Original Clinical Science—General

Age and Early Graft Function Relate With Risk-Benefit Ratio of Allogenic Islet Transplantation Under Antithymocyte Globulin-Mycophenolate Mofetil-Tacrolimus Immune Suppression

Dae Hae Lee, MD,[1,2] Bart Keymeulen, MD, PhD,[2] Robert Hilbrands, MD, PhD,[2] Zhidong Ling, MD, PhD,[2] Ursule Van de Velde, MSCN,[2] Daniel Jacobs-Tulleneers-Thevissen, MD, PhD,[2] Geert Maleux, MD, PhD,[3] Bruno Lapauw, MD, PhD,[4] Laurent Crenier, MD, PhD,[5] Christophe De Block, MD, PhD,[6] Chantal Mathieu, MD, PhD,[1] Daniel Pipeleers, MD, PhD,[2] and Pieter Gillard, MD, PhD[1,2]

Background. Induction therapy with a T cell–depleting agent followed by mycophenolate mofetil and tacrolimus is presently the most frequently used immune suppression (IS) regimen in islet transplantation. This study assesses its safety and tolerability in nonuremic type 1 diabetic recipients. **Methods.** Fifty-one patients (age, between 29 and 63 years) with high glycemic variability and problematic hypoglycemia received intraportal islet grafts under anti-thymocyte globulin-mycophenolate mofetil-tacrolimus protocol. They were followed up for over 48 months for function of the implant and adverse events. **Results.** Severe hypoglycemia and diabetic ketoacidosis were absent in patients with functioning graft. Immune suppressive therapy was maintained for 48 months in 29 recipients with sustained function (group A), whereas 16 patients stopped earlier due to graft failure (group B) and in 6 for other reasons. Group A was significantly older at the time of implantation and achieved higher graft function at posttransplantation month 6 under similar dose of IS. Prevalence of IS-related side effects was similar in groups A and B, occurring predominantly during the first year posttransplantation. IS-related serious adverse events (SAE) were reported in 47% of patients, with 4 presenting with cytomegalovirus infection and 4 (age, 42-59 years) diagnosed with cancer. Except in 1 patient with cancer, all SAEs resolved after appropriate treatment. **Conclusions.** These risk/benefit data serve as a basis for clinical decision-making before entering an intraportal islet transplantation protocol. A longer benefit is observed in recipients of higher age (≥40 years), but it is not associated with more side effects and SAE.

(*Transplantation* 2017;101: 2218–2227)

For type 1 diabetic patients with problematic hypoglycemia, islet transplantation represents a possibility of therapy.[1-3] However, the risk-benefit ratio needs to be determined. This is not easy in view of the heterogeneity of the recipients and in the transplant protocols. Single-center protocols usually have standardized follow-up of graft function, metabolic control, and safety outcome measurements.[2,4-7] Because of the limited number of patients per center, analysis is often conducted in a combination of preuremic and kidney transplant recipients and of patients under different immunosuppressive regimens.[8] In Belgium, a collaborative Islet Transplant Network was

formed, with islets being isolated in the central isolation facilities of the Diabetes Research Center of the Vrije Universiteit Brussel and transplantations being performed by a collaborative team in 2 Belgian academic centers.[6] The present study examines risk-benefit ratio of well-characterized human islet cell grafts in nonuremic type 1 diabetic patients receiving immune suppressive therapy that consists of antithymocyte globulin (ATG) as induction and mycophenolate mofetil (MMF) plus tacrolimus (Tac) as maintenance. This combination of T cell depletion and MMF-Tac is currently the most frequently used regimen in islet transplantation[9] and has been used in our program for more than 15 years.[10]

MATERIALS AND METHODS

Graft Recipients

Data were collected from a total of 51 islet β cell recipients with type 1 diabetes mellitus complicated by hypoglycemia unawareness, transplanted between December 2001 and March 2011. Baseline patient characteristics are shown in Table 1. The recipients were included according to the following criteria: (1) Nonsmokers aged between 18 and 65 years; (2) C-peptide negativity defined as plasma C-peptide < 0.09 ng/mL at glycemia of 120 to 200 mg/dL; (3) signs of chronic diabetic complications (microalbuminuria despite optimal dose of angiotensin-converting enzyme inhibitor, retinopathy, or hypoglycemic unawareness); (4) large within-person between-day variation in fasting self-monitored plasma glucose (defined by coefficient of variation fasting glycemia [CVfg] ≥25% and HbA1c ≥7% or 53 mmol/mol despite 4 daily subcutaneous insulin injections or pump therapy. Female patients of childbearing potential were excluded. Ethics committee of the Belgian Diabetes Registry and the participating university hospitals approved the protocol. This study was performed according to the Declaration of Helsinki and institution review board approval was obtained (institutional review board protocol BK/3 and BK/136; clinicaltrials.gov NCT00623610 and NCT00798785). A written informed consent was obtained from every patient.

Preparation of Islet β Cell Grafts and Transplantation

Isolation and preparation of islet β cell allografts was as described previously.[6] The technique to access the portal vein depended on the expertise of the site where patients were transplanted, either by laparoscopy (University Hospital Brussels[11]) or by percutaneous fluoroscopic-guided transhepatic injection (University Hospital Leuven[12]). In total, 91 grafts were transplanted, of which 41 grafts (n = 22 patients) were transplanted by laparoscopic and 50 grafts (n = 29 patients) by percutaneous cannulation of the portal vein. Twelve patients received 1 implant, and 38 patients received 2 implants within 3 months (interquartile range [IQR], 2.4-3.5 months) after the first implantation and 1 patient received 3 implants (at 2.5 and 5 months posttransplantation [PT]). Based on a possible beneficial effect of intravenous heparin infusion peritransplant as reported by Koh et al,[13] heparin was administered in 3 patients undergoing laparoscopic implantation at 70 U/kg using continuous infusion of heparin containing saline (10 U/mL) at 500 U/h (50 mL/h) up to 24 hours PT. After intra-abdominal bleeding in 2 of the 3 cases, heparin administration was removed from the protocol. Doppler ultrasonography of the portal vein and liver was performed within 24 hours PT to rule out bleeding and thrombosis.

