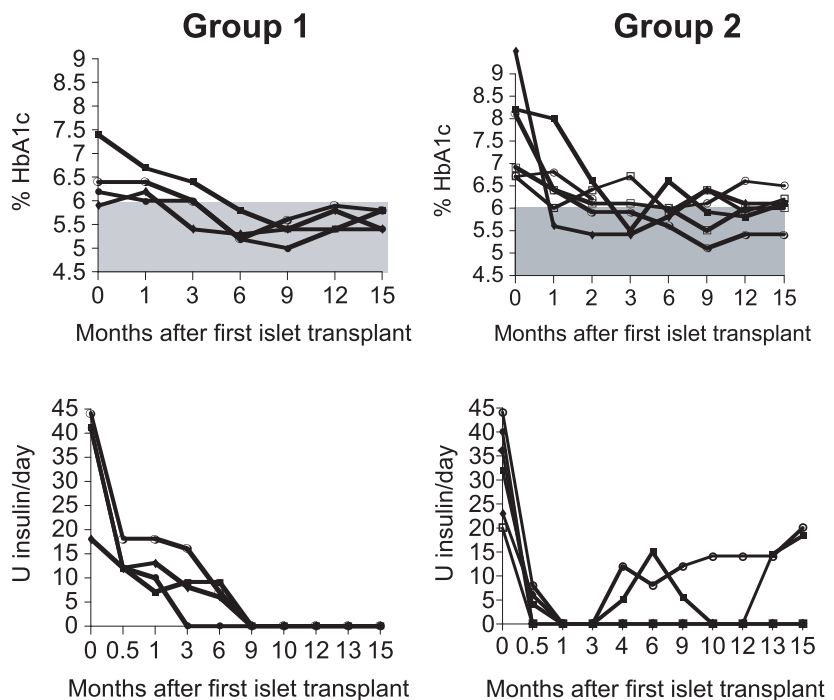

Figure 3: HbA1c levels for both groups during 15 months follow-up (top panels). Exogenous insulin requirement (U insulin/day) during 15 months follow-up for both groups (lower panels). All patients became insulin- independent when adequate islets were provided.
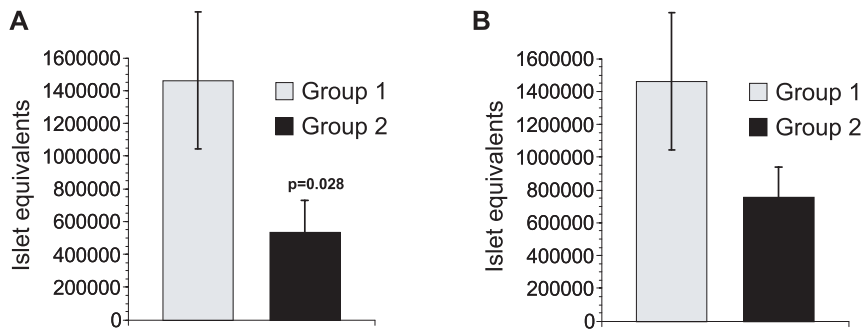
Figure 4: Total number of islets expressed as Islet Equivalent required to achieve insulin-independence in both groups. (A) Total number of islets transplanted in both groups during the entire study period (B).

296.7 ± 44.2 µmol/L at baseline to 244.8 ± 36.6 µmol/L (P = 0.01) after transplant.

Oral glucose tolerance test: At 12 months posttransplant, one subject in Group 1 showed diabetic oral glucose tolerance, one subject had borderline glucose intolerance, and two subjects showed a normal glucose tolerance. In Group 2, three subjects had impaired glucose tolerance, and two had diabetic glucose tolerance. One subject resumed insulin and was not tested.

Mixed meal test: The MMT showed a nondiabetic response to glucose stimulation testing with appropriate C-peptide response in all Group 1 subjects and in 4/5 of Group 2 subjects.

Glucagon stimulation test: At entry into the study, all subjects demonstrated absence of C-peptide (< 0.3 ng/mL) in response to GST and MMT. Posttransplant, all subjects became C-peptide positive and maintained normal levels of C-peptide for the entire study duration. In Group 1 all subjects showed significant increase of C-peptide after glucagon stimulation. In Group 2, 4 subjects underwent GST. One subject had a low response, though initial glucose level was low, which is known to reduce C-peptide response. All subjects demonstrated increased insulin production in response to glucagon stimulation.

