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Outcomes of Pancreatic Islet Allotransplantation Using the Edmonton Protocol at the University of Chicago

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Objective. The aim of this study was to assess short-term and long-term results of the pancreatic islet transplantation using the Edmonton protocol at the University of Chicago. **Materials and Methods.** Nine patients underwent pancreatic islet cell transplantation using the Edmonton Protocol; they were followed up for 10 years after initial islet transplant with up to 3 separate islet infusions. They were given induction treatment using an IL-2R antibody and their maintenance immunosuppression regimen consisted of sirolimus and tacrolimus. **Results.** Nine patients received a total of 18 islet infusions. Five patients dropped out in the early phase of the study. Greater than 50% drop-out and noncompliance rate resulted from both poor islet function and recurrent side effects of immunosuppression. The remaining 4 (44%) patients stayed insulin free with intervals for at least over 5 years (cumulative time) after the first transplant. Each of them received 3 infusions, on average 445 000 islet equivalent per transplant. Immunosuppression regimen required multiple adjustments in all patients due to recurrent side effects. In the long-term follow up, kidney function remained stable, and diabetic retinopathy and polyneuropathy did not progress in any of the patients. Patients' panel reactive antibodies remained zero and anti-glutamic acid decarboxylase 65 antibody did not rise after the transplant. Results of metabolic tests including hemoglobin A1c, arginine stimulation, and mixed meal tolerance test were correlated with clinical islet function. **Conclusions.** Pancreatic islet transplantation initiated according to Edmonton protocol offered durable long-term insulin-free glycemic control in only highly selected brittle diabetics providing stable control of diabetic neuropathy and retinopathy and without increased sensitization or impaired renal function. Immunosuppression adjustments and close follow-up were critical for patient retention and ultimate success.

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Impaired counter-regulatory responses caused by repetitive episodes of iatrogenic hypoglycemia in patients with type 1 diabetes mellitus (T1DM) results in hypoglycemia associated autonomic failure.[1] Frequently, they suffer from hypoglycemia-related altered mental status or seizure, which are potentially life-threatening. These patients live in constant fear of sudden death, and their quality of life is severely compromised.[2]

Pancreas transplantation is the only effective option for those selected brittle T1DM patients who experience hypoglycemic unawareness despite optimized insulin regimen.[3,4] Pancreas transplantation restores glycemic control and hypoglycemic awareness instantly in patients with a functional graft. Currently, 50% to 80% of patients are still insulin free 5 years after pancreas transplant with good control of secondary diabetic complications.[5] However, the morbidity and mortality associated with the surgery and the adverse effects of immunosuppression limit the use of this surgical option only to a small patient population.[6,7] In contrast, islet transplantation is a minimally invasive procedure with much lower morbidity. The successful results presented by the group from Edmonton in 2000 prompted us to test the same novel approach in our center.[8] Soon afterward, we initiated a similar clinical study to test the safety and effectiveness of the Edmonton protocol in patients with brittle T1DM. In this communication, we report the short-term and long-term outcomes including the challenges related to patient selection, compliance, and side effects of immunosuppression.

MATERIALS AND METHODS

Study Design

In 2004, we initiated an FDA-approved phase 1/2 clinical study at University of Chicago to test the safety and effectiveness of the human pancreatic islet transplantation for prevention of severe hypoglycemia in brittle T1DM patients. Safety was quantified based on the incidence, timing, and severity of adverse events as well as their relationship to the islet procedure and other protocol-specific products (immunosuppressive agents). Effectiveness was assessed based on the ability of transplanted allogeneic islets to counter hyperglycemia as measured by insulin independence, avoidance of hypoglycemic unawareness, hemoglobin A1c (HbA1C), c-peptide production, mean amplitude of glycemic excursion (MAGE), and responses to provocative testing: arginine stimulation test and mixed meal tolerance test (MMTT). Subjects were considered to have completed the study, if they received the islet transplants (up to 3 infusions and total maximum of 30 000 islet equivalents (IEQ)/kg) with the goal to achieve and maintain insulin independence. Patients were seen for follow-up (f/u) examinations weekly for 2 weeks, then every 2 weeks for 6 weeks, then monthly for the first 5 years and every 3 months after that. Neurological and eye evaluations were performed once a year.

Patient Selection

Screening intake questionnaire was distributed to all potential candidates who inquired about islet cell transplantation. Clarke score of 4 or higher was used to screen for hypoglycemia unawareness.[9] Individuals were selected based on our inclusion/exclusion criteria (Table 1), endorsement from their primary care physician/endocrinologist, and on history of medical compliance. Selected patients were invited to our clinical research center where they were provided with details of the study, and informed consent was obtained thereafter. Those who agreed were sent for laboratory testing and endocrine and cardiac evaluation. Patients who completed the evaluation were placed on the United Network for Organ Sharing waiting list for the islet transplant.

TABLE 1.

Inclusion and exclusion criteria for the study

Inclusion criteria	Exclusion criteria

• Age 18-58 years
  •Type I diabetes mellitus for at least five years
  •Undetectable fasting C-peptide
  •Patients must be on an intensive regimen of glucose monitoring and exogenous insulin injection (defined as greater than or equal to three checks and injections per day)
• Despite this intensive therapy, patients must have at least one of the following:
  ○ Brittle diabetes (metabolic instability), as defined by elevated mean amplitude of glycemic excursion
  ○ Hypoglycemic unawareness, with at least one episode in the past two years in which hypoglycemia required the assistance of another person (e.g., family member, EMT, etc.), was associated with a FSBG of < 50 mg/dl and prompt recovery after administration of oral glucose, intravenous glucose, or glucagon
  ○ Progressive complications of diabetes (nephropathy manifested by proteinuria, retinopathy documented by an ophthalmologist after dilated eye exam, or neuropathy as determined by a neurologist)
• Patients must be able to give informed consent
	• Failure to meet inclusion criteria
	• PRA > 50%
	• Creatinine clearance < 80 mL/min
	• Prior organ transplant
	• Portal hypertension
	• Abnormal liver function tests
	• History of malignancy
	• Active peptic ulcer disease
	• Pregnancy, or inability to comply with contraceptive regimen
	• Severe unremitting gastroparesis or diarrhea
	• Active infection or serologic positivity for HIV and/or hepatitis
	• Chest radiographic abnormality consistent with neoplastic or infectious disease
	• Major ongoing psychiatric illness and/or substance abuse
	• Noncompliance with current medical regimen
	• Obesity (BMI > 28)
	• Any other medical condition precluding safe transplantation and immunosuppression
	• Ejection fraction <30 %
	• MI within the past 6 months
	• Known allergies or hypersensitivity to immunosuppressive agents used in this protocol
	• Inability to provide informed written consent.

BMI, body mass index, EMT, emergency medical technician; FSBG, fingerstick blood glucose; PRA, panel reactive antibody.

Islet Isolation and Transplantation

All islets were isolated from brain dead donors. Donors were excluded, if their HbA1C was greater than 6% or if they were considered high risk according to the Centers for Disease Control.[10] The pancreas was retrieved during multiorgan procurement and preserved in cold storage with the standard preservation solutions: (Organ Recovery System, USA) or histidine-tryptophan-ketoglutarate (Köhler Chemie GmbH, Germany). Islets were isolated in a good manufacturing practice facility at the University of Chicago using the Edmonton protocol with standard semiautomated procedure.[11] In 3 cases, islets were isolated in the University of Illinois Good Manufacturing Practice facility according to the same protocol.