Immune Suppression and Monitoring

The immune suppression (IS) regimen consisted of induction therapy with ATG (Fresenius; Fresenius, HemoCare, Redmond, WA) and maintenance therapy with MMF (Cellcept; Roche, Basel, Switzerland, n = 46) or mycophenolic acid (Myfortic; Novartis, Basel, Switzerland, n = 5) and Tac (Prograf(t); Astellas Pharma Europe, Staines, UK). First dose of ATG was given at day 4 (n = 21) or day 1 (n = 30) before the first implantation (9 mg/kg) and continued at 3 mg/kg for 6 days if T-lymphocyte count was above 50/mm³. No additional ATG was administered at the second or third implantation. MMF (2 g/d) or mycophenolic acid (1440 g/d) was started together with the first ATG injection and maintained at this dose, unless clinically necessary. All 5 patients who were started with mycophenolic acid were switched over to MMF within the first year after transplantation, according to availability. Patients received Tac from day 2 (n = 33) or day 5 (n = 18) at a dose to achieve serum trough levels of 8 to 10 ng/mL first 24 months and 6 to 8 ng/mL thereafter. Two hours before the first ATG administration and before each β cell graft, 500 mg methylprednisolone was given intravenously.

Anti-infectious prophylaxis consisted of valganciclovir (Valcyte 900 mg daily; Roche) and sulfamethoxazole/trimethoprim (Bactrim Forte 800 mg/160 mg daily, Roche) during 100 days after each implantation.

Assessment of Side Effects and Adverse Events

Outpatient follow-up was performed weekly until PT week 6, and every 2 weeks between PT weeks 6 and 12 and monthly thereafter. At each visit, a questionnaire was completed with a listing of possible adverse events (AEs) and side effects, followed by a physical examination. Blood work was done for hematology, kidney and liver function, metabolic parameters, Tac trough levels (Tacrolimus II, IMx Abbott; Abbott Laboratories, Wiesbaden, Germany) and cytomegalovirus polymerase chain reaction (Amplicor cytomegalovirus [CMV] test; Roche Diagnostics). AEs were assessed and recorded following the Common Terminology Criteria for Adverse Events v3.0 (CTCAE), as recorded in the Clinical Islet Consortium,[14] with the grading scale of 1, mild; 2, moderate; 3, severe; 4, life-threatening; and 5, death. Serious AEs (SAE) were defined as fatal, life-threatening, causing disability, causing or prolonging hospitalization, occurrence of malignancy or congenital anomaly, and requiring intervention to prevent permanent impairment or damage.[15] The relationship between each AEs and transplant protocol was categorized by causality; definitely related, probably related, possibly related, probably not related, not related, and unknown. Events reported from grade 2 to 5 were included in our analysis.

Assessment of Metabolic Control and Acute Diabetic Complications

Plasma C-peptide, glycemia, and HbA1c concentrations were measured in the central laboratory of the Belgian Diabetes Registry.[6] All patients performed 4-point to 7-point glycemia profiles of home blood glucose monitoring. CVfg was calculated using the glycemia measured from home blood glucose monitoring defined as the ratio of the standard deviation (δ) to the mean (μ): CVfg = δ/μ. Insulin dose-adjusted A1c (%) was calculated using HbA1c (%) + (4 × insulin dose [U/kg per day]).[16]

Graft failure was defined as consecutive random plasma C-peptide less than 0.2 ng/mL measured at glycemia of 120

Patient and graft characteristics

Immune therapy	48 mo	Stop during 48 mo	
		Graft failure	Other reasons
n	29	16	6
General			
Male/female	17/12	9/7	3/3
Body weight, kg	66 (63-70)	70 (67-77)	69 (63-76)
BMI, kg/m^2	23 (22-25)	24 (22-26)	25 (23-28)
Age at first islet transplantation, y	46 (40-54)	37 (32-43)[a]	50 (44-55)
Diabetes			
Age at clinical onset, y	15 (12-26)	11 (8-22)	30 (14-37)
Duration of disease, y	28 (24-38)	23 (20-29)	17 (12-42)
Positivity for ICA/GADA/IA2A/I(A)A	5/13/7/26	3/3/6/14	0/2/2/6
≥ 1 Autoantibody positivity (%)	97	94	100
Autoantibody negative (%)	3	6	0
HbA1c, %	7.5 (6.9-8.2)	8.1 (7.4-8.8)	7.8 (7.3-8.3)
Insulin dose, IU/d	34 (28-42)	42 (32-58)	39 (32-54)
Insulin dose, IU/kg per day	0.55 (0.42-0.63)	0.58 (0.52-0.79)	0.56 (0.49-0.72)
IDAA1c, %	9.4 (9.0-10.4)	10.6 (9.5-11.3)[b]	10.1 (9.4-10.8)
CV fasting glycemia, %	46 (40-50)	47 (41-55)	49 (37-54)
Microvascular complications			
Diabetic retinopathy (yes)	25	12	3
Microalbuminuria (yes)	4	4	3
Macrovascular complication[c]	14	4	2
Graft			
Culture time, d	5 (3-10)	5 (3-10)	4 (2-7)
No. islet infusions	2	2	2
β-cell number (10^6/kg body weight)	2.6 (2.3-3.1)	2.7 (2.0-3.3)	2.6 (2.3-3.4)
Cellular composition, %			
β cells	29 (22-37)	26 (20-35)	24 (24-33)
α cells	8 (5-11)	7 (5-13)	9 (6-14)
Nongranulated cells	49 (41-61)	52 (41-59)	46 (39-49)
Acinar cells	1 (1-6)	2 (1-5)	3 (1-6)
Dead cells	8 (6-11)	9 (7-11)	8 (7-12)

Data are shown as median (IQR).
Coefficient of variation of fasting glycemia defined by 100 × (standard deviation/mean).
Statistical difference between IS continue group versus graft failure group or stopped due to other reasons group (Mann-Whitney U) [a]$P < 0.005$, [b]$P < 0.05$.
[c] Macrovascular complication is defined as a combined endpoint (carotid stenosis, coronary heart disease, and arterial stenosis of the lower limbs.
IDAA1c, Insulin dose-adjusted A1c; BMI, body mass index.

to 220 mg/dL. Hypoglycemic events were defined as blood glucose levels < 70 mg/dL and were assessed by records of home blood glucose self-monitoring. Severe hypoglycemia was defined as an event requiring assistance of a third party to actively administer carbohydrate, glucagon, or other resuscitative actions that may be associated with sufficient neuroglycopenia to induce seizure or coma.

Diabetic ketoacidosis was defined as hyperglycemia (blood glucose >200 mg/dL) with metabolic acidosis (venous pH <7.3 or plasma bicarbonate < 15 mmol/L) and the presence of ketones in blood or urine. This was documented with the use of patient reports and hospitalization records.