Intravenous glucose tolerance test: IVGTT showed a similar glucose disappearance pattern in all patients. However, insulin response varied among individuals, with most presenting an intermediate acute insulin response (10).

Hypoglycemia: All subjects entered the study with multiple episodes of severe hypoglycemia without awareness. During the follow-up period, there was no recurrence of severe hypoglycemia. We applied Ryan's formula to quantify pretransplant scores. After islet transplantation, we calculated actual HYPO scores from subjects' daily blood sugar records. Pretransplant HYPO score was 841.8 ± 1217.9 and 812.8 ± 941.7 for Group 1 and 2 respectively. Posttransplant HYPO score was 0 and 33.5 ± 50.0 for Group 1 and 2 respectively (p < 0.01, compared to pretransplant values, Table 4 and Figure 5C). Two subjects in Group 2 presented with mild hypoglycemia after exenatide injection.

Glycemia: As shown in Figure 5A, before transplant subjects in both groups exhibited wide variability in daytime glucose levels. After transplantation, both groups clearly showed tighter, more regular glucose control (Figure 5B).

Challenge tests with and without exenatide: To further study the metabolic effect of exenatide on islet performance, we compared frequently sampled intravenous glucose tolerance tests (FSIVGTT), C-peptide, proinsulin, amylin and glucagon with and without exenatide.

Frequently sampled intravenous glucose tolerance tests: In Group 2 the AIRg (postglucose acute insulin response) increased from 2.2 ± 1.2 to 2.7 ± 1.6 after taking exenatide (p = 0.4). In Group 1 the AIRg in was 2.4 ± 0.9 (p = 0.8) without exenatide. In our subjects, we did not find any intrapatient relationship between the response to IVGTT and OGTT (Table 5).

Intravenous glucose tolerance testing: On IVGTT conducted after subjects injected 5 µg of exenatide, C-peptide increased from 1.9 ± 0.2 to 2.5 ± 0.6 (p = 0.3). Proinsulin increased from 5.7 ± 1.6 to 7.8 ± 2.3 (p = 0.3) and amylin increased from 7.2 ± 2.3 to 14.7 ± 6.6 (p = 0.1).

Proinsulin to insulin ratio: The proinsulin to insulin ratio decreased after exenatide in all subjects (7.1 ± 2.1 to 4.3 ± 1.9, p = 0.02), pointing toward more efficient proinsulin to insulin processing with exenatide.

Glucagon: Glucagon levels decreased significantly in all subjects. The delta decrease was 18.7 fold more significant after exenatide in all subjects (Δ change 8.9 ± 18.4 vs. 165.7 ± 46.4 w/o and with exenatide, respectively; p = 0.04).

Autoantibodies: Two subjects in Group 1 and 5 subjects in Group 2 tested positive for anti-GAD 65. Of the 10 subjects, only 2 (both from Group 2) were positive for anti-ICA 512. Neither subject remained insulin-independent.

Long-term follow-up
The study was originally designed for a 15-month posttransplant follow-up. We offered subjects an additional

5-year follow-up which 8/10 subjects accepted. At the time of writing this report, two of the four subjects from Group 1 remain insulin-independent with an average follow-up of 30.3 ± 0.9 months. These two subjects maintain HbA1c values between 5.5 and 5.7 at almost 3-year follow-up. Of the two subjects taking insulin, one was withdrawn from immunosuppression because of lobular breast carcinoma after 19 months. Subject 1 in Group 1 developed breast cancer after 19 months follow-up and insulin-independence. This patient was 64 years old at the time of diagnosis and underwent surgery and chemotherapy and she is doing well with recovery and no signs of distant disease and no local recurrence more than 1 year later. Subject 4 in Group 1, experienced graft failure 18 months posttransplant for unknown reasons, requires 27–30 units of insulin daily, and maintains HbA1c at 6.7. All subjects in Group 2 maintain HbA1c values between 5.8 and 6.3 with average follow-up of 21.2 ± 4.1 months. Four of the six subjects in Group 2 remain off insulin. Of the two subjects on insulin, subject 5 withdrew from the study and subject 9 was unable to tolerate exenatide and resumed 15–20 U of insulin daily by pump while awaiting a third transplant.

Discussion

This study demonstrates that the addition of exenatide and etanercept to the Edmonton protocol is associated with a significantly lower number of islets required initially to achieve insulin-independence. In our cohort, recipients of the Edmonton protocol received either two or three sequential islet transplantations at least 2 weeks apart to achieve insulin-independence. By comparison, the subsequent six subjects who were treated under the UIC protocol with the addition of exenatide and etanercept achieved insulin-independence after only one islet preparation, though during the follow-up, two subjects required an additional transplant to maintain insulin-independence and one of these subjects resumed insulin 5 months after the second transplant.

