

ORIGINAL ARTICLE

International Trial of the Edmonton Protocol for Islet Transplantation

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ABSTRACT

BACKGROUND

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Islet transplantation offers the potential to improve glycemic control in a subgroup of patients with type 1 diabetes mellitus who are disabled by refractory hypoglycemia. We conducted an international, multicenter trial to explore the feasibility and reproducibility of islet transplantation with the use of a single common protocol (the Edmonton protocol).

METHODS

We enrolled 36 subjects with type 1 diabetes mellitus, who underwent islet transplantation at nine international sites. Islets were prepared from pancreases of deceased donors and were transplanted within 2 hours after purification, without culture. The primary end point was defined as insulin independence with adequate glycemic control 1 year after the final transplantation.

RESULTS

Of the 36 subjects, 16 (44%) met the primary end point, 10 (28%) had partial function, and 10 (28%) had complete graft loss 1 year after the final transplantation. A total of 21 subjects (58%) attained insulin independence with good glycemic control at any point throughout the trial. Of these subjects, 16 (76%) required insulin again at 2 years; 5 of the 16 subjects who reached the primary end point (31%) remained insulin-independent at 2 years.

CONCLUSIONS

Islet transplantation with the use of the Edmonton protocol can successfully restore long-term endogenous insulin production and glycemic stability in subjects with type 1 diabetes mellitus and unstable control, but insulin independence is usually not sustainable. Persistent islet function even without insulin independence provides both protection from severe hypoglycemia and improved levels of glycated hemoglobin. (ClinicalTrials.gov number, NCT00014911.)

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DESPITE SUBSTANTIAL IMPROVEMENTS in insulin therapy and the care of patients with type 1 diabetes mellitus, a subgroup of patients is disabled by refractory hypoglycemia. Cell-based therapy with islet transplantation offers the possibility of improved glycemic control. The past three decades have witnessed substantial progress in islet transplantation.¹⁻³ Before the year 2000, few centers performing islet transplantation achieved high rates of sustainable insulin independence after this procedure among patients with type 1 diabetes mellitus.¹⁻³ In 2000, Shapiro et al.⁴ reported their initial findings with up to a year of follow-up in seven consecutive subjects treated with glucocorticoid-free immunosuppressive therapy combined with infusion of an adequate mass of freshly prepared islets from two or more pancreases from deceased donors.⁵ In all seven subjects, insulin independence was achieved, with tight glycemic control and correction of glycosylated hemoglobin levels. This treatment became known as the Edmonton protocol. The goal of our study was to explore the feasibility and reproducibility of this protocol for islet preparation and management after transplantation, including immunosuppression.

METHODS

STUDY DESIGN

The nine international centers — six in North America and three in Europe — that participated in the study used a common protocol (the Edmonton protocol) of islet preparation and post-transplantation care. We required that investigators at each site demonstrate a consistent ability to prepare human islets under Good Manufacturing Practice conditions and apply standardized criteria for islet enumeration and product release. Investigators at each of the participating sites underwent intensive training in the preparation process and used common batch lots of collagenase enzyme. The level of previous experience in clinical islet transplantation varied among the participating centers from substantial to none.

We designed the study to be a single-group, phase 1-2 trial. The study was organized by the Immune Tolerance Network, initiated by the National Institutes of Health, with a goal of establishing centers of excellence to conduct future tolerance-based trials (details are available at www.immunetolerance.org).⁶ Our target enroll-

ment was 36 subjects, with 4 subjects per site, on the basis of available funding. Up to three islet infusions were permitted per subject until insulin independence was reached, on condition that partial islet function persisted after the preceding transplantation. The study had a planned follow-up of 3 years for all subjects after their last transplantation.

STUDY DEFINITIONS

We defined insulin independence as freedom from the need to take exogenous insulin, with adequate glycemic control, as defined by a glycosylated hemoglobin level of less than 6.5%, with a glucose level after an overnight fast not exceeding 140 mg per deciliter (7.8 mmol per liter) more than three times in any week (based on the morning fasting glucose level) and not exceeding 2-hour postprandial levels of 180 mg per deciliter (10 mmol per liter) more than four times per week. We recognize that applying more stringent measures for glycemic control might have altered the outcome.

We defined partial graft function as a C-peptide level of at least 0.3 ng per milliliter and a requirement for insulin or inadequate glycemic control. Complete graft loss was defined as primary nonfunction (an initial C-peptide level of <0.3 ng per milliliter), early graft loss (an initial increase in the C-peptide level but a decrease to less than 0.3 ng per milliliter within 2 months), or withdrawal from further treatment, with cessation of immunosuppression imputed from 13 weeks after withdrawal. A severe hypoglycemic event in the year after the last transplantation was defined as an episode of neuroglycopenia with unawareness severe enough for the subject to require assistance; such episodes were ascertained both by chart review and interviews for each subject.

STUDY END POINTS

The primary end point was defined as insulin independence with adequate glycemic control 1 year after the final transplantation. Secondary end points included insulin independence with adequate glycemic control throughout follow-up; improved values for levels of glycosylated hemoglobin, the mean amplitude of glycemic excursions, and basal and stimulated blood C-peptide levels in response to arginine challenge; and a reduction in the need for insulin, as compared with baseline. Written informed consent was obtained from subjects and from the families of deceased donors.

Table 1. Baseline and Procedural Characteristics and Arginine-Stimulated C-Peptide at 1 Year after the Last Transplantation.*

Subject No.	Baseline				Transplantation			Follow-up at 1 Yr		
	Age	Time since Diagnosis of Diabetes years	Weight kg	BMI	Insulin Requirement U/kg/day	Total No.	Total Islet Equivalents $\times 10^{-3}$		Stimulated C Peptide ng/ml	
1	48	44	59.3	21	0.74	44	3	1108	18,721	2.9
2	43	16	53.1	21	0.60	32	3	1093	19,814	1.8
3	48	40	59.4	26	0.40	24	1	320	5,387	EGL
4	35	24	67.0	25	0.54	36	1	338	5,189	PNF
5	32	26	60.0	21	0.48	29	3	1297	22,482	1.5
6	41	34	56.1	22	0.52	29	3	1144	20,710	2.3
7	43	22	50.4	19	0.56	28	3	1135	20,662	0.4
8	41	24	59.2	22	0.46	27	2	719	12,644	2.4
9	49	38	67.5	22	0.68	46	1	657	9,840	2.2
10	42	25	55.9	21	0.68	38	3	1009	18,107	1.3
11	59	29	56.8	20	0.35	20	3	1055	19,885	2.6
12	56	51	65.0	24	0.34	22	1	564	8,997	2.1
13	34	21	75.0	24	0.64	48	3	1248	17,053	2.2
14	27	23	61.0	23	0.62	38	3	1173	19,553	1.8
15	56	43	61.0	19	0.57	35	3	1203	20,606	1.8
16	37	25	63.3	22	0.74	47	3	1156	17,633	WD†
17	32	11	59.0	22	0.51	30	2	749	13,206	WD‡
18	34	18	60.0	23	0.50	30	2	800	13,912	2.3
19	45	45	72.7	24	0.62	45	2	847	11,789	3.3
20	50	20	61.7	20	0.29	18	1	469	7,682	2.4
21	50	21	60.0	20	0.50	30	2	779	13,458	2.8
22	43	39	72.0	22	0.58	42	3	1087	15,524	1.8
23	30	15	62.0	23	0.60	37	3	846	13,141	1.1
24	24	12	64.0	23	0.56	36	1	315	5,006	EGL
25	44	28	69.1	22	0.29	20	2	720	10,329	1.6
26	46	23	58.4	21	0.33	19	1	410	6,723	1.6
27	38	20	55.3	22	0.47	26	1	324	5,894	2.5
28	55	38	56.0	21	0.51	29	2	715	13,333	2.3
29	35	21	57.0	24	0.49	28	3	1043	20,261	2.5