Implant function and metabolic control data were compared between 2 patient groups, those who remained under IS until 48 months (group A) and those who stopped IS during the follow-up due to graft failure (group B).

Statistical Methods

Data collected were for intention-to-treat analysis. All values are expressed as median and IQR or mean ± standard deviation when indicated. For the comparison of the baseline patient characteristics, Pearson χ^2 test was used for categorical data and Kruskal-Wallis for continuous data in 3 different patient groups. To assess the difference between different timepoints during the follow-up, Fisher exact test was used for categorical data and Mann-Whitney U test was used for continuous data. All analyses were performed using SPSS (version 22.0) and the graphics were computed by using Graph Pad Prism (version 5.0). All reported P values are 2-sided, and a P value less than 0.05 was considered statistically significant.

RESULTS

Baseline Patient Characteristics of Patients With Continued and Discontinued Immune Suppressive Therapy

Of the 51 patients, 29 continued IS for 48 months (Figure 1). The treatment was stopped earlier in 22 patients

(median duration, 18 months; IQR, 13-34 months), in 16 because of a failing graft and in 6 for another reason in presence of a functioning graft. The latter group consisted of 2 patients diagnosed with cancer (malignant melanoma at month 13 PT; gastric adenocarcinoma with hepatic metastasis at month 27 PT), 1 suffering from therapy-resistant cytomegalovirus infection between months 6 and 13 PT, 1 who died at age 61 years as a consequence of acute cerebral hemorrhage at month 4 PT, and 2 who were disappointed that insulin-independence was not achieved (months 7 and 10 PT).

All 51 patients exhibited high pretransplant glycemic variability above 25% (Table 1). The majority had microvascular complications, with clinical macroangiopathy in 20 patients.

There were no differences in sex, body weight or body mass index in the recipients who continued or discontinued IS. Patients with graft failure were significantly younger ($P = 0.001$) and exhibited worse glycemic control at time of transplantation, shown as corrected insulin dose (higher insulin dose-adjusted A1c[16]) ($P = 0.01$).

SAEs

Of the 51 patients, 24 (47%) experienced SAE (Table 2). Three cardiovascular SAE were considered not to be study related.

SAE related to the implant procedure were uncommon; they occurred in 3 of 91 implant procedures (3%): 1 percutaneous implant caused severe thoracic pain associated with pleuroperitoneal irritation at the site of puncture (Table 2). No significant hemorrhage (defined as a drop in hemoglobin of more than 2 g/dL, or the need for transfusion or surgery) was seen after percutaneous islet transplantation. Two laparoscopic procedures were complicated by intra-abdominal bleeding when heparin injection was administered (2/3). Both cases received a blood transfusion without need for reintervention. No portal vein thrombosis or puncture of the gallbladder occurred in either of the 2 approaches.

Thirty-one cases of IS-related SAE were reported in 19 patients. Four patients presented 2 or more SAE. Two patients exhibited signs of Tac-related toxicity: 1 with encephalopathy and 1 with nephrotoxicity confirmed by renal biopsy, both resolving after dose reduction. The most frequently reported SAE caused by infection was PT CMV infection, observed in 5 patients (10%) and successfully treated with antiviral medication and/or reduction of MMF. Four of these 5 patients were CMV antibody negative before transplantation. The other CMV positive patient had reactivation of CMV at PT month 4. Four of 5 patients developed CMV infection after prophylaxis was stopped. One recipient with de novo CMV infection needed an additional course of oral valganciclovir because of persisting CMV PCR titers after IV ganciclovir. Gastrointestinal infections, possibly related to the intake of IS, were the most common cause of hospitalization, resolving with either antibiotics or supportive therapy.

There were 4 cancers reported; 1 metastasized gastric adenocarcinoma at age 56 years with a fatal outcome at PT month 45, 1 acral lentiginous malignant melanoma at age 43 years with complete remission after excision and cessation of MMF-Tac, 1 invasive prostate carcinoma at age 61 years with complete resolution after total prostatectomy, and 1 invasive rectosigmoid adenocarcinoma at age 47 years with remission after laparoscopic sigmoidectomy and cessation of MMF. Basocellular or squamous cell skin cancers or other nonskin cancers were not observed.

Side Effects Are Most Prominent During the First 12 Months PT

During the first 12 months when the IS dose was the highest, half of the patients experienced side effects but significantly decreased to 25% thereafter (Table 3).

The most prominent symptoms observed the first year were gastrointestinal symptoms, mostly pyrosis, nausea and vomiting, which significantly decreased from the second year after transplantation (Table 4). Neurological symptoms, mostly memory impairment and headache were observed in 22% of the patients the first year, but significantly decreased at the second year and remained present in around 5% of the patients. Respiratory, musculoskeletal symptoms, as well as asthenia and skin symptoms, were prominent during the first year, but also showed a decreasing trend from the second year until 48 months.
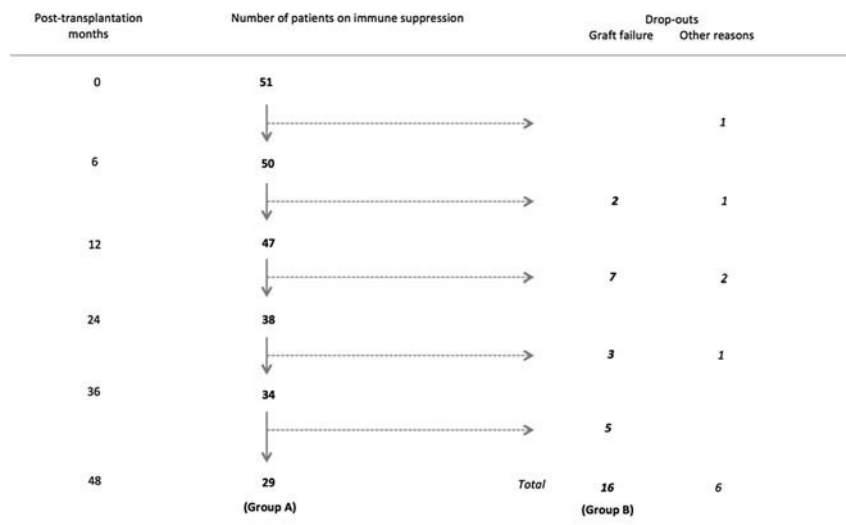

FIGURE 1. Flowchart of number of patients on immune suppression therapy after β cell transplantation.

TABLE 2.