Briefly, collagenase (Liberase; Roche, Indianapolis, IN) solution was infused through the main pancreatic duct, and the organ was digested in the Ricordi Chamber (Biorep Technologies, Miami, FL). After digestion, all tissue was collected, and islets were purified with a continuous density gradient in the COBE 2991 Cell Processor (Caridian BCT, Lakewood, CO). Blood group compatibility, negative crossmatch, Gram stain, and endotoxin level with viability over 85% were confirmed before the transplant. Islets were suspended in the Transplant Media (Mediatech, Herndon, VA) with 70 U/kg body weight of heparin and infused within 8 hours after isolation into the portal vein, which was percutaneously accessed under local anesthesia by an interventional radiologist. Patients received fractionated heparin subcutaneously for 14 days after the procedure.

Patients were followed up once weekly for the first 2 weeks, and then every 2 weeks for 6 weeks. Thereafter, all patients had a monthly f/u for 5 years at which point the patients' f/u frequency was every 2 to 3 months.

Immunosuppression

Initial immunosuppression consisted of Daclizumab (Zenapax; Hoffman-La-Roche, Nutley, NJ) for induction, sirolimus (Rapamune; Wyeth Pharmaceuticals, Philadelphia, PA), and low dose (through 3- 6 ng/ml) of tacrolimus (Tacro) (Prograf, Astellas, Deerfield, IL) for maintenance according to the Edmonton Protocol as previously described.[8] The target range for sirolimus through levels was 12 to 15 ng/mL for 3 months, and 7 to 10 ng/mL thereafter. Immunosuppression was modified whenever clinically necessary.

Assessment of Glycemic Control and Islet Graft Function

Patient monitoring included finger-stick glucose levels, plasma fasting glucose, exogenous insulin requirements, and HbA1c. In addition, patients were asked to complete 7 capillary glucose readings per day in 2 days to calculate the MAGE score.[12] The β cell score was calculated,[13] and hypoglycemic episodes were recorded.[14] Insulin independence was recorded in those patients, who did not require insulin support to maintain fasting blood glucose less than 126 mg/mL and postprandial glucose less than 180 mg/mL with A1c ≤6.1. Partial islet function was recognized in patients requiring insulin support, when serum c-peptide was greater than 0.5 ng/ml.

Assessment for Peripheral Neuropathy

Subjects underwent a neurological examination directed at detecting early distal neuropathy before the transplant and yearly thereafter. A neuropathy score was calculated based on: weakness in the distal foot muscles, vibratory sensation at the big toes and ankles using a 128-Hz tuning fork (in seconds), pinprick sensation at the distal lower limits (in centimeters from the tip of the big toe if there was any loss), and ankle reflexes. The subjects also underwent a nerve conduction study at the same time intervals by the same examiner (K.R.) using a Teca Synergy machine. That consisted of assessing the amplitude and conduction velocities of bilateral sural, right radial, and ulnar nerves, as well assessment of the motor nerve amplitudes, velocities, distal, and F wave latencies of the right peroneal and ulnar nerves.

Statistical Analysis

Paired *t* test was used to compare the β cell score, MAGE score, and HbA1c levels before islet transplantation and the most recent value. Significance was taken at a *P* value less than 0.05.

RESULTS

Patient Screening and Selection

Of 975 individuals who inquired about the study and received screening questionnaire, 285 (29%) patients filled out and returned questionnaire (Figure 1A). Two hundred forty-four were subsequently excluded (Figure 1B): 152 (62%) based on inclusion/exclusion criteria; another 67 (28%) were excluded lacking endorsement from primary physician or local endocrinologist due to medical noncompliance, and finally 25 (10%) patients decided not to pursue islet cell transplantation. The remaining 41 patients signed informed consent and proceeded with further evaluation (Figure 1C). Half of them, 20 (48.7%) patients, were excluded by our study endocrinologist because their glucose control substantially improved after optimization of insulin therapy. Twelve patients voluntarily withdrew or were excluded, leaving 9 patients who proceeded with at least 1 islet transplant. The breakdown of the different reasons for patient exclusion during selection process is presented in Figure 1.

Islet Transplantation

Characteristics of all 9 patients who received at least 1 islet transplantation are presented in Table 2. The median age was 42 (19-57) years, and the mean body mass index was 22.5 (19-27). All together, 18 islets infusions were performed. Average islet mass was 7400 IEQ/kg per infusion. Because 5 of 9 patients dropped out in early phase of the study and only 4 individuals accomplished long-term f/u, they will be presented separately (patients A, B, C, D in Table 2).

Patient Withdrawals

Five individuals did not complete the transplant protocol and were removed in early phase of the study. Four of those patients received single infusion and withdrew within first year and 1 of them received 2 infusions and dropped out in the second year after the first islet transplant. Average IEQ was 406 518 per infusion, whereas IEQ/kg was 5738.

Three of the patients chose to withdraw from the study secondary to persistent adverse effects of sirolimus and poor islet function. Two of them were terminated from the study personnel after the first infusion due to noncompliance (Figure 2).

