

C-Peptide Levels and Insulin Independence Following Autologous Nonmyeloablative Hematopoietic Stem Cell Transplantation in Newly Diagnosed Type 1 Diabetes Mellitus

Carlos E. B. Couri, MD, PhD

Maria C. B. Oliveira, MD

Ana B. P. L. Stracieri, MD, PhD

Daniela A. Moraes, MD

Fabiano Pieroni, MD, PhD

George M. N. Barros, MD

Maria Isabel A. Madeira, MD

Kelen C. R. Malmegrim, PhD

Maria C. Foss-Freitas, MD, PhD

Belinda P. Simões, MD, PhD

Edson Z. Martinez, PhD

Milton C. Foss, MD, PhD

Richard K. Burt, MD

Júlio C. Voltarelli, MD, PhD

ACCUMULATED CLINICAL EXPERIENCE indicates that there is an inverse association between beta-cell function and chronic complications of type 1 diabetes mellitus (DM)—the higher the C-peptide levels (an indirect measure of viable beta-cell function), the lower the incidence of microvascular complications of type 1 DM.¹ Since the establishment of the autoimmune etiology of type 1 DM in the late 1970s, many clinical trials analyzing the effects of different types of immune interventions demonstrated that beta-cell preservation is an achievable target in different degrees.^{2,3}

Based on the theory of possible reconstitution of immune tolerance after “immunologic reset” with autologous nonmyeloablative hematopoietic stem cell transplantation (HSCT),⁴ in 2007, we reported a phase 1/2 trial evaluating the safety and metabolic ef-

Context In 2007, the effects of the autologous nonmyeloablative hematopoietic stem cell transplantation (HSCT) in 15 patients with type 1 diabetes mellitus (DM) were reported. Most patients became insulin free with normal levels of glycated hemoglobin A_{1c} (HbA_{1c}) during a mean 18.8-month follow-up. To investigate if this effect was due to preservation of beta-cell mass, continued monitoring was performed of C-peptide levels after stem cell transplantation in the 15 original and 8 additional patients.

Objective To determine C-peptide levels after autologous nonmyeloablative HSCT in patients with newly diagnosed type 1 DM during a longer follow-up.

Design, Setting, and Participants A prospective phase 1/2 study of 23 patients with type 1 DM (aged 13-31 years) diagnosed in the previous 6 weeks by clinical findings with hyperglycemia and confirmed by measurement of serum levels of anti-glutamic acid decarboxylase antibodies. Enrollment was November 2003-April 2008, with follow-up until December 2008 at the Bone Marrow Transplantation Unit of the School of Medicine of Ribeirão Preto, Ribeirão Preto, Brazil. Hematopoietic stem cells were mobilized via the 2007 protocol.

Main Outcome Measures C-peptide levels measured during the mixed-meal tolerance test, before, and at different times following HSCT. Secondary end points included morbidity and mortality from transplantation, temporal changes in exogenous insulin requirements, and serum levels of HbA_{1c}.

Results During a 7- to 58-month follow-up (mean, 29.8 months; median, 30 months), 20 patients without previous ketoacidosis and not receiving corticosteroids during the preparative regimen became insulin free. Twelve patients maintained this status for a mean 31 months (range, 14-52 months) and 8 patients relapsed and resumed insulin use at low dose (0.1-0.3 IU/kg). In the continuous insulin-independent group, HbA_{1c} levels were less than 7.0% and mean (SE) area under the curve (AUC) of C-peptide levels increased significantly from 225.0 (75.2) ng/mL per 2 hours pretransplantation to 785.4 (90.3) ng/mL per 2 hours at 24 months posttransplantation ($P < .001$) and to 728.1 (144.4) ng/mL per 2 hours at 36 months ($P = .001$). In the transient insulin-independent group, mean (SE) AUC of C-peptide levels also increased from 148.9 (75.2) ng/mL per 2 hours pretransplantation to 546.8 (96.9) ng/mL per 2 hours at 36 months ($P = .001$), which was sustained at 48 months. In this group, 2 patients regained insulin independence after treatment with sitagliptin, which was associated with increase in C-peptide levels. Two patients developed bilateral nosocomial pneumonia, 3 patients developed late endocrine dysfunction, and 9 patients developed oligospermia. There was no mortality.

Conclusion After a mean follow-up of 29.8 months following autologous nonmyeloablative HSCT in patients with newly diagnosed type 1 DM, C-peptide levels increased significantly and the majority of patients achieved insulin independence with good glycemic control.