Number and type of SAE

	No. patients with SAE (%)	No. SAE (%)	Relationship to study	Description	Posttransplantation	Treatment	Outcome
Total	24 (47)	37 (100)					
Procedure-related	3 (6)	3 (8)	Definitely	Abdominal bleeding postlaparoscopy, diaphragm irritation postpercutaneous puncture	Days 1, day 1, day 5	Transfusion (1)	Resolution
Immune suppression-related	19 (37)	31 (84)	Definitely	Tacrolimus encephalopathy, tacrolimus nephropathy	Month 26, month 30	Stop tacrolimus Dose reduction	Resolution
			Probably	CMV infection	Month 1, month 17, month 4, month 6, month 23	IV ganciclovir PO valganciclovir, MMF reduction IV ganciclovir, PO valganciclovir, MMF reduction Stop MMF	Resolution
				Anemia	Months 0, 1, 4, 15	Transfusion	Resolution
				Opportunistic infection—*Candida oesophagitis*—Microsporidium	Months 5, 10	Fluconazole Albendazole and MMF stop	Resolution
				Leucopenia of unknown origin	Month 9	MMF dose reduction	Resolution
			Possibly	Gastrointestinal, gastroenteritis, left fossa pain, chronic diarrhea	Months 0, 1, 1, 3, 6, 9, 10, 17, 19 Month 2 Month 39	Antibiotics (2) Antibiotics and stop MMF	Resolution
				Other viral infections	Months 23, 25		Resolution
				Diffuse liver metastasis of gastric adenocarcinoma	Month 27	Chemotherapy	Death
				Acral lentiginous melanoma	Month 13	Resection, stop immune suppression	Resolution
				Invasive prostate adenocarcinoma	Month 24	Total prostatectomy	Resolution
				Invasive sigmoid adenocarcinoma	Month 34	Resection, stop MMF	Resolution
Other	2 (4)	3 (8)	Probably not	Instable angina	Month 1	CABG	Resolution
				Cerebral hemorrhage	Month 4	Drainage	Death
				Exercise-induced angina	Month 18	PTCA	Resolution

No specific therapy is mentioned when symptoms resolved with conservative management.
CABG, coronary artery bypass graft; PTCA, percutaneous transluminal coronary angioplasty.

TABLE 3.

Percent (%) of patients experiencing at least one adverse event under immune suppression therapy during 48 months follow-up

	Total 48 months follow-up	Month			
		0-12	12-24	24-36	36-48
Number of patients under IS	51	51	38	34	29
Serious adverse events	47%	33%	18%	15%[a]	3%[a]
Side effects (signs and symptoms)	57%	51%	26%[a]	21%[a]	21%[a]
Depletion of blood cells[b]	100%	100%	66%[c]	47%[c]	31%[c]

Statistically significant reduction of adverse events compared with first year PT (PT month 0-12), [a]$P < 0.05$.
[b] Neutropenia and/or lymphocytopenia and/or anemia and/or thrombocytopenia.
Statistically significant reduction of adverse events compared with first year PT (PT month 0-12), [c]$P < 0.001$.

Laboratory Abnormalities Are Most Common During the First 6 Months

Abnormal liver function tests were observed during the first month PT, with increases in aspartate transaminase (AST > 2.5 times upper limit of normal) in 27% of all patients from median day 6, quickly resolving at day 9 and alanine aminotransferase (ALT > 2.5 times upper limit of normal) in 43% from day 6, resolving at day 12. These abnormalities were not seen after the second islet infusion ($P < 0.001$ vs after first implant).

All patients developed a decrease in blood cell counts (Table 3), consisting of lymphocytopenia (100%), anemia (67%) and thrombocytopenia (8%) in the first weeks after first implantation. Blood cell counts normalized thereafter, except for lymphocytopenia, which remained present in a third of the recipients under IS until month 48 PT (Figure 2). Twenty-five percent of the patients received transfusion for anemia, but no erythropoietin treatment was required. One patient required therapy with granulocyte colony-stimulating factor. No platelet transfusions were given.

Implant Function With Metabolic Control Is Sustained in 29 Graft Recipients

Maximal basal C-peptide levels were reached at 3 to 6 months PT in both the group who continued IS for 48 month (A; n = 29) and those who stopped IS (B; n = 16) during follow-up due to graft failure (Figure 3). In this period, C-peptide levels were twice as high in group A than those in group B, although after the first implantation (before month 3), a similar degree of β-cell implant function was measured in both groups. The insulin dose per kilogram in group A was reduced to half the dosage at this time, reaching nearly a third of the dose per kg of those in group B at 12 months ($P = 0.002$) (Table 5). After month 12 PT, median C-peptide levels decreased at a similar pace in both groups.

A striking inverse relationship was observed between implant function and both glycemic variability and insulin dose-adjusted A_1c levels. None of the patient experienced ketoacidosis or severe hypoglycemic events during the entire duration of the study, whereas this was the case for 6 (group A) and 20 (group B) patients in the year preceding the transplantation.

Tac trough levels as well as MMF dose were decreased with time, as defined in our protocol, and there was no significant difference between groups A and B observed (Table 5).

DISCUSSION

This study shows that clinical benefits of islet transplantation outweigh the AEs of chronic IS in a large group of nonuremic type 1 diabetic patients. Patients with a functioning graft experience less glycemic variability with absence of severe hypoglycemia or episodes of ketoacidosis, without exposing them to unacceptable risks of chronic IS. This positive risk-benefit ratio is in line with the results of other groups [2,4,8,17-23] which studied more heterogeneous groups of islet-alone and islet-after-kidney transplantation recipients and/or using different IS regimens.

Our patient group received cultured islet grafts[6] that were standardized in terms of β cell number, purity and time frame of a second transplant. To achieve these objectives, islet β cells from more than 1 donor were combined in each implant. Immunosuppression consisted of ATG induction and MMF-Tac maintenance therapy, which is currently the most frequently used worldwide.[9]

Of the 51 islet recipients, more than half of the patients experienced at least 1 side effect during the 48-month follow-up. Both implantation procedures (laparoscopy[11] and transcutaneous[12]) were rarely associated with acute complications so that a negative impact on long-term islet function[24] was

TABLE 4.