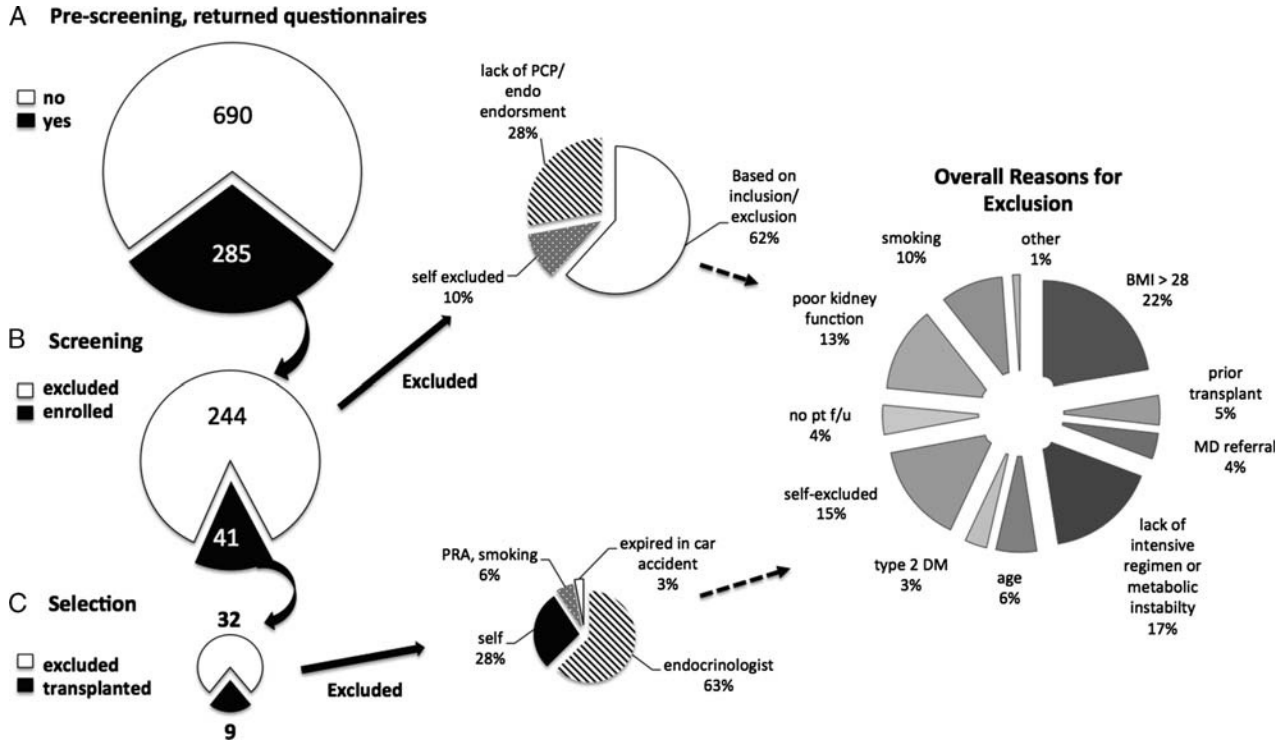


FIGURE 1. Patient screening and selection for the study. A, Prescreening, returned questionnaires. B, Screening. C, Selection. BMI, body mass index; endo, endocrinologist; MD, medical doctor; PCP, primary care physician.

Patients Who Accomplished the Study

Four (44%) of 9 transplanted patients completed the protocol and the length of their f/u is as follows: patient A for nearly 11 years (131 months), patient B for nearly over 10 years (116 months), patient C for 7 years (84 months), and patient D for 9 years (110 months) after their first islet infusion (Figure 3A). All those patients received 3 separate islet infusions. Average dose was 445 000 (225 000-719 000) IEQ per transplant or 7400 (4400-11 000) IEQ/kg per dose (Figure 3B). All islets for transplants were isolated from a single donor pancreas besides transplant numbers 2 and 3 for patient D, where islets from 2 donors were combined.

Glycemic Control and Islet Graft Function

Five patients who withdrew from the study during the early phase did not experience improved glucose control. They had very poor islet function with undetectable or minimal c-peptide, continued to experience severe hypoglycemic episodes as well as side effects related to immunosuppression. The remaining 4 patients presented dramatically improved glycemic control with elimination of hypoglycemic unawareness. We present rates of prevention of severe hypoglycemic episodes when maintaining HbA1c less than 7 mg% 1 and 5 years after initial infusion as well as 1 year after last infusion (Table 3). Insulin independence rates and partial islet graft function were assessed at the same time points. All 4 patients experienced long-term insulin independence and partial islet function with low insulin requirements before subsequent islet infusions. Cumulative time of insulin independence was 10.5, 8.8, 5.3, and 5.9 years for patients A, B, C, D, respectively (Figure 3C). At the same time, the patients still did not experience any severe hypoglycemic episodes with resumed hypoglycemic awareness: they presented alerting symptoms in case of infrequent postprandial blood glucose drop.

TABLE 2.

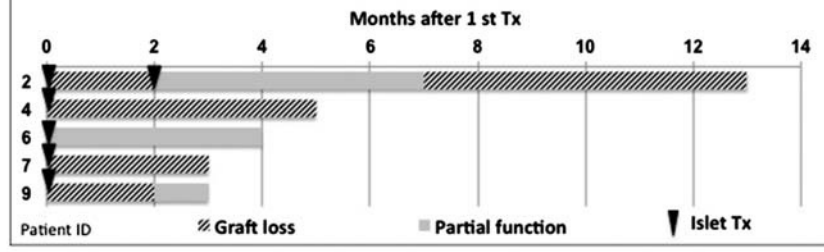
Characteristics of patients enrolled into the study

	Median	Range	1/A	2	3/B	4	5/C	6	7	8/D	9
Age	42	(19-57)	42	25	36	19	48	35	52	51	57
Sex	5 F/4 M		F	F	M	M	F	M	F	F	M
Years of T1DM	22.5	(14-38)	25	20	14	15	43	17	32	38	—
BMI	22.5	(19-27)	22	19	20	22	20	—	26	24	27
Weight	68	(53-94)	61	54	67	68	53	79.5	70	75	94
Insulin U/24 h	35	(20-54)	23	38	28	54	20	39	28	35	40
U/kg per day	0.45	(0.38-0.69)	0.38	0.69	0.42	0.79	0.38	0.48	0.44	0.47	0.43
HbAC1	8.1	(6.9-8.9)	8.2	8.9	8.7	8.9	6.9	6.9	8.1	7.7	7.9
PRA	0	0	0	0	0	0	0	0	0	0	0

Patients 1, 3, 5, and 8 completed the study and will be presented as A, B, C, and D in the tables and figures, respectively.

F, female; M, male.

A



B

Patient ID	Islet Mass		Complications
	IEQ	IEQ/kg	
2	1st Tx: 244,534 2nd Tx: 558,294	13,260 (Total)	PNF(Primary Non-function) after 1st Tx, constant, chronic mouth ulcers, Sirolimus to MMF- nausea, vomiting, diarrhea, muscle ache, 2nd graft loss, **disappointment**
4	367,839	5088	Teenager- SHE- mouth, heal ulcers, headache, Abdominal wall cellulitis, tremor, **noncompliance**
6	444,624	5495	Abdominal pain, cramps, mouth ulcers, headache, marihuana
7	319,133	5009	Nausea, vomiting tremor, PNF and **disappointment**
9	504,683	5581	Upset stomach, poor islet function, hypoglycemia, **disappointment**

FIGURE 2. Results in 5 patients (no. 2, 4, 6, 7, 9) who dropped the study. A, Islet graft function in relation to time after the transplant. B, Islet mass transplanted and list of complications and reasons for dropout. PNF, primary nonfunction; MMF, mycophenolate mofetil; SHE, severe hypoglycemic events; Tx, transplant.

In patients who became insulin-independent after islet graft infusions, HbA1c stayed below 6.1, whereas in patients with suboptimal or partial islet function requiring insulin supplementation, their HbA1c was around 7 (Figure 4).

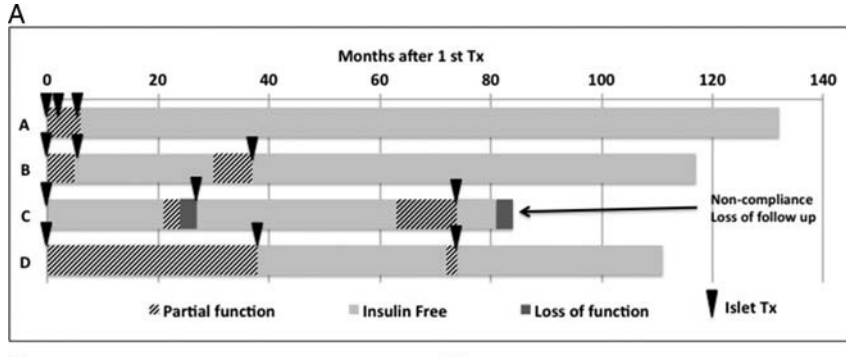
The results of MAGE, β score, and metabolic tests, which included arginine and MMTTs, corresponded well with the clinical islet function (Figure 4). For patients off insulin, MMTT fasting glucose oscillated below 115 mg/dL and peak 180 to 200 mg/dL, whereas c-peptide stimulation index varied between 2 and 4 for patients A and B, 4 to 7 for patients C and D. In the same settings of insulin independence, arginine-stimulated c-peptide varied in range of 0.35 to 1.2 ng/mL, whereas insulin release was in the range of 6 to 16 μU/mL in all 4 patients.