The salutary effect of exenatide on decreasing initial islet requirement is largely unknown. However, evidence exists that exenatide can increase insulin synthesis under elevated glucose levels (23) and has antiapoptotic and β-cell proliferative effects in both rodent and human β-islets in *in vivo* and *in vitro* experiments (24,25). Moreover, the antiinflammatory effects of etanercept (26) likely contributed to early islet function and improved engraftment. In fact, the University of Minnesota group has successfully used the peri-operative administration of etanercept to achieve insulin-independence after transplant of a marginal dose of islets from a single donor (5). In addition, another recent report has confirmed the efficacy of IL-1 receptor antagonist in preventing the inflammatory β-cell destruction in diabetes type II (27). We found that acute insulin response to glucose was increased, although not significantly, in recipients of the UIC protocol considering they received fewer

**Table 4:** Outcome of the study 15 months posttransplantation

| | Recipient No. | | | | | | | | | | | | | |
| | Group 1 | | | | | Group 2 | | | | | | | |
Variable	1	2	3	4	Mean (SD)	5	6	7	8	9*	10*	Mean (SD)
Body weight, kg	51.3	68.9	57.6	63.0	60.2(6.5)	69.4	54.9	56.7	61.8	58.5	55.8	59.5(4.9)
BMI (kg/m²)	21.3	22.4	20.0	23.1	21.7(1.2)	22.0	21.1	17.5	22.3	21.0	19.8	20.6(1.6)
Total islet mass/kg (No. of transplants)	24 981 (3)	14 757 (2)	13 541 (2)	44 262 (3)	24 385 (14 209)	5840 (1)	6180 (1)	21 133 (2)	7273 (1)	20 587 (2)	7884 (1)	11 483(7302)
Daily insulin, U/kg	0	0	0	0	–	20	0	0	0	18.3	0	–
HbA1c	5.4	5.4	5.8	5.8	5.6(0.2)	5.4	6.0	6.1	6.1	6.5	6.2	6.1(0.3)
Hyposcore	0	0	0	0	–	83	0	6	1	111	0	–
OGTT	IGT	NL	DM	NL	–	–	DM	DM	IGT	IGT	IGT	–
Alloantibodies (class I & II PRA)	0	0	0	0	–	0	0	51	0	0	0	–
Autoantibodies:												
• Anti-GAD651	6.2	1.0	1.0	1.6	2.5 (2.2)	117.2	1.1	1.2	6.3	100.7	1.1	37.9 (50.5)
• Anti-ICA512	–	–	–	–	–	+	–	–	–	+	–	–

BMI = body mass index; PRA = panel reactive antibodies; OGTT = oral glucose tolerance test; IGT = impaired glucose tolerance; NL = normal; DM = diabetes mellitus. *These two subjects did not tolerate exenatide (<2 months taken).