Prevalence of side effects[a] in nonuremic type 1 diabetic recipients of long-term cultured islet β cell allograft under immune suppression

Follow-up	Patients	Side effects n (%)					
PT months	n	Gastrointestinal	Neurological	Respiratory	Musculoskeletal	Asthenia	Skin
0-1	51	11 (22)	5 (10)	3 (6)	4 (8)	3 (6)	0 (0)
1-12	51	15 (29)	11 (22)	8 (16)	7 (14)	6 (12)	6 (12)
12-24	38	4 (11)[b]	1 (3)[b]	2 (5)	4 (11)	3 (8)	3 (8)
24-36	34	3 (9)[b]	2 (6)	3 (9)	3 (9)	1 (3)	2 (6)
36-48	29	1 (3)[b]	2 (7)	2 (7)	3 (10)	1 (3)	2 (7)

[a] Grade ≥ 2 symptoms in > 5 percent of patients.
Statistically significant reduction of patients experiencing side effects compared with first year PT (PT month 0-12), [b]$P < 0.05$.
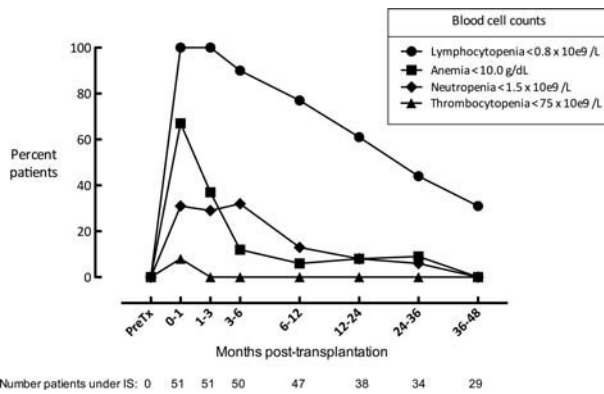
FIGURE 2. Depletion of blood cells in nonuremic type 1 diabetic recipients of long-term cultured islet β cell allograft under immune suppression. Pre-Tx: pretransplantation.

not seen in our cohort. AEs related to IS were reported in 57% of patients. They occurred predominantly during the first year after transplantation, when the patients were treated with higher doses of MMF-Tac, together with the cumulative effect of ATG induction therapy and concomitant prophylactic medication. As observed in other transplant protocols,[25-29] gastrointestinal and neurological symptoms, well-recognized side effects of respectively MMF and/or Tac, were the most frequent in this study. MMF-related gastrointestinal symptoms rarely needed hospitalization and resolved after short-term supportive therapy. The majority of these AE resolved after dose reduction of immune therapy and side effects of grade 2 or more were observed in only a quarter of patients after the first year. In line with other reports,[30-32] the majority of patients in the present study found the side effects less debilitating when compared with severe hypoglycemic episodes and unstable metabolic control and were satisfied with the transplantation.

In all patients, depletion of blood cells was observed in the first month after the first implant, caused by ATG induction, start of maintenance IS and the administration of valganciclovir and trimethoprim-sulfamethoxazole. Frequent blood sampling in the peritransplant period may have aggravated anemia. Most patients presented with neutropenia during the first month, but this was not associated with serious infections or high numbers of opportunistic infections. All patients had prolonged lymphocytopenia. The use of lymphocyte-depleting therapies is a well-recognized risk factor for cytomegalovirus infection,[33-35] explaining the comparable CMV disease incidence in our cohort as in the study of Gala-Lopez et al[35] (9% vs 9.5%). It is also in line with lower CMV disease incidence observed in other islet transplant cohorts not using T cell depletion.[4,36] An additional important risk factor for CMV disease is the use of seropositive organ donors in seronegative recipients,[37-39] which was the case in 4 of 5 patients in our study. Except for 1 patient, CMV disease occurred more than 100 days after the transplantation, when prophylactic valganciclovir was stopped according to our protocol. Extending the duration of prophylactic therapy might prevent additional cases as shown after kidney transplantation.[40]

Cancer is a well-established risk of chronic IS.[41] None of the patients in our cohort developed lymphoma or related diseases. Four patients with a well-functioning islet graft were diagnosed with cancer, 3 of them after year 2 PT. In

3 patients, diagnosis was made at an early stage, with immediate reduction (prostate cancer, colon cancer) or cessation (melanoma) of IS and follow-up has been so far uneventful. One patient died from liver metastasized gastric carcinoma. Although we cannot rule out the contribution of IS in the occurrence of this late stage gastric cancer, it is probable that an underlying pathology was already present before transplantation. This is probably also the case for the 3 SAEs due to macrovascular events because they occurred in the early stages after transplantation (1, 4, and 18 months PT) in a patient population with already a high incidence of preexisting macrovascular disease. Nevertheless, our findings emphasize the need for frequent monitoring of islet transplant recipients.

Another well-known risk of calcineurin inhibitor–based IS is deterioration of native kidney function in recipients of a nonrenal organ.[42] In a previous study, we already showed that the use of Tac caused an initial 20% reduction in
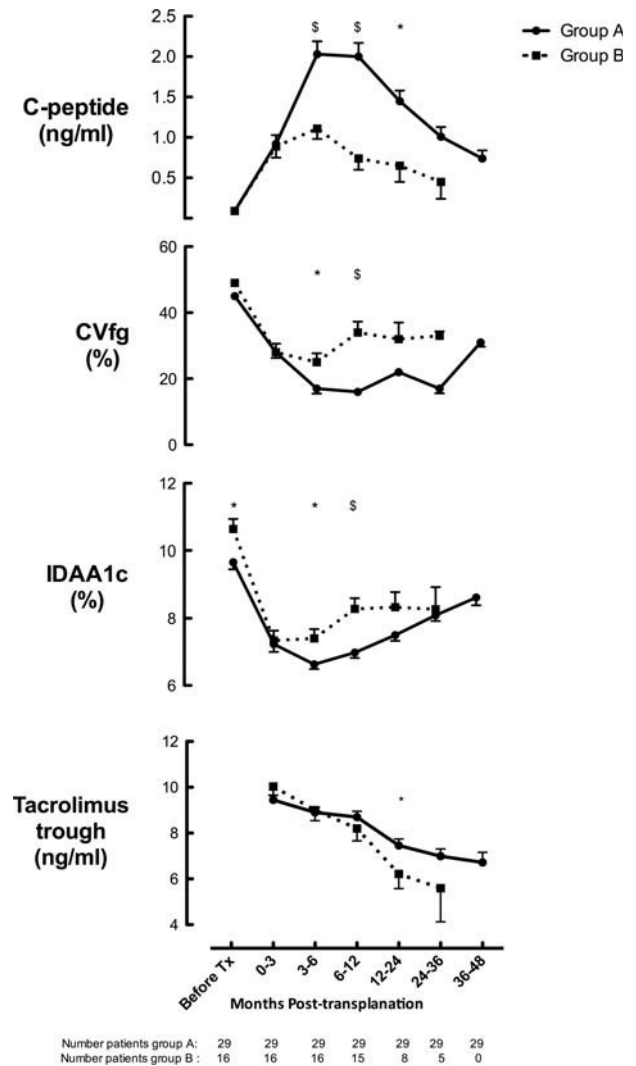

FIGURE 3. Implant function and metabolic control in diabetic recipients of long-term cultured islet β-cell allograft. Data are shown as mean ± SEM. Random C-peptide measured when glycemia between 120 and 220 mg/dL. Group A: under immune suppression for 48 months, Group B: immune suppression stopped before 48 months due to graft failure. Statistically significant compared with Group B *$P < 0.05$, $P < 0.005$. IDAA1c, Insulin dose-adjusted hemoglobin A1c.