A significant reduction in HbA1c was observed, when comparing overall pretransplant values to baseline (baseline, 8.3 ± 1.1%; posttransplant, 6.0 ± 0.29%; $P < 0.05$; Figure 4). Also, MAGE score showed a significant improvement, when compared with pretransplant (baseline, 4.8 ± 1.2; posttransplant, 1.6 ± 0.30; $P < 0.01$). Substantial increase was observed in the β cell score of 4 patients after islet transplantation when insulin free (baseline, 2.0 ± 0.6; posttransplant, 6.0 ± 0.5; $P < 0.01$).

Adverse Events Related to Islet Procedure

Altogether, there were 2 immediate complications out of all 18 (11%) intrahepatic islet infusions. Patient D developed an intraperitoneal bile leak after her first islet transplant after

A



B

	1st Tx IEQ	2nd Tx IEQ	3rd Tx IEQ	Total IEQ	Total IEQ/kg
A	318,724	256,095	560,471	1,135,290	19,424
B	315,548	719,998	383,702	1,419,248	22,040
C	337,251	500,000	224,715	1,061,966	21,723
D	655,894	560,000	504,872	1,720,766	26,383

C

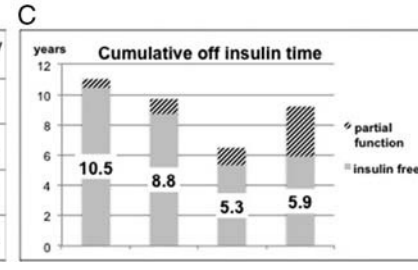


FIGURE 3. Results in 4 patients, who maintain islet function and remain in follow-up. A, Islet graft function in relation to time after the transplant. B, Islet mass transplanted.

TABLE 3.

Prevention of severe hypoglycemic episodes and insulin independence rates

Endpoint	Follow-up time		
	1 y after first Tx	5 y after first Tx	1 y after last Tx
Prevention of severe hypoglycemic episodes with HbA1c <6.5	4/9 (44%)	4/9 (44%)	4/9 (44%)
Insulin independence	3/9 (33%)	4/9 (44%)	4/9 (44%)

Tx, transplantation.

percutaneous intrahepatic portal vein approach for islet infusion. She required endoscopic retrograde cholangiopancreatography with temporary bile duct stenting for recovery. Patient C experienced subcapsular hematoma, which resolved on its own without blood transfusion.

Immunosuppression and Other Adverse Events

All patients required substantial adjustments in immunosuppression due to adverse effects. Patient A developed recurrent mouth ulcers and diarrhea (target sirolimus level 12-15 ng/mL), and she was switched to Tacro (through 5-10 ng/mL) and mycophenolate mofetil (MMF) (Cellcept, Roche, Nutley, NJ) after 1 month. However persistent chronic diarrhea and abdominal cramps led to subsequent multiple adjustments, and patient has remained on sirolimus (through concentration, 4-6 ng/mL: since 1 year after the first transplant), and azathioprine (Imuran, GlaxoSmithKline, Greenford, GB) 75 mg with addition of prednisone 5 to 10 mg for the last 8 years. Other severe adverse events, included deep vein thrombosis, ovarian cyst, bilateral breast carcinoma in situ treated with bilateral mastectomy 10 years after first transplant and small basal cell skin cancer was excised soon after. Patient B also experienced transient azotemia, recurrent mouth ulcers and diarrhea, and after 4.5 years, his immunosuppression was finally changed to Tacro and myfortic when he underwent a surgery for tendonitis. At that time, Tacro was increased to maintain through of 6 to 8 ng/mL for 2 years and reduced to 4 to 6 ng/mL for the next 3 years up to now with stable islet graft function and off insulin. Patient C also developed renal insufficiency, and Tacro was replaced with MMF a year and a half after the first islet cell infusion. Three years later, the patient developed severe pneumonitis, sirolimus was replaced with Tacro (through 5-10 ng/mL) and MMF. Few months later, the same patient developed hemoptysis from a cavitary pulmonary lesion, was treated for nontypical mycobacterium, and put on chronic fungal prophylaxis with posaconazole. The addition of posaconazole required Tacro dose reduction to 0.2 mg of oral suspension twice a day secondary to drug/drug interaction, but when patient decided to stop posaconazole on her own, Tacro level became undetectable and patient lost islet function and decided to drop out of the study at this point. Patient D developed recurrent mouth ulcers and severe pruritus involving hands, the axilla, and the groin area (sirolimus target through 8-12 ng/mL), so patient D was converted to Tacro (through 5-8 ng/mL) and MMF 6 months after the transplant. She continues to tolerate this regimen well. She developed chronic headaches, which she tolerates well with antimigraine medications (butalb/acetaminophen/caffeine), after second islet transplant with negative diagnostic investigation.

All together, the original Edmonton immunosuppression protocol needed to be modified due to severe side effects in all 4 patients, but those modifications did not affect islet function, which remained well preserved in the long term (Figure 4). Patient C received thymoglobulin induction during her third transplant, which did not affect overall outcomes because the patient