Trial Registration clinicaltrials.gov Identifier: NCT00315133

JAMA. 2009;301(15):1573-1579

www.jama.com

fects of autologous nonmyeloablative HSCT in 15 patients with newly diagnosed type 1 DM.⁵ The majority of pa-

Author Affiliations are listed at the end of this article.
Corresponding Author: Julio C. Voltarelli, MD, PhD, Regional Blood Center (Hemocentro), Campus USP, 14051-140 Ribeirão Preto, Brazil (jcvoltar@fmp.usp.br).

tients became insulin free with no further immune suppressive medications. However, it was suggested that subsequent insulin independence was a prolonged honeymoon period due to dietary and exercise changes associated with close posttransplant medical observation.^{6,7}

Herein, we report updated results of the extended group of 23 patients transplanted from January 2004 through April 2008, followed up from 7 to 58 months (mean, 29.8 months and median, 30 months) after treatment, and monitored for C-peptide levels to determine if posttransplant insulin independence was due to improved endogenous beta-cell function independent of dietary or lifestyle alterations.

METHODS

Study Patients

Between November 2003 and April 2008, 160 patients were screened for enrollment. Of those patients, 71 fulfilled the inclusion criteria and were personally interviewed, 23 patients opted to participate, and all 23 were subsequently enrolled and observed until December 2008 at the Bone Marrow Transplantation Unit of the School of Medicine of Ribeirão Preto, Ribeirão Preto, Brazil. The majority of screened patients were male and most patients interested in the study were excluded for not fulfilling protocol criteria or for not accepting the potential adverse effects of the study protocol. Inclusion criteria were patients of both sexes, aged 12 to 35 years, with a clinical and laboratory diagnosis of type 1 DM during the previous 6 weeks confirmed by measurement of serum levels of anti-glutamic acid decarboxylase antibodies.

Exclusion criteria included positive serology for human immunodeficiency virus; hepatitis B or C; underlying hematologic, nephrologic, cardiac, psychiatric, or hepatic disease; or pregnancy. After the first patient was enrolled, selection criteria were adjusted to transiently exclude diabetic ketoacidosis onset and to avoid glucocorticoid use in the immunosuppression regimen.

Race/ethnicity was self-reported and was assessed because of the diversity of the Brazilian population along with the prevalence of black and white biracialism. The study protocol was approved by both Committees of Ethics in Research of the University Hospital of the School of Medicine of Ribeirão Preto and of the Brazilian Ministry of Health. An informed consent according to the Declaration of Helsinki was signed by all patients and by their parents when the patients were younger than 18 years.

Study Design

The main outcome measure was to determine C-peptide levels during the mixed-meal tolerance test, before, and at different times following transplantation. Other end points included morbidity and mortality from transplantation, temporal changes in exogenous insulin requirements (daily dose and duration of usage), and serum levels of glycated hemoglobin A_{1c} (HbA_{1c}).

Blood samples for C-peptide were collected in the fasting state and every 30 minutes during a 2-hour mixed-meal tolerance test. The morning and evening doses of insulin were withheld the day before the test, which was repeated at pretreatment, 6 months, 12 months, and then yearly following autologous nonmyeloablative HSCT. Serum C-peptide levels were measured by radioimmunoassay using commercial kits (Diagnostic Systems Laboratories Inc, Webster, Texas). Blood samples for HbA_{1c} were collected after 8-hour fasting at pretreatment and every 3 months, and measured by low-pressure liquid chromatography.

All patients were encouraged to self-monitor blood glucose at least twice daily (before and 2 hours after different meals and/or at 3 AM) indefinitely after discharge from the hospital. Insulin titration was based on fasting glucose levels before meals and 2 hours after meals with target blood glucose levels of less than 120 mg/dL and less than 140 mg/dL, respectively (to convert to millimoles per liter, multiply by 0.0555). The dose of insulin was reduced by 1 to 2 IU/mL (to convert to

picomole per liter, multiply by 6.945) if patients presented clinical findings of hypoglycemia and/or blood glucose levels of less than 90 mg/dL.

Standard recommendations for lifestyle modification (performing physical activities and a low-sugar diet) after autologous nonmyeloablative HSCT were made to all patients irrespective of exogenous insulin use. Intensive insulin therapy was the treatment of choice for all patients who needed exogenous insulin. All changes in insulin doses were ordered by one of the endocrinologists of the team (C.E.B.C.).

Stem Cell Mobilization Regimen

Peripheral hematopoietic stem cells were mobilized with cyclophosphamide and granulocyte colony-stimulating factor (Leucin, Laboratory Bergamo, São Paulo, Brazil). Cyclophosphamide (2 g/m²) was infused in 2 doses 12 hours apart in 250 mL of saline solution over 1 hour. Uroprotection was achieved with intravenous saline infusion at 250 mL/h, initiated 4 hours before cyclophosphamide infusion and continued for 16 hours. Mesna (sodium 2-mercaptoethanesulfonate, 4 g/m²) was infused over 24 hours to bind toxic cyclophosphamide metabolites in the bladder. Granulocyte colony-stimulating factor (10 ug/kg/d) was injected subcutaneously starting 1 day after cyclophosphamide infusion and continuing until leukapheresis was completed.