TABLE 5.
Implant function and metabolic control in diabetic recipients of long-term cultured islet β cell allograft

Follow-up PT months			Immune suppression										Metabolic control							
	Patient number, n		Tacrolimus trough, ng/mL		MMF dose,[a] mg/d		C-peptide,[b] ng/mL		CVfg, %		HbA1c, %		Insulin dose, IU/kg per day		% of patients with insulin independence		% patients with detectable c-peptide (≥0.2 ng/mL)	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Before Tx	29	16					0.09 (0.09-0.09)	0.09 (0.09-0.09)	46 (40-50)	47 (41-55)	7.6 (7.0-8.3)	8.1 (7.5-8.8)	0.55 (0.42-0.63)	0.58 (0.52-0.79)				
0 - 3	29	16	9.5 (8.8-10.2)	10.0 (9.0-10.2)	2000 (2000-2000)	2000 (2000-2000)	0.81 (0.45-1.21)	0.77 (0.48-1.22)	26 (0-36)	26 (20-31)	5.4 (5.0-6.2)	5.7 (5.1-5.9)	0.39 (0.30-0.52)	0.41 (0.22-0.56)	3	6	97	94
3 - 6	29	16	8.9 (8.0-10.0)	9.2 (8.0-10.5)	1500 (1000-2000)	1500 (1500-2000)	2.06[a] (1.46-2.50)	1.00 (0.67-1.60)	16[c] (12-22)	25 (17-34)	6.0 (5.5-6.2)	6.1 (5.6-7.0)	0.16 (0.02-0.29)	0.27 (0.20-0.48)	48	19	100	100
6 - 12	29	15	8.7 (8.0-9.1)	7.3 (6.5-10.0)	1500 (1000-1500)	1500 (1500-2000)	1.85[a] (1.07-2.74)	0.53 (0.28-0.97)	16[d] (11-20)	36 (23-46)	6.4[c] (5.9-6.8)	6.7 (6.2-7.5)	0.13[d] (0-0.25)	0.33 (0.20-0.48)	52	13	100	93
12-24	29	8	8.0[c] (6.9-8.0)	6.5 (4.4-7.6)	1250 (1000-1500)	1500 (1250-1500)	1.39[c] (0.52-1.93)	0.45 (0.21-1.22)	22 (18-28)	31 (18-42)	6.7 (6.3-7.1)	7.2 (6.4-7.7)	0.20 (0.09-0.30)	0.36 (0.07-0.45)	38	14	100	88
24-36	29	5	7.0 (6.3-8.0)	6.5 (2.2-8.6)	1000 (1000-1500)	1250 (1000-1250)	0.80 (0.52-1.34)	0.19 (0.10-0.95)	26 (23-33)	34 (30-35)	7.0 (6.5-7.5)	7.1 (5.8-7.7)	0.29 (0.17-0.39)	0.43 (0.19-0.53)	14	0	97	40
36-48	29	0	7.0 (5.0-8.4)		1000 (1000-1500)		0.66 (0.31-1.01)		33 (25-36)		7.2 (6.2-8.0)		0.31 (0.23-0.42)		7		93	

[a] Total 4 patients (3 in IS continue and 1 in IS stop group) were temporarily under mycophenolic acid (Myfortic) during the first year and switched to mycophenolate mofetil (Cellcept) thereafter.
[b] Median C-peptide with glycemia between 120 and 220 mg/dL.
Data are shown as median (IQR).
Statistically significant compared with group B [c] P < 0.05, [d] P < 0.005.
A: Under immune suppression for 48 months, B: immune suppression stopped before 48 months due to graft failure.

estimated glomerular filtration rate which was reversible after its discontinuation.[43] Moreover, the risk-benefit ratio of IS regimen using sirolimus with lower levels of Tac is not better than IS regimens using MMF with higher levels of Tac.[44] Our previous study using sirolimus-Tac or sirolimus alone showed in patients with preexisting microalbuminuria, a worsening of albuminuria that was caused by sirolimus,[45] supporting further use of a MMF-Tac–based regimen in this patient population.[43]

The most relevant clinical benefit for the patients was the absence of severe hypoglycemia PT. All patients also had significantly better (insulin dose-adjusted) HbA1c and a significant reduction in glycemic variability, which are well-known risk factors both for long-term complications and hypoglycemia.[46-49] Although C-peptide levels were comparable to patients with optimal graft function in other studies,[2,7,9] maintenance of insulin independence was similar[2] or lower in our cohort. Although this can be a sign of more rapid decline in β-cell mass, our approach has always been to rapidly restart insulin if glucose values become abnormal preprandial or postprandial, leading to early reintroduction of insulin.

The patient group where IS was stopped because of loss of graft function had similar initial graft function (ie, 0-3 months) as the group that maintained graft function during 48 months. The last group exhibited a twofold rise in graft function during the 3 to 6 months PT, whereas no increase in graft function was measured in the patient group that failed within the first 4 years. This is in line with the publication by Vantyghem et al,[7] emphasizing the importance of optimal graft function 1 month after the last implant in maintaining long-term graft function with the Edmonton protocol.

The patient group who became C-peptide negative during follow-up was significantly younger. There are no surrogate markers that allow us to examine the possible reasons for this difference in outcome. MMF and Tac levels were not different with the first year PT but the patients with less favorable outcome were significantly younger and had a significantly worse baseline glucose control. Because HbA1c was not different at 0 to 3 months PT, we hypothesize that immune-related factors rather than metabolic factors are responsible for the worse outcome. It is well established in other organ transplantation settings that older age is associated with improved transplant survival and lower rates of rejection.[50] Another reason for worse graft function in younger patients might be less compliance with the immunosuppressive regimen.[51,52] Future islet transplant protocols can explore the risk-benefit ratio of more potent immune suppressive regimen, especially in younger subjects.