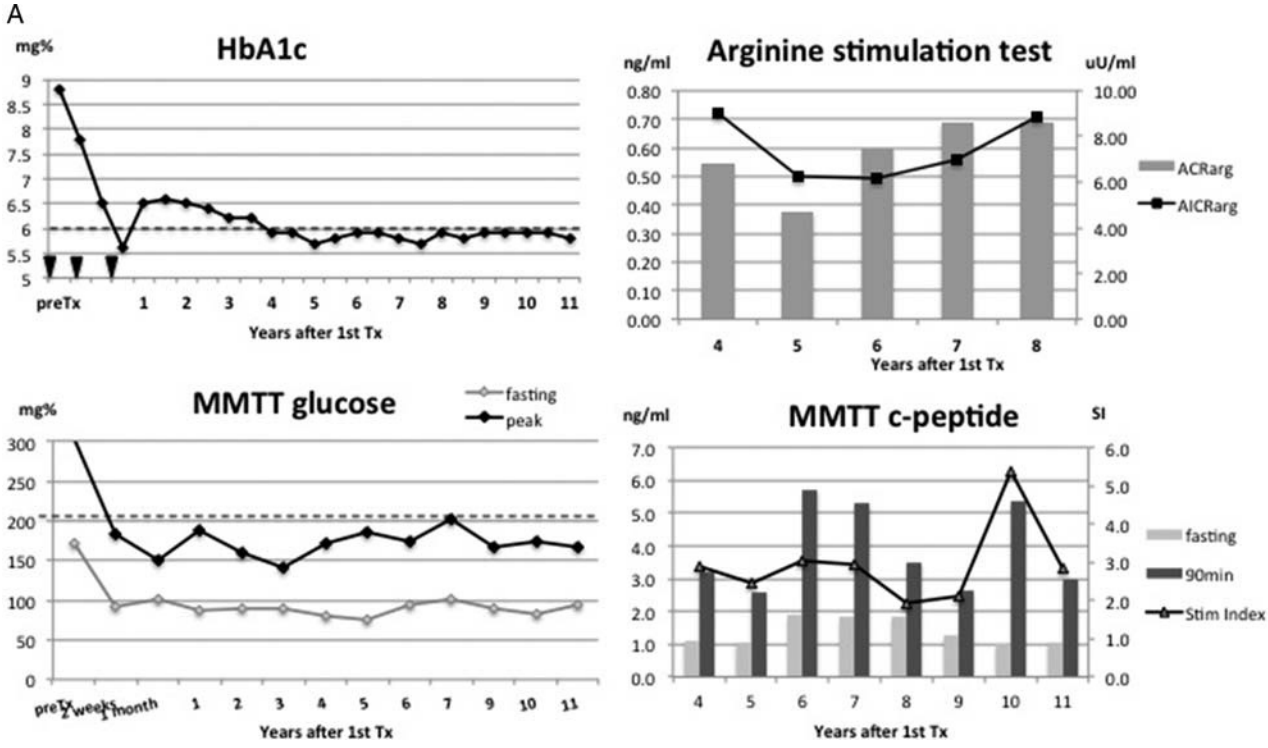


FIGURE 4. A-D, Overview of endocrine islet function during the follow up for each patient (subjects A, B, C, D), who completed the study, respectively. AIRarg, acute insulin response to arginine; ArgST, arginine stimulation test; Stim index, stimulation index.

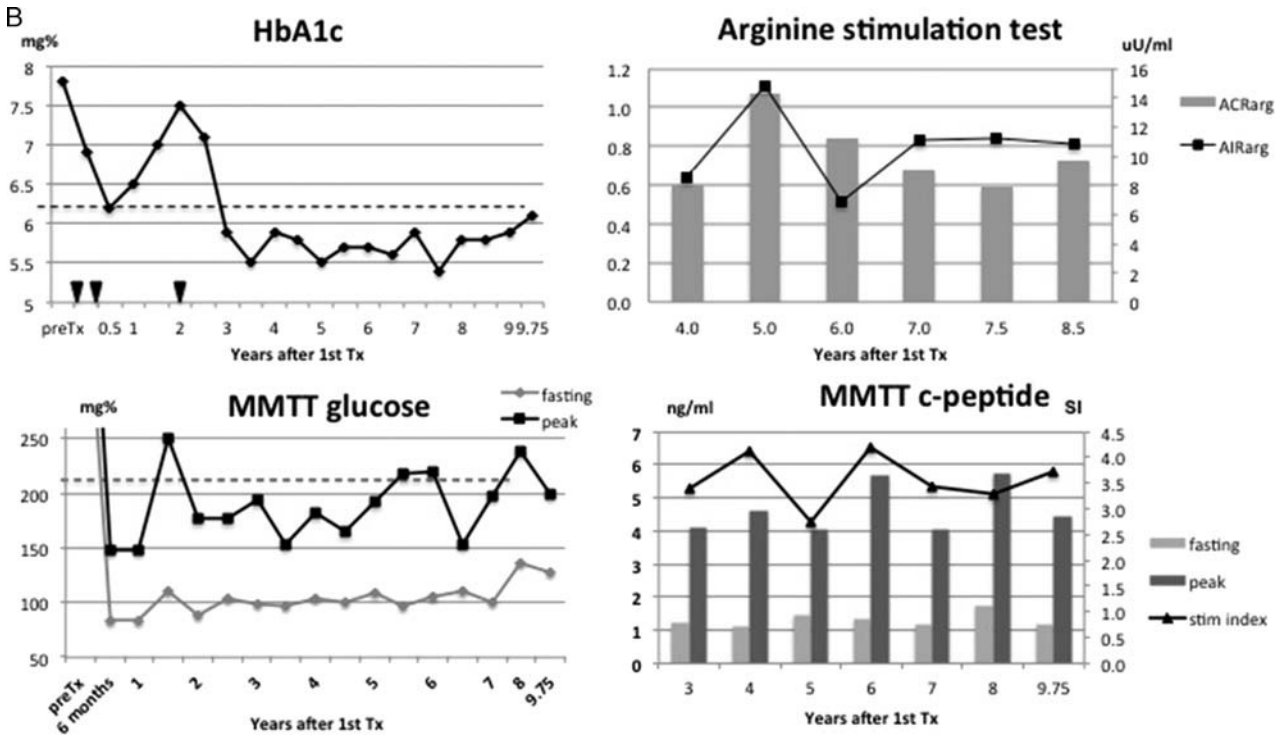


FIGURE 4. (Continued).

lost her graft function a few months later due to noncompliance as described above. Patient D also received thymoglobulin for induction during her second islet transplant, subsequently developing severe cytokine release syndrome with severe headache. Addition of the thymoglobulin as induction agent in those 2 cases was based on the published results from Edmonton, indicating the beneficial effect of thymoglobulin during the supplemental islet infusion.[15]

Other Side Effects Related to Immunosuppression

Two patients developed hyperlipidemia, a known side effect of rapamycin (Rapa), and required medications for

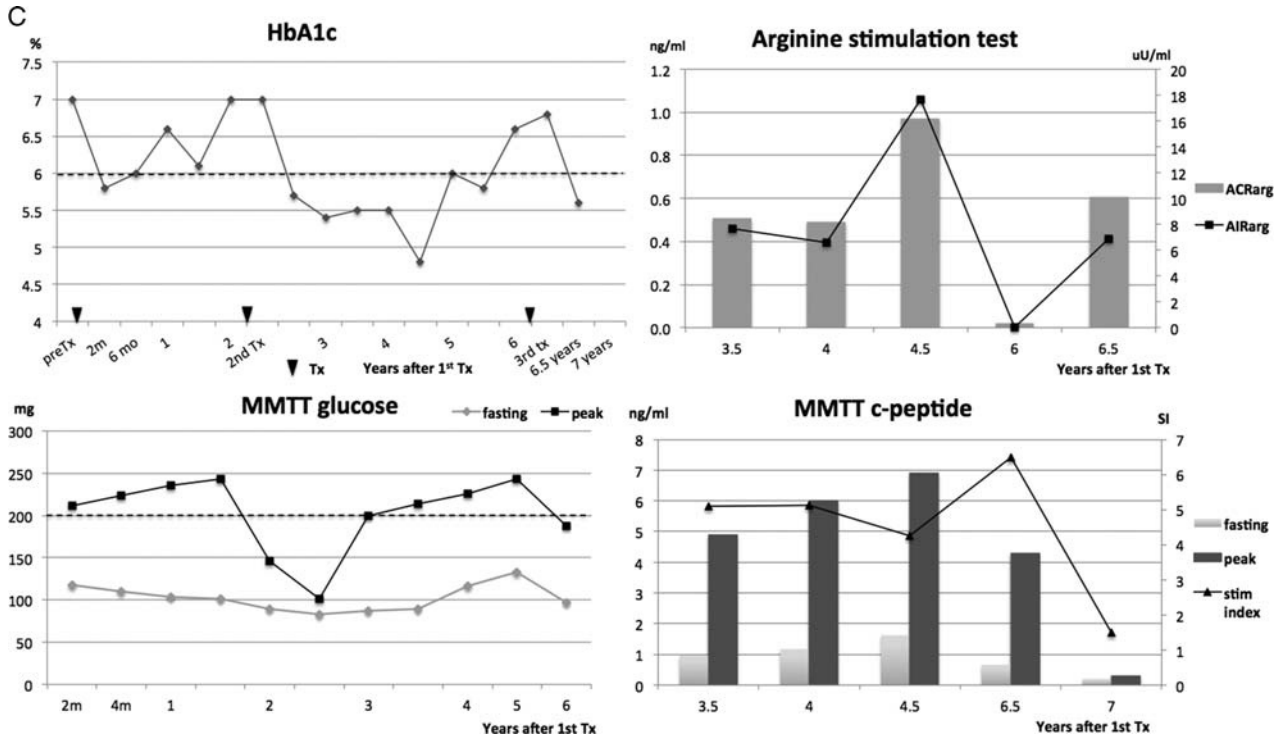


FIGURE 4. (Continued).