30	38	16	69.0	23	0.61	42	1	425	6,297	PNF
31	35	31	62.1	25	0.50	31	1	415	7,606	PNF
32	33	23	51.8	20	0.39	20	1	286	5,677	PNF
33	30	29	66.2	23	0.45	30	3	1013	15,526	3.0
34	51	42	62.4	22	0.50	31	2	689	11,968	WD§
35	45	30	70.4	25	0.45	32	3	1318	19,745	1.7
36	23	11	58.1	23	0.64	37	2	591	10,667	WD¶
Mean	41	27	61.6	22	0.52	32	2	807	13,473	NA
Median	42	25	60.5	22	0.51	31	2	790	13,269	NA
SE	2	2	1.0	<1	0.02	1	<1	54	923	NA

* BMI denotes body-mass index (the weight in kilograms divided by the square of the height in meters), EGL early graft loss, PNF primary nonfunction of the graft, WD withdrawn voluntarily with subsequent complete graft loss, and NA not applicable.

† The subject withdrew from the study on the basis of a personal decision.

‡ The subject withdrew from the study because of an immunosuppressive side effect (headache).

§ The subject withdrew from the study because of immunosuppressive side effects (mouth ulcers, diarrhea).

¶ The subject withdrew from the study because of compliance issues.

Formal approval was obtained from the investigational review board at each site.

RECIPIENT SELECTION

Eligible subjects were between the ages of 18 and 65 years, had undetectable C-peptide levels, and had had type 1 diabetes mellitus for more than 5 years with recurrent neuroglycopenia, including reduced awareness of their hypoglycemic episodes or severe glycemic lability. To confirm eligibility, an endocrinologist or diabetologist assessed subjects independently of the islet-transplantation team. Appropriate attempts to optimize intensive insulin therapy and glycemic monitoring had failed in all subjects. Major exclusion criteria were noncorrectable coronary artery disease; a body-mass index (the weight in kilograms divided by the square of the height in meters) of more than 26; a weight of more than 70 kg (154 lb) for women or 75 kg (165 lb) for men; an insulin requirement of more than 0.7 U per kilogram of body weight per day; a glycated hemoglobin level of more than 12%; inadequate renal reserve, which was defined as a serum creatinine level of more than 1.5 mg per deciliter (133 μ mol per liter), a creatinine clearance of less than 80 ml per minute per 1.73 m² of body-surface area, or an albumin level of more than 300 mg per 24-hour period (macroalbuminuria); and negative results on serologic analysis for Epstein-Barr virus at the time of assessment (to avoid reactivation of the virus after transplantation).

DONOR SELECTION

Pancreases were obtained from brain-dead multiorgan donors ranging in age from 15 to 70 years. The organs were transported in chilled University of Wisconsin solution without the use of perflurodecalin, for a maximum cold-storage time of less than 12 hours. Standard criteria for donor exclusion were applied to minimize the risk of transmission of donor-derived infection or cancer.

IMMUNOSUPPRESSIVE REGIMEN

The immunosuppressive regimen was based on that previously described in the Edmonton protocol.⁴ Five doses of daclizumab at a dose of 1 mg per kilogram were administered intravenously over a period of 8 weeks after each transplantation. Sirolimus was administered once daily to achieve a target trough therapeutic range of 12 to 15 ng per milliliter for 3 months after transplantation, af-

ter which the target trough range was lowered to 7 to 12 ng per milliliter. Tacrolimus was administered twice daily and adjusted to achieve a target trough level of 3 to 6 ng per milliliter.

ISLET PREPARATION AND TRANSPLANTATION

Islets were prepared locally in Good Manufacturing Practice–grade facilities at each of the nine sites, according to identical standard operating procedures. The pancreas was distended by controlled ductal perfusion with the use of common batch lots of Liberase human islet enzyme (Roche Diagnostics), previously validated at the participating sites.⁷ The pancreas was digested in a Ricordi chamber and purified on continuous Ficoll gradients on a cooled apheresis system (model 2991, Cobe Laboratories). The islets were then washed and resuspended in transplant medium (Mediatech), and the manufactured islet-cell product was infused into the portal vein without culture within 2 hours after completion of the isolation and purification.^{4,8,9}

The final criteria for islet product release included an islet infusion compatible with the ABO blood group, an islet mass of 5000 islet equivalents per kilogram or more (on the basis of the weight of the recipient), an islet purity of 30% or more, a membrane-integrity viability of 70% or more, a packed-tissue volume of less than 10 ml, negative Gram's staining, and an endotoxin content of 5 endotoxin units per kilogram or less (on the basis of the weight of the recipient).

A cumulative islet mass of 10,000 islet equivalents per kilogram or more was delivered with at least two islet infusions, unless insulin independence was achieved with a single transplant. A third islet infusion was offered if circulating C peptide was detectable and insulin independence was not achieved after two infusions. The percutaneous transhepatic approach for portal venous access was used in all cases, with Doppler ultrasonography performed on days 1 and 7 after transplantation.^{10,11}

STATISTICAL ANALYSIS

On the basis of an enrollment of 36 subjects, we set the predicted proportion reaching the primary end point at 70%, with a 95% confidence interval (CI) of 57% to 83%. Event rates are expressed as percentages and the 95% CI is reported for specified outcomes. We used Fisher's exact test to assess the homogeneity of the rate of success accord-

ing to the research site and the chi-square test to assess the rate of success according to the level of experience at the site. With four subjects per site, there was adequate power (80%, with an alpha of 0.05) to detect extreme differences in proportions (0.01 to 0.99). Continuous measures, presented as means with the standard deviation or 95% CI, were compared by t-test analysis of variance, generalized estimating equations, or nonparametric testing. Kaplan–Meier estimates for outcome measures were made for the overall data and for strata-defined variables and were compared by means of the log-rank chi-square test. All reported P values are two-sided.