In our cohort under IS during 48 months, a decline in implant function started already after the first year. It is unknown whether this decline is due to a reduction in Tac and/or MMF and/or a limited graft β cell mass that is vulnerable to metabolic stress. Studies are ongoing that aim to reduce the important loss of functional β cells immediately after intraportal transplantation[53-56] (ClinicalTrials.gov identifier: NCT00789308). A phase 1 study with embryonic stem cell derived endocrine cells[57] has also been started (ClinicalTrials.gov identifier: NCT02239354); this new cell source may overcome the barrier of the limited amount of cells that is available for transplantation when deceased donors are used.

In conclusion, islet transplantation using ATG in combination with MMF-Tac is safe to use in the long term, with side effects mostly limited to the first year with the maintenance of metabolic benefit in those with good initial graft function.

REFERENCES

1. Pepper AR, Gala-Lopez B, Ziff O, et al. Current status of clinical islet transplantation. *World J Transplant*. 2013;3:48–53.
2. Lablanche S, Borot S, Wojtusciszyn A, et al. Five-year metabolic, functional, and safety results of patients with type 1 diabetes transplanted with allogenic islets within the Swiss-French GRAGIL network. *Diabetes Care*. 2015;38:1714–1722.
3. Choudhary P, Rickels MR, Senior PA, et al. Evidence-informed clinical practice recommendations for treatment of type 1 diabetes complicated by problematic hypoglycemia. *Diabetes Care*. 2015;38:1016–1029.
4. Ryan EA, Paty BW, Senior PA, et al. Five-year follow-up after clinical islet transplantation. *Diabetes*. 2005;54:2060–2069.
5. Froud T, Ricordi C, Baidal DA, et al. Islet transplantation in type 1 diabetes mellitus using cultured islets and steroid-free immunosuppression: Miami experience. *Am J Transplant*. 2005;5:2037–2046.
6. Keymeulen B, Gillard P, Mathieu C, et al. Correlation between beta cell mass and glycemic control in type 1 diabetic recipients of islet cell graft. *Proc Natl Acad Sci U S A*. 2006;103:17444–17449.
7. Vantyghem MC, Kerr-Conte J, Arnalsteen L, et al. Primary graft function, metabolic control, and graft survival after islet transplantation. *Diabetes Care*. 2009;32:1473–1478.
8. Brooks AM, Walker N, Aldibbiat A, et al. Attainment of metabolic goals in the integrated UK Islet Transplant Program with locally isolated and transported preparations. *Am J Transplant*. 2013;13:3236–3243.
9. Scientific Summary of the Collaborative Islet Transplant Registry (CITR) 2012 (Eighth) Annual Report. 2015;1.
10. Keymeulen B, Ling Z, Gorus FK, et al. Implantation of standardized beta-cell grafts in a liver segment of IDDM patients: graft and recipients characteristics in two cases of insulin-independence under maintenance immunosuppression for prior kidney graft. *Diabetologia*. 1998;41:452–459.
11. Movahedi B, Keymeulen B, Lauwers M-H, et al. Laparoscopic approach for human islet transplantation into a defined liver segment in type-1 diabetic patients. *Transpl Int*. 2003;16:186–190.
12. Maleux G, Gillard P, Keymeulen B, et al. Feasibility, safety, and efficacy of percutaneous transhepatic injection of beta-cell grafts. *J Vasc Interv Radiol*. 2005;16:1693–1697.
13. Koh A, Senior P, Salam A, et al. Insulin-heparin infusions peritransplant substantially improve single-donor clinical islet transplant success. *Transplantation*. 2010;89:465–471.
14. CITT. htm [Internet]. www2.niddk.nih.gov [cited 2016 May 4] Available from: http://www2.niddk.nih.gov/Research/ScientificAreas/Pancreas/EndocrinePancreas/CITT.htm.
15. Commissioner OOT. Reporting Serious Problems to FDA - What is a Serious Adverse Event? http://www.fda.gov/Safety/MedWatch/HowToReport/ucm053087.htm. Updated 2016. Accessed September 26, 2016.
16. Mortensen HB, Hougaard P, Swift P, et al. New definition for the partial remission period in children and adolescents with type 1 diabetes. *Diabetes Care*. 2009;32:1384–1390.
17. Hafiz MM, Faradji RN, Froud T, et al. Immunosuppression and procedure-related complications in 26 patients with type 1 diabetes mellitus receiving allogeneic islet cell transplantation. *Transplantation*. 2005;80:1718–1728.

18. Takita M, Matsumoto S, Shimoda M, et al. Safety and tolerability of the T cell depletion protocol coupled with anakinra and etanercept for clinical islet cell transplantation. *Clin Transplant*. 2012;26:E471–E484.

19. Al-Adra DP, Gill RS, Imes S, et al. Single-donor islet transplantation and long-term insulin independence in select patients with type 1 diabetes mellitus. *Transplantation*. 2014;98:1007–1012.

20. Qi M, Kinzer K, Danielson KK, et al. Five-year follow-up of patients with type 1 diabetes transplanted with allogeneic islets: the UIC experience. *Acta Diabetol*. 2014;51:833–843.

21. Ludwig B, Reichel A, Kruppa A, et al. Islet transplantation at the Dresden Diabetes Center: five years' experience. *Horm Metab Res*. 2015;47:4–8.

22. Rickels MR, Liu C, Shlansky-Goldberg RD, et al. Improvement in β-cell secretory capacity after human islet transplantation according to the CIT07 Protocol. *Diabetes*. 2013;62:2890–2897.

23. Consortium AIT. Multicenter Australian Trial of Islet Transplantation: improving accessibility and outcomes. *Am J Transplant*. 2013;13: 1850–1858.

24. Caiazzo R, Vantyghem MC, Raverdi V, et al. Impact of procedure-related complications on long-term islet transplantation outcome. *Transplantation*. 2015;99:979–984.

25. Savvidaki E, Papachristou E, Kazakopoulos P, et al. Gastrointestinal disorders after renal transplantation. *Transplant Proc*. 2014;46:3183–3186.

26. Gruessner RW. Tacrolimus in pancreas transplantation: a multicenter analysis. Tacrolimus Pancreas Transplant Study Group. *Clin Transplant*. 1997; 11:299–312.