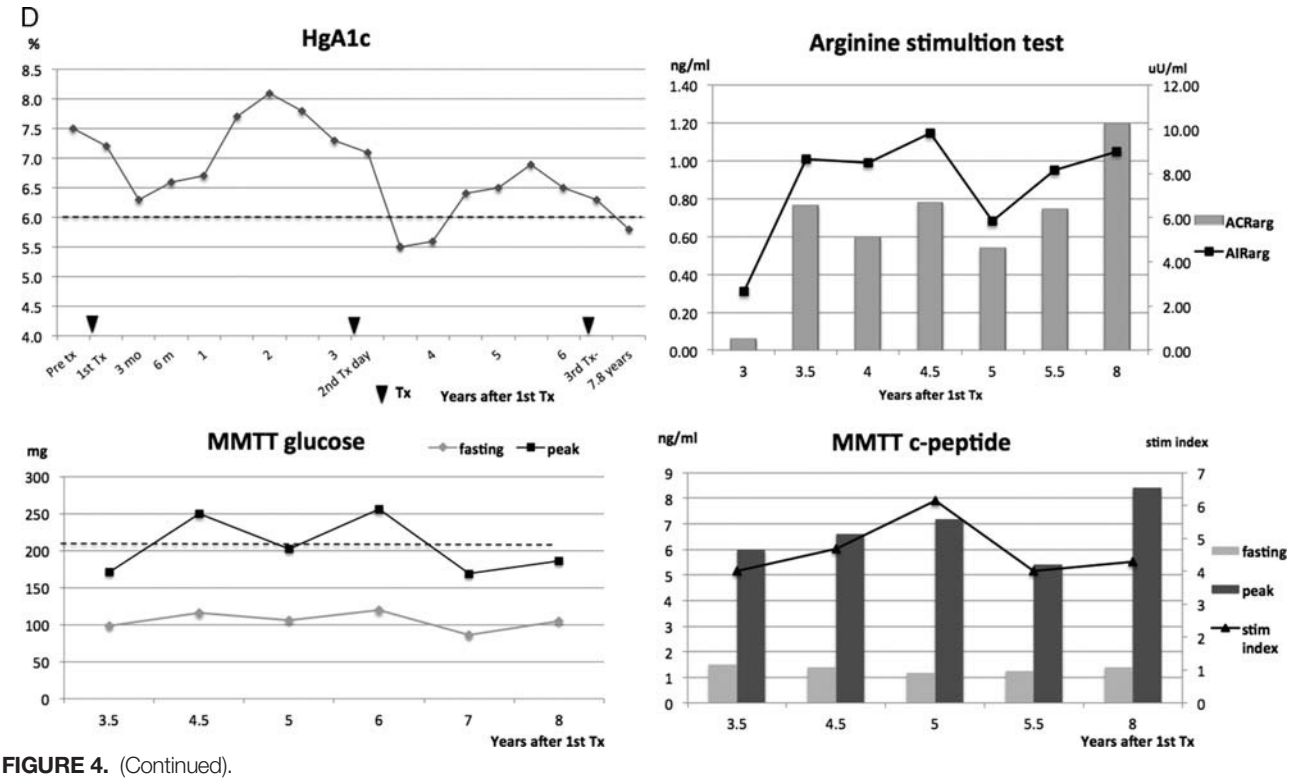


FIGURE 4. (Continued).

lipid control. Also, 3 patients are currently on antihypertensive medication. Patient D did not develop any chronic medication-related side effects (Table 4).

Renal Function

Despite potential nephrotoxicity of Tacro and Rapa, renal function in all patients was very well preserved with stable serum creatinine levels and estimated glomerural filtration rate (Figure 5). Three of them did not develop any albuminuria, whereas 1 patient, who has been on Rapa for 10 years, developed transient minimal microalbuminuria (urine albumin/creatinine ratio 32 μg/mg at 9 years after the transplant; normal (8 μg/mg) at 11 years f/u).

Influence of Islet Transplantation on Retinopathy and Neuropathy

All islet cell transplant recipients had annual examinations with a vitreoretinal specialist. There was no progression of the proliferative diabetic retinopathy in any of the 4 patients. Two individuals (B, D) have never developed diabetic retinopathy for over 8 years f/u (Table 5). Furthermore, no patient developed diabetic macular edema while in this study.

All of the subjects also had repeated neurological assessments and nerve conduction studies. One of the 4 subjects developed a mild axonal neuropathy between 1 and 2 years after start of the study which remained stable. One patient did not develop a neuropathy, and neuropathy score and nerve conduction parameters showed partial improvement in the other 2 patients over the course of the study (Table 5).

Autoimmune Antibodies and Panel of Reactive Antibody

Autoimmune antibodies were not routinely tested. Anti-glutamic acid decarboxylase (GAD)65 antibody was found to be negative in 3 patients with long-term f/u. Remaining insulin-free patient (patient B) has had persistent anti-GAD65 antibody however at a lower titer than before the first transplant (Table 6).

All of the patients have had undetected anti-HLA antibodies before and after the transplant. Panel-reactive antibody (PRA) has remained zero (Table 6). There was no specific pattern of main HLA mismatches observed between donors and recipients. They were from 1 to 4 mismatches for HLA-A and -B and 0 to 4 for DR (Table 6).

TABLE 4.

Long-Term complications and applied treatment for each patient

Patient ID	A	B	C	D
Duration on sirolimus	10 y	4.5 y	4.5 y	6 mo
Duration on tacrolimus	9 mo	9.5 y	4 y	9 y
Long-term complications				
Pretransplant	None	None	Hypertension (ARB)	None
Posttransplant (medications)	Hypertension (ARB) Hyperlipidemia (statins)	Hypertension (CaCB)	Hypertension (BB) Hyperlipidemia (statins)	None

ARB, angiotensin II receptor blockers; CaCB, calcium channel blockers; BB, B-blockers.