RESULTS

SUBJECTS

We screened approximately 2000 prospective subjects centrally to determine eligibility for enrollment. Of these subjects, only 149 (7%) fulfilled the initial stringent screening criteria and were referred to the sites. All nine sites enrolled subjects (seven sites with four subjects each, one site with five subjects, and one site with three subjects). All 36 subjects had one or more primary diabetes-related indications for enrollment: 35 (97%) had severe recurrent hypoglycemia, 20 (56%) had severe glycemic lability, and 19 (53%) had progressive secondary complications of type 1 diabetes mellitus (neuropathy, retinopathy, or nephropathy). Table 1 shows the demographic and clinical characteristics of the subjects, including baseline insulin requirements and the duration of disease, the transplanted islet mass, and stimulated C-peptide levels at 1 year.

NUMBER OF TRANSPLANTS AND FOLLOW-UP

Enrollment took place between May 2001 and January 2003, and in all 36 subjects, the primary end point was determined by June 2005. The 36 subjects received a total of 77 islet infusions, with 11 subjects (31%) receiving 1 infusion, 9 (25%) receiving 2 infusions, and 16 (44%) receiving 3 infusions. We evaluated 35 subjects at 2-year follow-up and 21 subjects at 3-year follow-up or later. The median follow-up time was 41 months (range, 37 to 50) from the time of the first transplantation.

OUTCOMES

One year after the final transplantation, 16 of 36 subjects (44%) had reached the primary end point

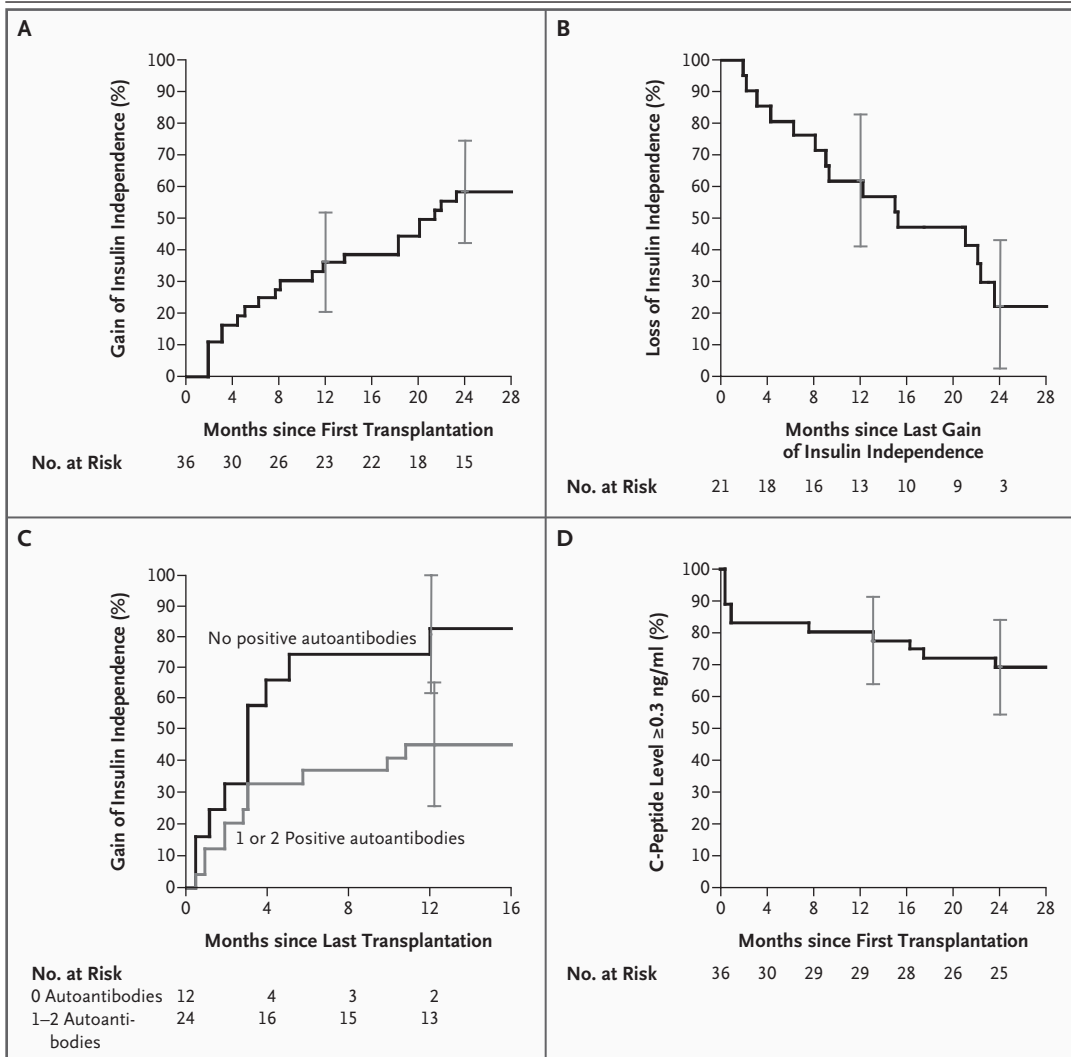


Figure 1. Kaplan–Meier Estimates of Event Rates after Islet Transplantation.

Panel A shows the interval between the first transplantation and insulin independence (attained in 21 of 36 subjects), and Panel B shows the subsequent loss of insulin independence among 16 of these 21 subjects during the next 28 months. Panel C shows insulin independence since the last transplantation according to the number of autoantibodies (glutamic acid decarboxylase 65, islet-cell autoantigen 512, or islet-cell autoantigen IA-2) detected before subjects underwent the last transplantation: 85% for the 12 subjects who had no positive autoantibodies and 46% for the 24 subjects who had one or two positive autoantibodies ($P=0.03$ by the log-rank test). Panel D shows the percentage of subjects who had a basal C-peptide level of at least 0.3 ng per milliliter after transplantation. After the first 2 months, a decrease in basal C peptide to levels below 0.3 ng per milliliter occurred only in subjects who stopped receiving immunosuppressive therapy, with a presumed subsequent loss of islet function. I bars denote 95% CIs.