A. Glycemic Control before Islet Transplant
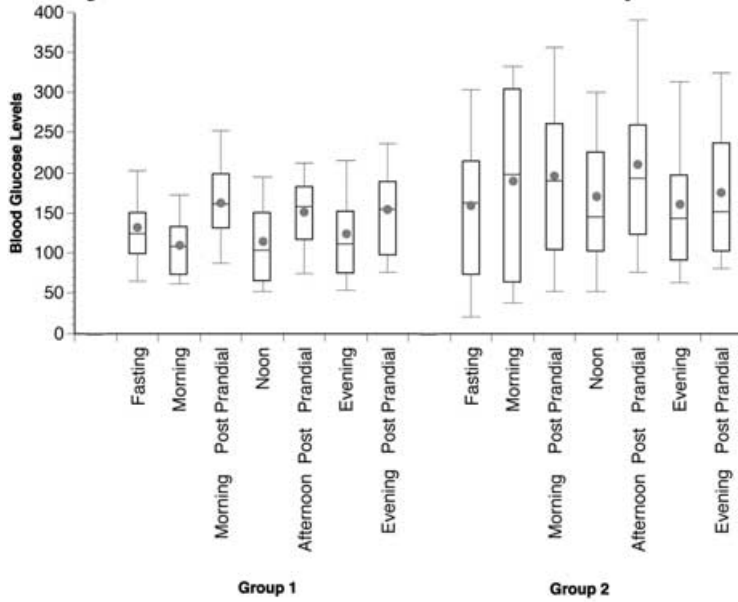
B. Glycemic Control after Islet Transplant
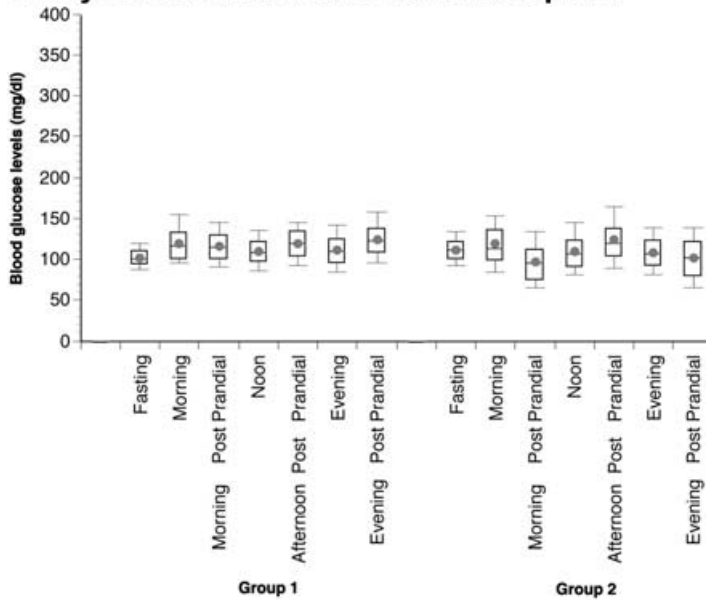
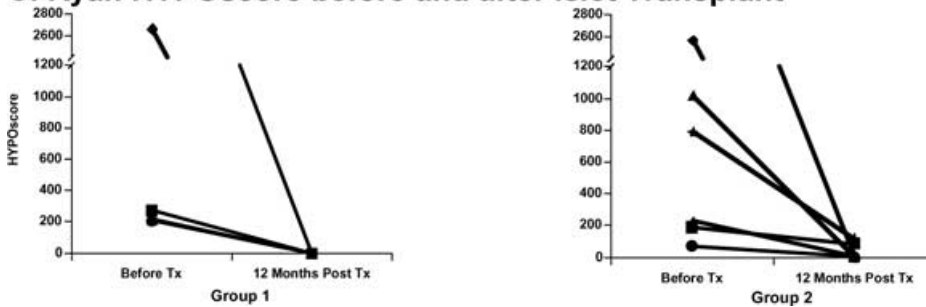## C. Ryan HYPOscore before and after Islet Transplant

Figure 5: Glycemic control before (Panel A) and after (Panel B) transplantation in both groups. The top, bottom and line through the middle of the box correspond to the 75th percentile, 25th percentile, and 50th percentile respectively. The whiskers on the bottom extend from the 10th percentile and top 90th percentile, • represents the arithmetic mean. Panel C shows the frequency and severity of hypoglycemia before and after transplantation assessed by Ryan HYPOscore. HYPOscore was significantly decreased after transplant for both groups (p < 0.01). Two subjects in Group 2 experienced occasional, nonsevere, postprandial hypoglycemia while under exenatide.

Table 5: Oral glucose tolerance test (OGTT) and Intravenous glucose tolerance test (IVGTT) results 12 months after the first transplant

	Recipient No.									
	Group 1				Group 2					
	1	2	3	4	5	6	7	8	9	10
OGTT	IGT	NL	DM	NL	–	DM	DM	IGT	IGT	IGT
IVGTT										
Acute insulin response (mU.L^{-1}.min)	–	33.33	3.67	18.66	10.33	3.66	17.66	15.3	7.33	18.66
Sensitivity index ((mU/L)$^{-1}$.min^{-1})	–	3.96	3.22	13.6	0.42	0.014	3.58	3.6	0.042	1.65