27. Gruessner RW, Sutherland DE, Drangstveit MB, et al. Mycophenolate mofetil in pancreas transplantation. *Transplantation*. 1998;66:318–323.

28. Scalea JR, Levi ST, Ally W, et al. Tacrolimus for the prevention and treatment of rejection of solid organ transplants. *Expert Rev Clin Immunol*. 2016;12:333–342.

29. Guethoff S, Meiser BM, Groetzner J, et al. Ten-year results of a randomized trial comparing tacrolimus versus cyclosporine a in combination with mycophenolate mofetil after heart transplantation. *Transplantation*. 2013; 95:629–634.

30. Benhamou PY, Milliat-Guittard L, Wojtusciszyn A, et al. Quality of life after islet transplantation: data from the GRAGIL 1 and 2 trials. *Diabet Med*. 2009;26:617–621.

31. Toso C, Shapiro AMJ, Bowker S, et al. Quality of life after islet transplant: impact of the number of islet infusions and metabolic outcome. *Transplantation*. 2007;84:664–666.

32. Poggioli R, Faradji RN, Ponte G, et al. Quality of life after islet transplantation. *Am J Transplant*. 2006;6:371–378.

33. Kaufman DB, Iii GWB, Bruce DS, et al. Prospective, randomized, multicenter trial of antibody induction therapy in simultaneous pancreas-kidney transplantation. *Am J Transplant*. 2003;3:855–864.

34. Huurman VAL, Kalpoe JS, van de Linde P, et al. Choice of antibody immunotherapy influences cytomegalovirus viremia in simultaneous pancreas-kidney transplant recipients. *Diabetes Care*. 2006;29:842–847.

35. Gala-Lopez BL, Senior PA, Koh A, et al. Late cytomegalovirus transmission and impact of T-depletion in clinical islet transplantation. *Am J Transplant*. 2011;11:2708–2714.

36. Hafiz MM, Poggioli R, Caulfield A, et al. Cytomegalovirus prevalence and transmission after islet allograft transplant in patients with type 1 diabetes mellitus. *Am J Transplant*. 2004;4:1697–1702.

37. Freeman RB, Paya C, Pescovitz MD, et al. Risk factors for cytomegalovirus viremia and disease developing after prophylaxis in high-risk solid-organ transplant recipients. *Transplantation*. 2004;78:1765–1773.

38. Humar A, Paya C, Pescovitz MD, et al. Clinical utility of cytomegalovirus viral load testing for predicting CMV disease in D+/R– solid organ transplant recipients. *Am J Transplant*. 2004;4:644–649.

39. Humar A, Mazzulli T, Moussa G, et al. Clinical utility of cytomegalovirus (CMV) serology testing in high-risk CMV D+/R- transplant recipients. *Am J Transplant*. 2005;5:1065–1070.

40. Humar A, Limaye AP, Blumberg EA, et al. Extended valganciclovir prophylaxis in D+/R- kidney transplant recipients is associated with long-term reduction in cytomegalovirus disease: two-year results of the IMPACT study. *Transplantation*. 2010;90:1427–1431.

41. Chapman JR, Webster AC, Wong G. Cancer in the transplant recipient. *Cold Spring Harb Perspect Med*. 2013;3:a015677.

42. Ojo AO, Held PJ, Port FK, et al. Chronic renal failure after transplantation of a nonrenal organ. *N Engl J Med*. 2003;349:931–940.

43. Gillard P, Rustandi M, Efendi A, et al. Early alteration of kidney function in nonuremic type 1 diabetic islet transplant recipients under tacrolimus-mycophenolate therapy. *Transplantation*. 2014;98:451–457.

44. Vantyghem MC, Defrance F, Quintin D, et al. Treating diabetes with islet transplantation: lessons from the past decade in Lille. *Diabetes Metab*. 2014;40:108–119.

45. Gillard P, Ling Z, Mathieu C, et al. Comparison of sirolimus alone with sirolimus plus tacrolimus in type 1 diabetic recipients of cultured islet cell grafts. *Transplantation*. 2008;85:256–263.

46. Gorst C, Kwok CS, Aslam S, et al. Long-term glycemic variability and risk of adverse outcomes: a systematic review and meta-analysis. *Diabetes Care*. 2015;38:2354–2369.

47. Weber C, Schnell O. The assessment of glycemic variability and its impact on diabetes-related complications: an overview. *Diabetes Technol Ther*. 2009;11:623–633.

48. Nathan DM, for the DCCT/EDIC Research Group. The diabetes control and complications trial/epidemiology of diabetes interventions and complications study at 30 years: overview. *Diabetes Care*. 2014;37: 9–16.

49. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. *N Engl J Med*. 1993;329:977–986.

50. Tullius SG, Tran H, Guleria I, et al. The combination of donor and recipient age is critical in determining host immunoresponsiveness and renal transplant outcome. *Ann Surg*. 2010;252:662–674.

51. Gaynor JJ, Ciancio G, Guerra G, et al. Graft failure due to noncompliance among 628 kidney transplant recipients with long-term follow-up: a single-center observational study. *Transplantation*. 2014;97:925–933.

52. Bosma OH, Vermeulen KM, Verschuuren EA, et al. Adherence to immunosuppression in adult lung transplant recipients: prevalence and risk factors. *J Heart Lung Transplant*. 2011;30:1275–1280.

53. Moberg L, Olsson A, Berne C, et al. Nicotinamide inhibits tissue factor expression in isolated human pancreatic islets: implications for clinical islet transplantation. *Transplantation*. 2003;76:1285–1288.

54. Goto M, Johansson H, Maeda A, et al. Low-molecular weight dextran sulfate abrogates the instant blood-mediated inflammatory reaction induced by adult porcine islets both in vitro and in vivo. *Transplant Proc*. 2004;36: 1186–1187.

55. Özmen L, Ekdahl KN, Elgue G, et al. Inhibition of thrombin abrogates the instant blood-mediated inflammatory reaction triggered by isolated human islets possible application of the thrombin inhibitor melagatran in clinical islet transplantation. *Diabetes*. 2002;51:1779–1784.

56. Cabric S, Sanchez J, Lundgren T, et al. Islet surface heparinization prevents the instant blood-mediated inflammatory reaction in islet transplantation. *Diabetes*. 2007;56:2008–2015.

57. Kroon E, Martinson LA, Kadoya K, et al. Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. *Nat Biotechnol*. 2008;26:443–452.