(5 with one transplant, 6 with two transplants, and 5 with three transplants), 10 subjects (28%) had partial graft function, and 10 subjects (28%) had complete graft loss (4 with primary nonfunction, 2 with early graft loss, and 4 who withdrew from further treatment). All subjects with residual islet function were completely protected from severe hypoglycemic episodes, as reported from

days 28 to 365 after transplantation. As of February 2006, 24 of 36 subjects (67%) had at least partial graft function (11 subjects at 3 years), and 6 subjects were insulin-independent (1 subject at 3 years). The time to insulin independence reflects the limitations of isolating sufficient islets from available pancreas donors in a multicenter trial (45% of isolations resulted in clinical transplants)

(Fig. 1A). Of the 21 subjects who reached insulin independence (58%), 16 subjects (76%) were dependent on insulin again at 2 years (Fig. 1B). There was a significant correlation between attainment of insulin independence and autoantibody status ($P=0.03$) (Fig. 1C). C-peptide secretion was detectable (≥ 0.3 ng per milliliter) in 70% of subjects at 2 years (Fig. 1D).

Subjects were evaluated for a reduction in the need for insulin, levels of fasting glucose and glycated hemoglobin, basal C-peptide secretion, and the mean amplitude of glycemic excursions over time; subjects with insulin independence or partial graft function had a substantial benefit in all measures during 2 years of follow-up, as compared with subjects with complete graft loss (Fig. 2A through 2E). Subjects who reached the primary end point had full protection from severe hypoglycemia or hyperglycemia, and those with partial function had a marked benefit in glycemic control, in contrast to their baseline status (Fig. 2F). Figure 3A shows site-to-site heterogeneity in the proportion of subjects who reached the primary end point (range, 0 to 100%; $P=0.05$ by Fisher's exact test). Experience with islet transplantation at various sites and the use of sirolimus in the 2 years preceding the start of the trial are shown in Figure 3B. A positive relation between previous experience with islet transplantation at a site and the attainment of the primary end point was observed. The primary end point was reached by 12 of 18 subjects (67%) at sites where four or more transplantations had been performed in the preceding 2 years, as compared with only 4 of 18 subjects (22%) at sites where fewer than four transplantations had been performed ($P=0.007$ by the chi-square test).

ADVERSE EVENTS

There were no reports of death, post-transplantation lymphoproliferative disease, cancer, or opportunistic infections among the study subjects. There was no disease related to cytomegalovirus or Epstein-Barr virus on the basis of clinical presentation or central monitoring.

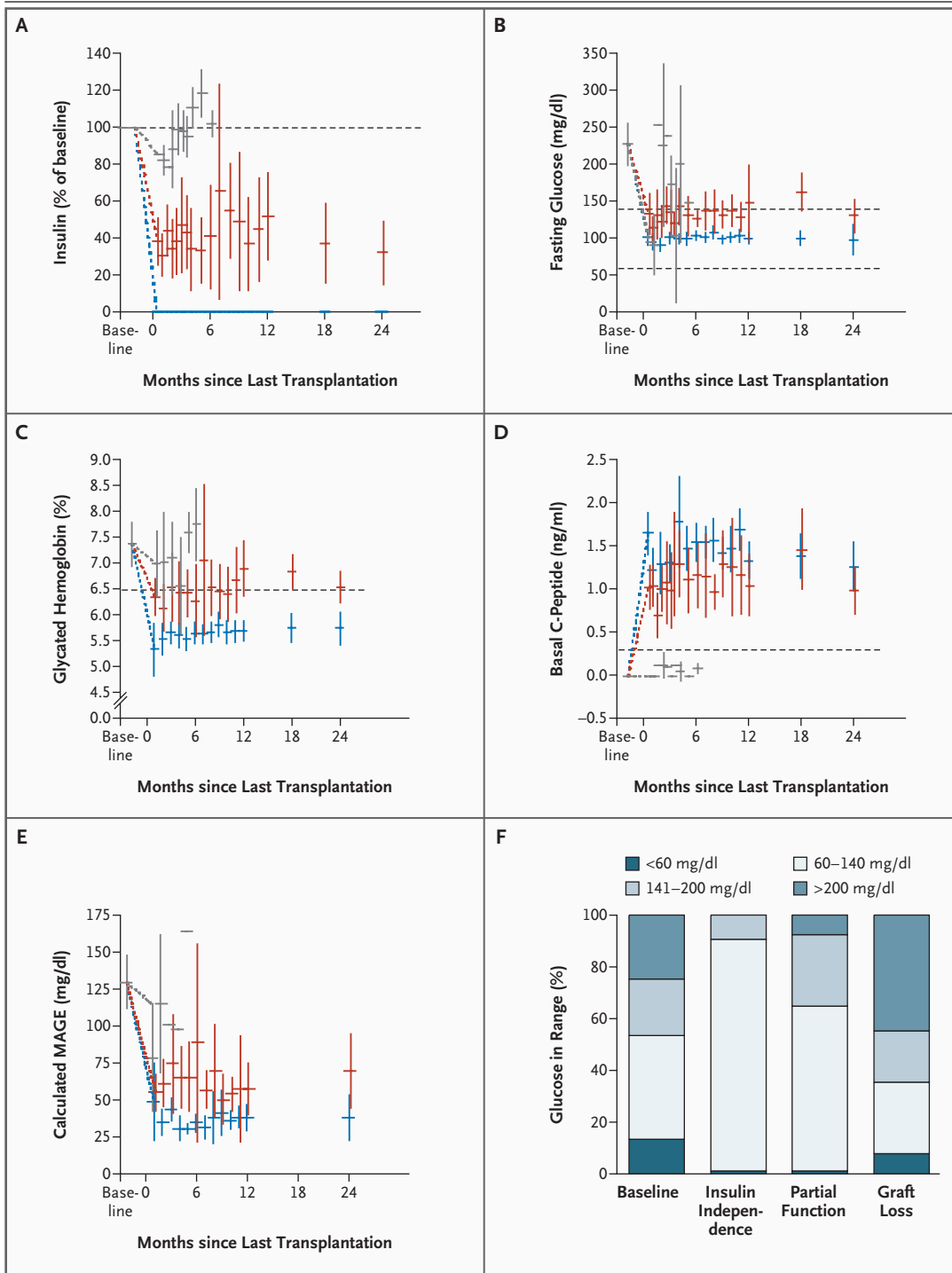
Of a total of 38 serious adverse events, 23 were considered to be related to the study therapy (18 of which were associated with hospitalization). Serious immunosuppression-related events included neutropenia (five cases), pneumonia, mouth ulcers, gastrointestinal conditions (two cases), fever, chest pain, pericardial effusion, pyelonephritis,

Figure 2 (facing page). Measures of Glycemic Control after Islet Transplantation.