islets than recipients of the Edmonton protocol. It is likely that a larger number of islets survived in subjects treated with the UIC protocol as compared to the amount of the islets in recipients of the Edmonton protocol. This suggests that the addition of etanercept and exenatide may improve islet engraftment. It would be interesting to see if administering exenatide for a longer period of time could enhance the 'trophic' effect of the drug. None of the patients treated with the UIC protocol achieved normal OGTT, showing IGT or DM responses, while normal OGTT was observed in 50% of the subjects treated with two to three transplants and the Edmonton protocol. The islet mass in UIC treated subjects is presumably adequate under normal condition. However, under stress and excess demand during OGTT, the islets in these subjects could not meet the demand, resulting in IGT or DM. This indicates that UIC protocol-treated patients have lower islet mass, which apparently did not increase under exenatide treatment. An alternative speculation may be considered as well: the finding of decreased pro-insulin to insulin ratio after exenatide in all subjects tested can point toward a more efficient pro-insulin to insulin conversion. We expected a more significant increase in C-peptide, proinsulin and amylin after exenatide due to the proposed effect of the GLP-1 analogues on augmentation of pancreatic β-cell response to glucose stimulation via receptors on the β-cells (28,29). However, in our subjects there was no significant increase, and only one subject, as well as a healthy, nondiabetic control showed an increase in the acute insulin response to an intravenous glucose challenge when exenatide was given before the test.

Decrease in glucagon is a known effect of GLP-1 and its analogues that was recently validated by Ionut et al. (30), who tested the effect in dogs. They infused glucose in combination with GLP-1 similar to the meal response. Results showed no augmentation of insulin stimulation. The failure of GLP-1 to augment the insulin secretory response in their experimental setting suggested a possible indirect effect such as reduction in glucagon and is consistent with our results. In fact, we found a significant decrease of glucagon levels in subjects receiving exenatide.

It is important to note that the majority of subjects receiving the UIC protocol did not demonstrate any evidence of a potential 'exhaustion effect' after prolonged administration of exenatide on β-cell capacity to secrete insulin.

The adverse effects of exenatide include mainly nausea and vomiting. Nausea is common with initial doses and tends to diminish over time. As a result of persistent nausea and vomiting, two of our subjects did not tolerate exenatide longer than 2 months. The side effects of etanercept include serious nervous system disorders such as multiple sclerosis and seizures, blood disorders, increased risk of lymphoma and injection site reactions. However, these have been reported in long-term treatment for autoimmune diseases such as rheumatoid arthritis, psoriasis and ankylosing spondylitis; Group 2 subjects received only four doses of etanercept during the first 10 days posttransplant.

In our study, two subjects developed serious adverse events: a 64-year-old patient in the first group, treated with Edmonton protocol, developed breast cancer 19 months posttransplant and another subject receiving the UIC protocol developed diabetic myonecrosis on her neck, which was complicated by muscle and bone infection. The pathophysiology of diabetic myonecrosis is not well understood. Thromboembolism superimposed on diabetic small-vessel disease and subsequent ischemia-reperfusion injury has been suggested as the major underlying mechanism. A few cases of diabetic muscle infarction have been reported after simultaneous pancreas-kidney transplants. Underlying diabetic microangiopathy and hypercoagulability have been proposed as contributing factors in development of myonecrosis in these patients. Furthermore, tacrolimus and cyclosporine have been shown to be associated with thrombotic microangiopathy and impaired endothelial function. In our patient, the immunosuppression therapy may have had a contribution to this late infectious complication.

As this was a pilot study, its major weakness is the relatively small sample size and potential for both type II and type I error. In addition, the lack of a concurrent control group as opposed to the sequential study design is less than ideal. Furthermore, while four of six subjects in the UIC protocol required fewer islets than those in the standard Edmonton protocol, two subjects did need to resume insulin therapy. Since two subjects in Group 2 did not tolerate exenatide, its effects may have been underestimated in this pilot study.

Despite these caveats, the results presented are extremely promising. The islet equivalents utilized to achieve

insulin-independence in the UIC protocol arm of the study were lower than the sequential Edmonton protocol group and among the lowest reported in the literature. Few adverse long-term effects were seen, and while subjects on exenatide did experience high rates of nausea, this usually abated within 1 to 2 weeks.

In summary, utilization of exenatide and a short course of TNF alpha blockade shows promise in decreasing the need for multiple donors and warrants further investigation. While the presented protocol allowed for consistently achieving insulin-independence with lower islet mass, it did not lead to a measurable increase in functional islet mass over time. Future studies will have to investigate whether the speculated 'trophic' effect of exenatide could be enhanced by higher drug levels and prolonged administration. In our opinion, this will require improving the pharmacokinetics of exenatide, avoiding peaks and allowing for extended release. Such pharmacokinetics may decrease the likelihood of the reported side effects, and may allow for achieving higher overall drug exposure. Therefore, we suggest that different treatment regimens with exenatide (e.g. extended release formulations) should be investigated in future studies.

Acknowledgments

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