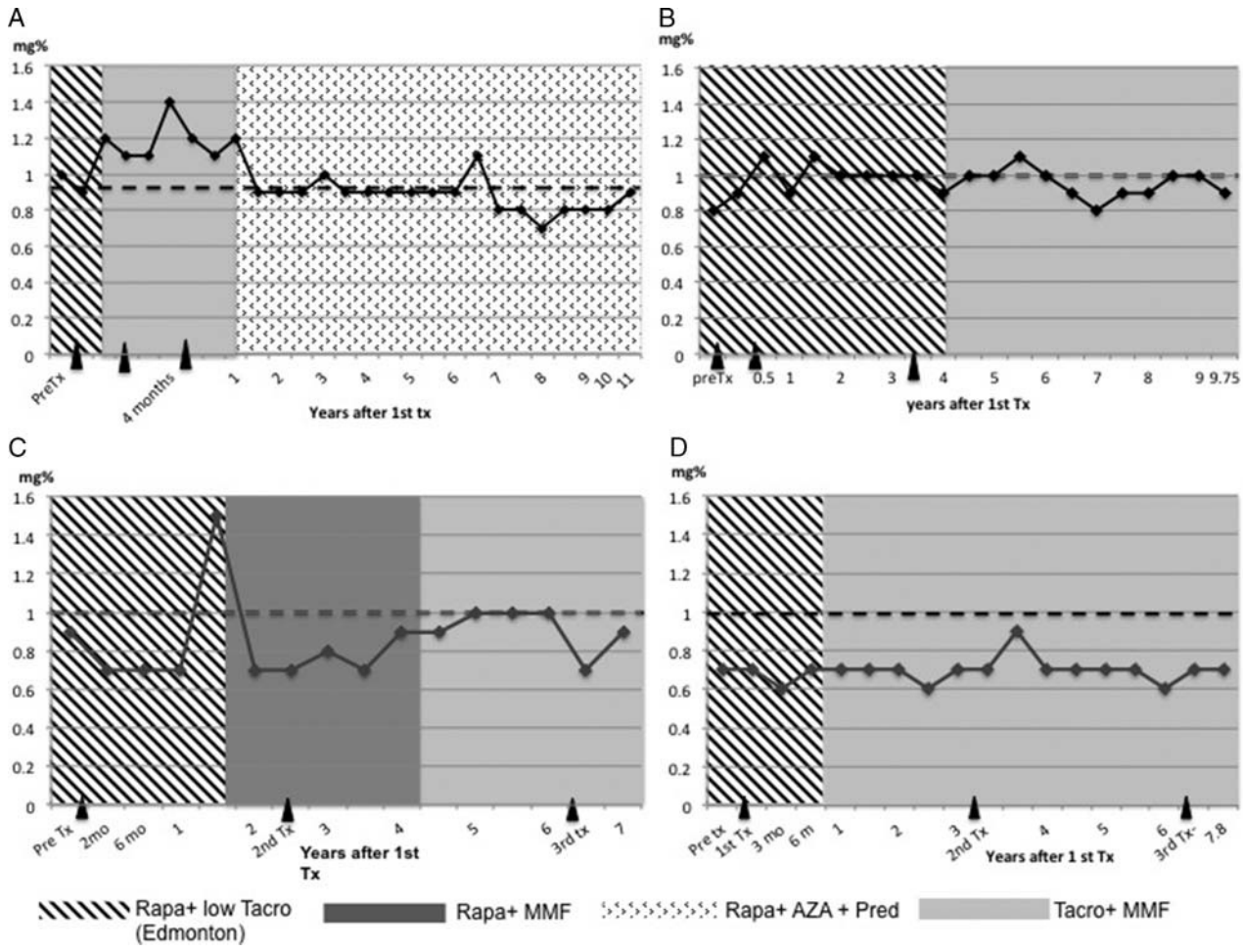


FIGURE 5. Summary of kidney function in patients (subjects A, B, C, D) over the years, represented by serum creatinine levels, respectively. Rapa, rapamacyn; Tacro, tacrolimus AZA, azathioprine, Pred, prednisone; PreTx, pretransplant.

Improvement in Quality of Life

At the most recent f/u all 4 patients confirmed that despite the burden of chronic immunosuppression and related side effects, they would again volunteer for the study. They would also recommend participation in the study to close family members if they suffered from "brittle" type 1 diabetes, that is, frequent lows that were difficult to manage. They felt that islet transplantation tremendously improved their life, as well as their close family members, removing constant fear of unexpected severe hypoglycemic episode and sudden death or brain damage. Being insulin-free is an additional great advantage of the procedure. They all would sign up for a fourth islet transplant if they would start requiring insulin again.

DISCUSSION

Islet transplantation was developed as a minimally invasive alternative to whole pancreas transplantation for treatment of "brittle" T1D. Although this term is of limited utility, the main common feature is hypoglycemic unawareness and frequent severe low blood sugars. Intrahepatic islet transplantation offers patients with T1DM chance for

TABLE 5.

Diabetic retinopathy and peripheral neuropathy before and after islet transplant

Patient ID		A	B	C	D
PDR	Pre-Tx status	PDR	No PDR	Early PDR	No PDR
	Follow-up period	8 y	7 y	7 y	8 y
	Recent Status	No progression	No PDR	No progression	No PDR
PN	Pre-Tx Status	PN	None	None	PN
	Follow-up period	10 y	8 y	7 y	7 y
Recent Status	PN improved in upper limbs	None	Developed mild PN 1-2 years after Tx and then stable	PN improved	

PDR, prolipherative diabetic retinopathy; PN, peripheral neuropathy.

TABLE 6.

HLA mismatch between donors and recipients for each transplant

Patient ID	A		B		C		D	
HLA	A, B	DR, DR	A, B	DR, DR	A, B	DR, DR	A, B	DR, DR
First Tx	3/4	3/4	4/4	4/4	2/4	2/4	1/4	2/4
Second Tx	3/4	3/4	3/4	0/4	2/4	1/4	4/8	4/8
Third Tx	3/4	2/4	3/4	3/4	3/4	1/4	7/8	3/8
	Pre-Tx	9 y	Pre-Tx	7 y	Pre-Tx	6 y	Pre-Tx	8 y
GAD (<0.02)	—	0	2.92	1.33	—	0	—	0
PRA	0	0	0	0	0	0	0	0

Autoantibodies and PRA levels before and after the transplant.

restoration of glucose counterregulation and endogenous glucose production in response to hypoglycemia via c-peptide suppression (absent before transplant), recovery of glucagon secretion, and improved epinephrine release.[16] Although the risk related to the procedure is limited, the burden of lifelong immunosuppression remains a major obstacle, preventing wider use of islet allotransplantation.[8,15,17] Therefore, in our study, we conducted a careful screening and patient selection process to identify and enroll only those patients who were medically and psychologically suitable, and who were logistically prepared with good social support. Only 1% of potential patients eventually received islet transplant, a total of 9 of over 900 individuals who inquired about the study. A multicenter trial testing effectiveness of the Edmonton protocol reported similar enrollment rate 36 (1.8%) of 2000.[18] Despite careful selection, we had a substantial dropout rate with 5 (55%) patients deciding to exit the study in the early phase. The reasons for withdrawal from the study were frequent side effects and poor islet transplant outcome. The debilitating side effects of high-level sirolimus likely further compromised the patients' quality of life and led to their withdrawal from the study. Rother and Harlan[19] report similar experience, which eventually led to termination of the program.