Panels A through E show mean values for glycemic control during the 24 months after the last transplantation for subjects in whom insulin independence was achieved (blue), those with partial graft function (red), and those with complete graft loss (gray). Horizontal lines indicate target limits for levels of glucose and glycated hemoglobin. I bars denote 95% CIs. Panel A shows insulin requirements as a percentage of the amount required before transplantation (baseline). $P<0.001$ for the comparison between the insulin-independence group and the partial-function group, and $P<0.001$ for the comparison between baseline and each follow-up time point in both groups. Panel B shows glucose levels after an overnight fast. $P<0.001$ for the comparison between the insulin-independence and partial-function groups, and $P<0.001$ for the comparison between baseline and each follow-up time point in both groups. Panel C shows glycated hemoglobin levels. $P<0.001$ for the comparison between the insulin-independence and partial-function groups, and $P<0.001$ for the comparison between baseline and each follow-up time point except 12 months in both groups. Panel D shows C-peptide levels. $P=0.17$ for the comparison between the insulin-independence and partial-function groups, and $P<0.001$ for the comparison between baseline and each follow-up time point in both groups. Panel E shows the mean amplitude of glycemic excursions (MAGE). $P=0.01$ for the comparison between the insulin-independence and partial-function groups at months 4 through 7 ($P>0.05$ for subsequent months), and $P<0.001$ for the comparison between baseline and each follow-up time point in both groups. For all these measures, $P<0.001$ for the comparison between patients with complete graft loss and those with insulin independence or partial graft function. Panel F shows categorical capillary glucose values (in milligrams per deciliter) at baseline and 1 year after transplantation. To convert values for glucose to millimoles per liter, multiply by 0.05551. All P values are based on generalized estimating equations with adjustment for repeated measures.

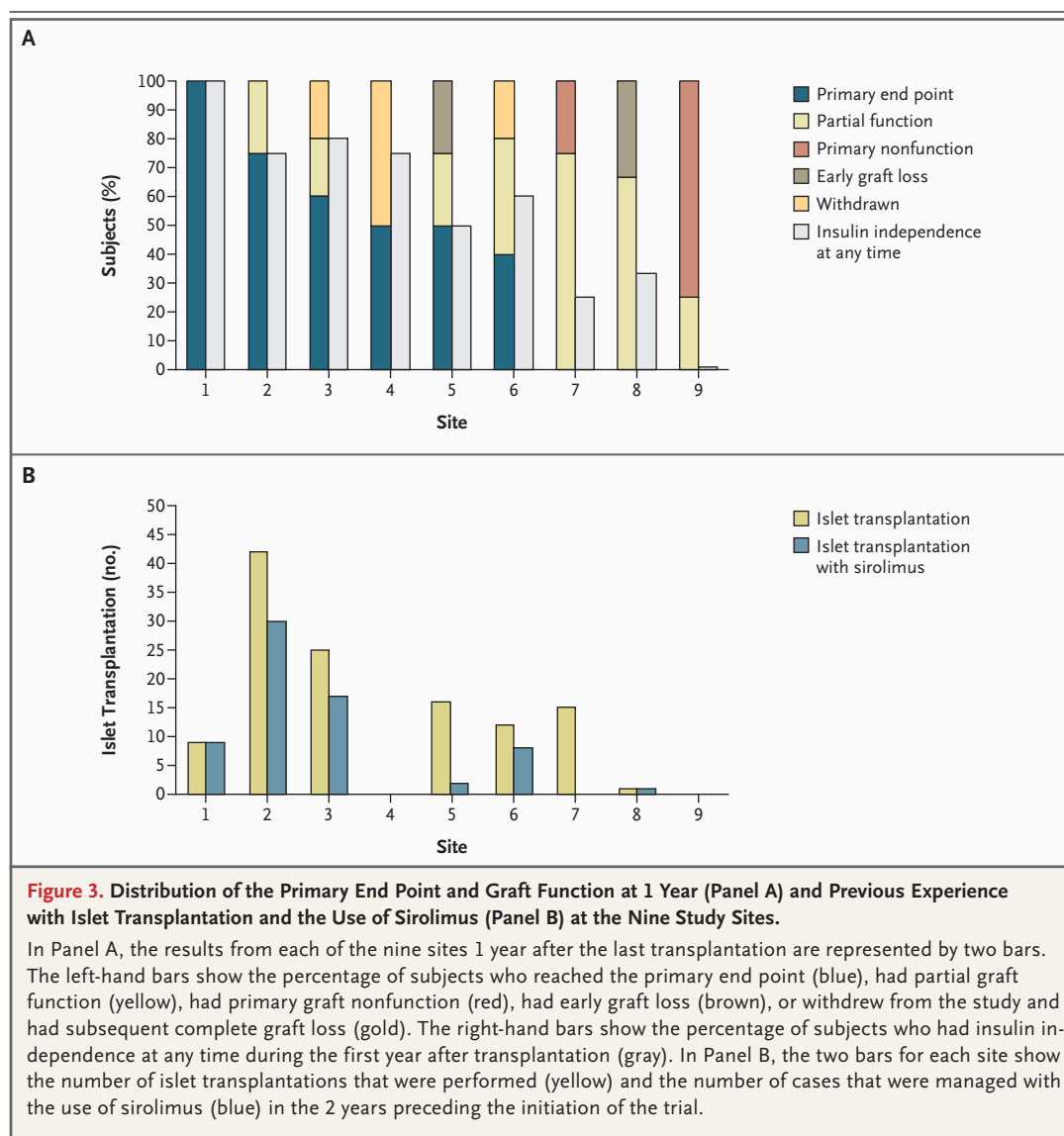
worsening genital herpes, and appendiceal abscess. Procedure-related events included acute intraperitoneal bleeding in 7 of 77 islet infusions (9%), in 4 cases requiring blood transfusion, and in 1 laparotomy. A second subject required laparotomy for a bile leak, which subsequently resolved. Severe hypoglycemia developed in one subject with primary graft nonfunction immediately after islet infusion. Complete thrombosis of the portal vein did not occur. Partial branch-vein occlusions were identified in 2 of 36 subjects (6%) and were treated successfully with temporary anticoagulation.

The 10 most common nonserious adverse events were mouth ulceration (in 92% of subjects), anemia (81%), leukopenia (75%), diarrhea (64%), head-



ache (56%), neutropenia (53%), nausea (50%), vomiting (42%), acne (39%), and fatigue (39%). Nine of 36 subjects (25%) were switched to a non-sirolimus-based alternative immunosuppressive regimen because of side effects: 8 subjects were switched to mycophenolate mofetil, and 1 sub-

ject to azathioprine. Mild hepatic steatosis was observed on routine magnetic resonance imaging 2 years after transplantation in 4 of 13 subjects (31%); it was not associated with clinical sequelae. In terms of renal function, a modest decline in creatinine clearance with a mild elevation in



serum creatinine levels was observed over time, which was associated in some cases with increased albuminuria (Fig. 4).

SENSITIZATION

Only five subjects had detectable levels of alloantibody during the study. Two subjects had alloantibodies without donor specificity before their first transplantation, and one of these two had primary nonfunction of the graft. The other reached insulin independence with only a single transplant. One subject had antidonor antibody before

receiving the first transplant but nonetheless had partial graft function and eventually became insulin-independent after a third islet infusion. New antidonor antibodies developed in two subjects at 4.5 and 6 months after the loss of islet function and subsequent withdrawal of immunosuppressive therapy.

DISCUSSION

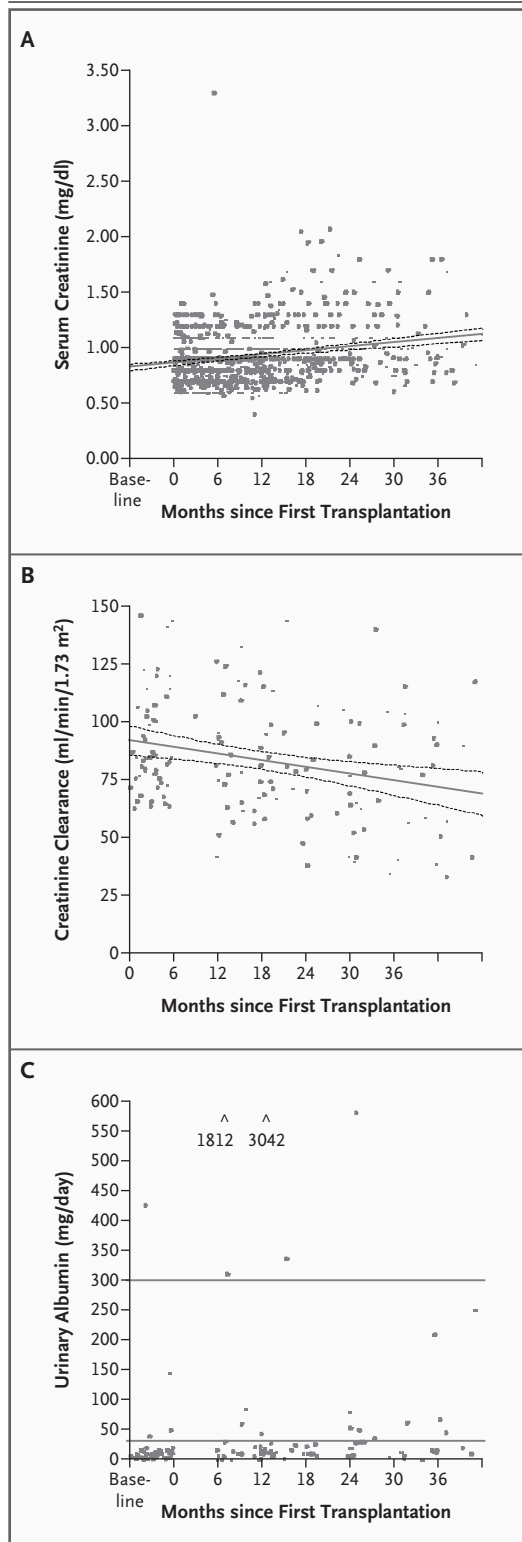
The results of this international, multicenter trial confirm previous experiences with the Edmon-

Figure 4. Measures of Renal Function after Islet Transplantation.

In Panels A and B, measurements are shown with dots, linear regression with solid lines, and 95% CIs with dashed lines. Levels of serum creatinine increased by 0.007 mg per deciliter per month ($P=0.01$) (Panel A), and creatinine clearance (as estimated by the Cockcroft–Gault formula) decreased by 0.45 ml per minute per 1.73 m^2 of body-surface area per month ($P=0.06$) (Panel B). In Panel C, the two horizontal lines denote levels of urinary albumin of 30 mg per day and 300 mg per day. At baseline, 2 of 36 subjects (6%) had urinary albumin levels between 30 mg and 300 mg per day (microalbuminuria), and 1 (3%) had urinary albumin levels that exceeded 300 mg per day (macroalbuminuria), which was a deviation from the protocol. The remainder of subjects had values below 30 mg per day. During follow-up, microalbuminuria developed in 13 subjects (36%); the condition resolved in 2 subjects and was sustained in 4 (11%). At 6 and 12 months, the urinary albumin levels were 1812 mg and 3042 mg per day, respectively, in one subject.

ton protocol at single centers and demonstrate the reproducibility and benefits of islet-alone transplantation in patients who have type 1 diabetes mellitus with unstable glycemic control.^{4,5,12,13} The trial succeeded in standardizing pancreas selection, islet processing, product-release criteria, recipient selection, and post-transplantation care under a Food and Drug Administration investigational new drug submission.

Investigators reported no deaths, cancer, or post-transplantation lymphoproliferative disease during the observation period. Although procedure-related complications were manageable, side effects related to immunosuppression prompted a change in therapy in 25% of subjects and occasionally precipitated withdrawal of subjects from the study. With the exception of the high frequency of mouth ulceration, anemia, and leukopenia, the frequency of immunosuppression-related side effects was similar to that typically seen in solid-organ transplantation. It was worrisome to observe a decline in renal function in some subjects, presumably reflecting the combined toxic effects of tacrolimus and sirolimus on preexisting diabetic nephropathy, which highlights a need for the development of less toxic immunosuppressive therapy. Acute bleeding from the percutaneous hepatic puncture site is now considered avoidable



if the track is sealed along its entire length with thrombogenic material.^{14,15}

One year after final transplantation, subjects who reached the primary end point (44%) had marked improvement in glycemic control, and subjects with partial graft function (28%) had substantial clinical improvement in all measures of diabetic control, as compared with subjects with no residual islet function (28%). In addition, subjects with residual islet function had no severe hypoglycemic episodes during the first year after transplantation.

The site-to-site variation in the clinical outcome that we observed was anticipated, given the baseline experience with human-islet processing and transplantation or with sirolimus-based immunosuppressive therapy, which ranged from none to substantial at the various centers. Achievement of the primary end point was significantly affected by the previous experience at each site. Regionalization of islet-processing facilities could potentially reduce the cost and the variation in outcome and improve efficiency in future trials if islets are cultured routinely.^{16,17}

A progressive loss of full islet function was observed in most subjects who became insulin-independent initially but had persistent C-peptide secretion. The transient nature of insulin independence after 1 year has been observed in single-center studies.^{13,18,19} More detailed immunologic and histologic studies will be needed for a full understanding of the pathophysiology underlying these observations. Allograft rejection may explain the graft deterioration observed, but a lack of HLA sensitization and the gradual and incomplete loss of graft function suggest that alternative mechanisms may be operative.