Most of our patients who dropped out, experienced poor islet function or primary nonfunction after initial infusion. Islet transplantation is a very complex procedure, and its success is dependent on the proficiency at every step of the process, including donor selection, pancreas procurement/transportation, meticulous islet isolation, and handling before and during the infusion, and patient selection and posttransplant medical management. As observed in a multicenter trial,[18] the outcomes at inexperienced programs (like ours in 2005) are usually inferior compared with centers with significant experience In addition to experience in islet processing and transplantation, proficiency in using and adjusting specific immunosuppression medications is critical to achieve a successful outcome.[18] Before starting the trial in 2005, we had not had any clinical experience with sirolimus, and it likely led to significant morbidity.

Our remaining 4 patients noticed benefit of improved glucose control immediately after the first islet infusions, maintaining partial graft function and eventually became insulin-free, so they were able to endure extensive side effects they experienced. It allowed the investigators to adjust the immunosuppressive regimen, ultimately limiting severity and frequency of adverse events. Eventually, none of those patients remained on original Edmonton protocol in the long-term f/u. The need for alternative immunosuppression regimen was reported in other centers.[15,17,20,21] In the recently published 10-year f/u multicenter study, only 1 patient out of 7 (13%) was able to tolerate sirolimus in long term with good clinical outcome.[22]

As noted, the pattern of achieving insulin independence varied between patients. The first patient required 3 islet infusions in a row within first year and then stopped insulin maintaining excellent glucose control for over 9 years. The second patient required 2 islet infusions to achieve insulin independence and soon after 2 years received the third transplant, which allowed him to enjoy insulin independence for over 6 years. The next patient achieved insulin independence after each infusion and but lost it after few years. In this patient, it seems like sequential islet infusions are necessary to achieve critical islet mass to maintain insulin independence, and some islet function deterioration persisted over time. The same pattern has been observed in other studies.[17,22-24] It is unlikely that acute rejection was responsible for the loss of islet function because all patients were maintained on stable immunosuppression. None of our patients developed PRA or donor-specific antibody, but we still do not have tools to exclude or confirm a cellular rejection with certainty. There is a long list of possible explanations for islet deterioration including autoimmunity, drug toxicity, chronic rejection, islet exhaustion, and again no diagnostic test is available to test the hypotheses.[17,22,23] Blood glucose control was clearly superior when islet cell recipients had stable and robust graft function, allowing them to be completely off insulin which corresponded to the results of MMTT, arginine stimulation test, and lower HgA1c HbA1c level. The first and immediate advantage of islet cell transplantation was that patients became aware of hypoglycemia as soon as they achieved at least stable partial islet function with lower insulin requirements.[25,26] Obviously, subsequent insulin independence improved the ultimate outcome and lowered the risk-to-benefit ratio. We also looked at whether side effects of long-term immunosuppression outweighed the beneficial effect of improved glucose control on preventing end-organ damage. Renal function was the biggest concern, as Tacro is a well-known nephrotoxic agent.[27,28] Renal function, as measured by serum creatinine, remained stable in all 4 patients. Only 1 patient developed minimal microalbuminuria after 9 years on sirolimus, which is a known side effect. Retinopathy and peripheral neuropathy remained stable overall, and 2 patients had partial improvement in neuropathy score and nerve conduction study parameters. The same observation regarding stable renal function, and retinopathy was confirmed in another 10-year f/u study, despite long-term exposure to Tacro or mammalian target of rapamycin (mTOR) inhibitor[29] as well as in the cross over study in Vancouver.[29,30] Kidney function was much less compromised over time in islet recipients than in islet transplant candidates on the waiting list.[29] Improved glucose control did not prevent most of our patients from developing hypertension and hyperlipidemia. Most patients on immunosuppression require antihypertensive and cholesterol-lowering medications.[20,31] Increased risk for infection and neoplasm is correlated with chronic immunosuppression. As reported in the multicenter study,[22] we observed 1 patient who developed small basal cell carcinoma after 10 years from the first transplant. Fortunately, we have not experienced severe infectious complications besides 1 patient who was exposed

to atypical mycobacterium before transplant and developed minor bleeding from a lung cavitary lesion afterward. Overall, sirolimus was the agent most frequently responsible for severe adverse events affecting our patients: recurrent mouth ulcers, diarrhea, pruritus, nephrotoxicity, and compromised tissue healing, ovarian cyst, which were confirmed in other studies.[17,22] Those side effects substantially contributed to drop out 5 of our patients. Tolerability of the immunosuppression improved substantially once sirolimus was replaced with antimetabolites, such as MMF or mycophenolic acid, and this is also a well-described observation.[17,20–23] In light of its side effects and weaker immunosuppressive effects, mTOR inhibitors had been replaced in recent protocols by Tacro with antimetabolites for the first-line maintenance immunosuppression for most organ transplants as well as islet transplantation.[20,32] However, with a recent finding of mTOR inhibitor's protolerogenic properties supporting regulatory T cells, sirolimus is being tested again in islet studies.[33]

High risk for patient immunological sensitization due to exposure to islets from multiple donors has been raised as a potential significant disadvantage of the procedure. On the contrary, a single blood transfusion or single organ transplant can also highly sensitize a patient in the setting of suboptimal or no immunosuppression. The Edmonton group also showed benefit of fourth and fifth islet infusions without an increased risk of developing a positive PRA as long as the patient maintains proper immunosuppression.[15,22] Results in our patients confirmed the same observation. No positive PRA in long-term f/u was found, despite patients receiving islets from 3 to 4 donors, without any special donor/recipient immunological matching. Recurrent autoimmunity is theorized as 1 of the causes of islet graft failure in selected patients, and it seems that an increasing level of anti-GAD65 is correlated with poor islet and pancreas graft survival.[23,34] In all our patients, anti-GAD65 antibody remains 0 or lower than before transplant, which would support the others' observation. New tetramer technology allows looking for autoantigen-specific T-cell and their impact in the future studies.[35,36]

Although, Edmonton protocol is no longer in use for islet cell transplantation, the data presented here still have a great clinical value. Most patients were converted to and exposed in long term to Tacro and antimetabolites, most common current maintenance immunosuppression, which allowed for not only stable long-term islet graft survival but also long-term preservation of the renal function, retinopathy, and neuropathy with limited risk for cardiovascular complications and immunologic sensitization. Those results address major clinical concerns related to currently used lifelong immunosuppression. The use of T depletion induction agents, anti-inflammatory agents, and effective maintenance therapy will likely result in better outcomes.

In conclusion, only a small fraction of patients presenting for evaluation were suitable candidates for islet transplantation. Despite thorough patient screening and selection, the drop-out rate was high and was due to combination of poor initial islet graft function and extensive side effects of sirolimus. Immunosuppressant medications must be frequently adjusted to facilitate long-term islet survival and overall health of the islet transplant recipients. Insulin independency was achieved by multiple infusions without detecting PRA. Overall, in properly selected subjects with type 1 diabetes and severe hypoglycemia with hypoglycemic unawareness, pancreatic islet transplantation offered a chance for long-term excellent glycemic control and prevention of progression of diabetic complications, including nephropathy, retinopathy, and neuropathy. We hope and think that there was improvement in hypoglycemic unawareness, but we did not study it directly. All patients who are still participating in the study emphasize enormous improvement in their quality of life despite significant immunosuppression-related complications.

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