Although recurrent autoimmunity may play a role, in our study, autoantibody levels did not correlate with the loss of insulin independence (data not shown). Other investigators have observed a relationship between outcome and autoantibody status in both islet and whole-pancreas transplantation with previous, less potent immunosuppressive regimens.²⁰⁻²² Most immunosuppressive drugs, including tacrolimus and sirolimus, are known to impair islet function.²³⁻²⁵ Prolonged exposure to these compounds, particularly in the portal-hepatic site, may enhance diabetogenic toxic effects,^{26,27} underscoring a need for alternative islet delivery sites^{1,2,28} and for more potent and less diabetogenic immuno-

suppressive therapy, including drugs with tolerance-inducing potential.^{2,29-32}

Metabolic exhaustion from chronic overstimulation of a marginal islet engraftment mass may be the most plausible explanation for the discrepancy between persistent C-peptide secretion and a gradual loss of insulin independence over time, but this hypothesis remains to be proved. A similar finding has been noted previously in large-animal models of islet autotransplantation.^{32,33}

Since 2000, approximately 550 islet transplantations have been performed in more than 40 institutions.¹⁹ Recent refinements in technique include the culture of islets, the use of oxygenated perfluorodecalin in the preparation, and "rescue" gradients (i.e., use of a more tailored osmotic gradient for a second centrifugation of the islet preparation); none of these procedures were used in our trial. Hering et al. reported high rates of insulin independence with single-donor islet infusions after modifications of the procedure for preserving the pancreas, the culture medium, and peritransplantation management, as well as alternative inductive and maintenance immunotherapies.^{31,34}

In summary, our trial confirmed that islet transplantation may successfully restore long-term endogenous insulin production and glycemic stability in subjects who have type 1 diabetes mellitus with unstable baseline control. However, normal endocrine reserve is rarely achieved, and insulin independence is gradually lost in most cases over time. Persistent islet function without insulin independence provides considerable benefit, with correction of glycemic lability, as indicated by protection from hypoglycemia and improved glycated hemoglobin levels, provided the subject is able to tolerate the immunosuppressive regimen. Therefore, islet transplantation may best be considered as an evolving therapy for use in highly selected patients with severe hypoglycemia or labile type 1 diabetes mellitus, provided all other attempts to stabilize glycemic control have been exhausted. For patients seeking long-term independence from insulin, whole-pancreas transplantation appears to offer more robust metabolic reserve at the present time.³⁵ Clinical trials in development will focus on enhanced islet engraftment,³⁶⁻³⁸ less toxic immunosuppressive therapy,^{29-31,34} reduced metabolic stress, reduced apoptosis, enhanced regeneration,³⁹ the use of living donors,⁴⁰ and the induction of immuno-

logic tolerance.² A combination of these strategies should further improve engraftment and result in more protracted or permanent independence from insulin. Given the enormous clinical burden of diabetes, the search for alternative sources of regulated insulin-secreting cells must continue, since the current supply of islets from deceased donors cannot meet the demand.

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APPENDIX

The following investigators and technicians participated in islet manufacture or contributed to clinical care at the sites: *University of Alberta, Edmonton, AB, Canada* — I. Larsen (coordinator), P. Dinyari (coordinator), D. McGhee-Wilson, T. Kin, R. Wilson, D. O'Gorman, S. Rosichuk, B. Richer, J. Oberholzer, P. Senior, B. Paty, T. McCready, R. Owen, K. O'Kelly, M. McCarthy, N. Kneteman, D. Bigam; *University of Miami, Miami* — V. Sotelo (coordinator), Y. Blanco-Jivanjee (coordinator), A. Kahn, I. Iglesias, L. Jones, N. Kenyon, G. Ponte, D. Baidal, P. Cure, R. Goldberg, A. Mendez; *University of Minnesota, Minneapolis* — K. Duderstadt (coordinator), K. Hodges (coordinator), J. Ansite, S. Clemmings, J. Oberbrockling, A. Friberg, J. Parkey, B. Lervik, P. Pakala, H.-J. Zhang, M. Nakano, I. Matsumoto, T. Sawada, S.-H. Ihm, B. Liu, D. Hunter; *Washington University, St. Louis* — K. Flavin (coordinator), L. O'Brien (coordinator), H. Robertson (coordinator), T. Mohanakumar, N. Desai, B. Olack, C. Swanson, N. Benshoff, N. White, M. Koch, L. Lopez-Rocafor; *University of Washington, Seattle* — M. McCulloch-Olson (coordinator), M. Horike (coordinator), C. Greenbaum, R. Wilburn, S. Matsumoto, G. Zhang, W. Wang, S. Qualley, K. Nelson, D. Youngs, J. Clever-Hendrix, Y. Tamura, L. Upshaw; *Harvard University, Boston* — S. Fritz (coordinator), A. Dea (coordinator), G. Weir, J. O'Neil, A. Omer; *University of Milan, Milan* — A. Del Maschio, M. Venturini, M. Cardillo, R. Nano, B. Antonioli, R. Melzi, M. Scirpoli, P. Maffi, F. De Taddeo; *University of Geneva, Geneva* — M.-C. Kempf (coordinator), D. Bosco, P. Bucher, A. Andres, C. Toso, R. Mage, J. Oberholzer; *Justus-Liebig University, Giessen, Germany* — M. Eckhard, D. Winter, D. Brandhorst, H. Brandhorst, B. Hussmann, S. Fast, A. Alt, U. Flechtner; *University of Colorado Health Sciences Center, Denver* — L. Yu, D. Miao; and *Emmes Corporation, Rockville, MD* — J. Mitchell, S. Sykes.

REFERENCES

- Hering B, Ricordi C. Islet transplantation for patients with type 1 diabetes. *Graft* 1999;2:12-27.
- Ricordi C, Strom TB. Clinical islet transplantation: advances and immunological challenges. *Nat Rev Immunol* 2004;4:259-68.
- Shapiro AM, Nanji SA, Lakey JR. Clinical islet transplant: current and future directions towards tolerance. *Immunol Rev* 2003;196:219-36.
- Shapiro AM, Lakey JR, Ryan EA, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* 2000;343:230-8.
- Ryan EA, Lakey JR, Rajotte RV, et al. Clinical outcomes and insulin secretion after islet transplantation with the Edmonton protocol. *Diabetes* 2001;50:710-9.
- Rotrosen D, Matthews JB, Bluestone JA. The immune tolerance network: a new paradigm for developing tolerance-inducing therapies. *J Allergy Clin Immunol* 2002;110:17-23.
- Lakey JR, Warnock GL, Shapiro AM, et al. Intraductal collagenase delivery into the human pancreas using syringe loading or controlled perfusion. *Cell Transplant* 1999;8:285-92.
- Lakey JR, Kobayashi N, Shapiro AM, Ricordi C, Okitsu T. Current human islet isolation protocol. Chuo-ku, Osaka, Japan: Medical Review, 2004.
- Ricordi C, Lacy PE, Scharp DW. Automated islet isolation from human pancreas. *Diabetes* 1989;38:Suppl 1:140-2.
- Goss JA, Soltes G, Goodpastor SE, et al. Pancreatic islet transplantation: the radiographic approach. *Transplantation* 2003;76:199-203.
- Owen RJ, Ryan EA, O'Kelly K, et al. Percutaneous transhepatic pancreatic islet cell transplantation in type 1 diabetes mellitus: radiologic aspects. *Radiology* 2003;229:165-70.
- Ryan EA, Lakey JR, Paty BW, et al. Successful islet transplantation: continued insulin reserve provides long-term glycemic control. *Diabetes* 2002;51:2148-57.
- 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;5:2037-46.
- Froud T, Yrizarry JM, Alejandro R, Ricordi C. Use of D-STAT to prevent bleeding following percutaneous transhepatic intraportal islet transplantation. *Cell Transplant* 2004;13:55-9. [Erratum, *Cell Transplant* 2004;13:475.]
- Villiger P, Ryan EA, Owen R, et al. Prevention of bleeding after islet transplantation: lessons learned from a multivariate analysis of 132 cases at a single institution. *Am J Transplant* 2005;5:2992-8.
- Benhamou PY, Oberholzer J, Toso C, et al. Human islet transplantation network for the treatment of Type 1 diabetes: first data from the Swiss-French GRAGIL consortium (1999-2000). *Diabetologia* 2001;44:859-64.
- Goss JA, Goodpastor SE, Brunicaudi FC, et al. Development of a human pancreatic islet-transplant program through a collaborative relationship with a remote islet-isolation center. *Transplantation* 2004;77:462-6.
- Ryan EA, Paty BW, Senior PA, et al. Five-year follow-up after clinical islet transplantation. *Diabetes* 2005;54:2060-9.
- Shapiro AM, Lakey JR, Paty BW, Senior PA, Bigam DL, Ryan EA. Strategic opportunities in clinical islet transplantation. *Transplantation* 2005;79:1304-7.
- Braghi S, Bonifacio E, Secchi A, Di Carlo V, Pozza G, Bosi E. Modulation of humoral islet autoimmunity by pancreas allotransplantation influences allograft outcome in patients with type 1 diabetes. *Diabetes* 2000;49:218-24.
- Jaeger C, Brendel MD, Hering BJ, Eckhard M, Bretzel RG. Progressive islet graft

- failure occurs significantly earlier in autoantibody-positive than in autoantibody-negative IDDM recipients of intrahepatic islet allografts. *Diabetes* 1997;46:1907-10.
22. Vantyghem MC, Fajardy I, Pigny P, et al. Kinetics of diabetes-associated autoantibodies after sequential intraportal islet allograft associated with kidney transplantation in type 1 diabetes. *Diabetes Metab* 2003;29:595-601.
23. Hyder A, Laue C, Schrezenmeir J. Effect of the immunosuppressive regime of Edmonton protocol on the long-term in vitro insulin secretion from islets of two different species and age categories. *Toxicol In Vitro* 2005;19:541-6.
24. Nanji SA, Shapiro AM. Islet transplantation in patients with diabetes mellitus: choice of immunosuppression. *BioDrugs* 2004;18:315-28.
25. Lopez-Talavera JC, Garcia-Ocana A, Sipula I, Takane KK, Cozar-Castellano I, Stewart AF. Hepatocyte growth factor gene therapy for pancreatic islets in diabetes: reducing the minimal islet transplant mass required in a glucocorticoid-free rat model of allogeneic portal vein islet transplantation. *Endocrinology* 2004;145:467-74.
26. Desai NM, Goss JA, Deng S, et al. Elevated portal vein drug levels of sirolimus and tacrolimus in islet transplant recipients: local immunosuppression or islet toxicity? *Transplantation* 2003;76:1623-5.
27. Shapiro AM, Gallant HL, Hao EG, et al. The portal immunosuppressive storm: relevance to islet transplantation? *Ther Drug Monit* 2005;27:35-7.
28. Robertson RP. Islet transplantation as a treatment for diabetes — a work in progress. *N Engl J Med* 2004;350:694-705.
29. Adams AB, Shirasugi N, Durham MM, et al. Calcineurin inhibitor-free CD28 blockade-based protocol protects allogeneic islets in nonhuman primates. *Diabetes* 2002;51:265-70.
30. Adams AB, Shirasugi N, Jones TR, et al. Development of a chimeric anti-CD40 monoclonal antibody that synergizes with LEA29Y to prolong islet allograft survival. *J Immunol* 2005;174:542-50.
31. Hering BJ, Kandaswamy R, Harmon JV, et al. Transplantation of cultured islets from two-layer preserved pancreases in type 1 diabetes with anti-CD3 antibody. *Am J Transplant* 2004;4:390-401.
32. Alejandro R, Cutfield RG, Shienvold FL, et al. Natural history of intrahepatic canine islet cell autografts. *J Clin Invest* 1986;78:1339-48.
33. Sutton R, Gray DW, Burnett M, McShane P, Turner RC, Morris PJ. Metabolic function of intraportal and intrasplenic islet autografts in cynomolgus monkeys. *Diabetes* 1989;38:Suppl 1:182-4.
34. Hering BJ, Kandaswamy R, Ansit JD, et al. Single-donor, marginal-dose islet transplantation in patients with type 1 diabetes. *JAMA* 2005;293:830-5. [Erratum, *JAMA* 2005;293:1594.]
35. Frank AM, Barker CF, Markmann JF. Comparison of whole organ pancreas and isolated islet transplantation for type 1 diabetes. *Adv Surg* 2005;39:137-63.
36. Bertuzzi F, Marzorati S, Maffi P, et al. Tissue factor and CCL2/monocyte chemoattractant protein-1 released by human islets affect islet engraftment in type 1 diabetic recipients. *J Clin Endocrinol Metab* 2004;89:5724-8.
37. Contreras JL, Eckstein C, Smyth CA, et al. Activated protein C preserves functional islet mass after intraportal transplantation: a novel link between endothelial cell activation, thrombosis, inflammation, and islet cell death. *Diabetes* 2004;53:2804-14.
38. Johansson H, Lukinius A, Moberg L, et al. Tissue factor produced by the endocrine cells of the islets of Langerhans is associated with a negative outcome of clinical islet transplantation. *Diabetes* 2005;54:1755-62.
39. King A, Lock J, Xu G, Bonner-Weir S, Weir GC. Islet transplantation outcomes in mice are better with fresh islets and exendin-4 treatment. *Diabetologia* 2005;48:2074-9.
40. Matsumoto S, Okitsu T, Iwanaga Y, et al. Insulin independence after living-donor distal pancreatectomy and islet allotransplantation. *Lancet* 2005;365:1642-4.

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