Leukapheresis using a continuous-flow blood cell separator was initiated when the rebounding CD34 cells reached 10 cells/μL of whole blood. Apheresis was continued daily until the number of harvested progenitor cells reached a minimum of 3.0 × 10⁶ CD34 cells per kilogram body weight. Unmanipulated peripheral blood stem cells were frozen in 10% dimethyl sulfoxide in a rate-controlled freezer and stored in the vapor phase of liquid nitrogen.

Conditioning (Immune Ablative) Regimen

Conditioning was achieved with cyclophosphamide and antithymocyte globulin. Cyclophosphamide was administered intravenously in divided doses of

50 mg/kg/d over 1 hour on days 5, 4, 3, and 2 before stem cell infusion. Rabbit antithymocyte globulin (thymoglobulin, Genzyme Polyclonals SAF, Marcy l'Etoile, France) was administered at a dose of 0.5 mg/kg/d on day 5 before, and at a dose of 1 mg/kg/d on days 4, 3, 2, and 1 before stem cell infusion. Except for the first patient, prophylaxis of antithymocyte globulin reactions was performed with dexchlorpheniramine (6 mg orally) avoiding the use of glucocorticoids. Stem cell infusion was performed on day 0 and granulocyte colony-stimulating factor (5 µg/kg/d) was administered subcutaneously from day 5 after stem cell infusion until neutrophil count was more than 1000/µL.

Statistical Analysis

Multiple comparisons of total area under the curve (AUC) of serum C-peptide levels measured during the mixed-meal tolerance test (during fasting and at 30, 60, 90, and 120 minutes) were made by using a model of multiple regression of mixed effects for periods 0, 6, 12, and 24 months posttransplantation. To present the mean variation of HbA_{1c} levels with time, a model of linear regression of random effects was constructed by using the following equation: $y = \beta_0 + \beta_1 \times \log(\text{time}) + \beta_2 \times [\log(\text{time})]^2$, in which each parameter represents a random effect in each patient. These models are characterized to present residuals that are normally distributed. Data analysis was completed by using PROC MIXED, SAS statistical software, version 8 (SAS Institute Inc, Cary, North Carolina). Statistical significance was set a priori at $P < .05$.

RESULTS

Twenty-three patients aged 13 to 31 years (mean, 18.4 years) were enrolled in the study between November 2003 and April 2008. Individual demographic characteristics and follow-up variables are shown in TABLE 1. This is a very selected group of patients, predominantly male, with a short duration of disease and mostly without previous diabetic ketoacidosis. All patients presented clinical findings of hypergly-

cemia associated with a mean blood glucose level of 398.6 mg/dL (range, 130-793 mg/dL) at diagnosis and mean anti-glutamic acid decarboxylase antibody level of 24.9 U/mL (range, 1.1-102.0 U/mL). Mean body mass index (calculated as weight in kilograms divided by height in meters squared) at diagnosis was 19.7 (range, 16.6-23.4). Nine patients presented both HLA haplotypes characteristic of increased risk for type 1 DM⁸ and 14 patients presented 1 of those haplotypes.

Mean time from diagnosis to mobilization was 37.7 days (range, 24-56 days) and mean duration of hospital stay for transplantation (from conditioning to discharge) was 18.6 days (range, 15-24 days). Mean number of infused CD34 cells was $10.52 \times 10^6/\text{kg}$ (range, $4.98\text{-}23.19 \times 10^6/\text{kg}$). Neutrophil engraftment ($>500/\mu\text{L}$) occurred between days 8 and 11 after transplantation (mean, 9.3 days) and platelet engraftment ($>20\,000/\mu\text{L}$) was detected between day 0 and day 18 after transplantation (mean, 10.4 days).

The majority of the adverse effects related to treatment were mild and included nausea, vomiting, fever, and alopecia (TABLE 2). With regard to severe adverse effects, 2 patients presented bilateral nosocomial pneumonia that completely responded to intravenous broad-spectrum antibiotics. There was no mortality. During long-term follow-up, there was 1 case each of Graves disease, transient hypergonadotropic hypogonadism, and autoimmune hypothyroidism. Nine patients had posttransplant oligospermia. Two patients fathered children 2 years after transplantation.

Of the 23 included patients, 20 experienced time free from insulin (12 continuously and 8 transiently). In the majority of patients, insulin suspension occurred soon before or after stem cell infusion, ranging from 6 days before to 34 days after the infusion (mean, +2 days). Patients remained continuously insulin free for a mean time of 31 months (range, 14-52 months) (Table 1). One patient went more than 4 years without exogenous insulin use, 4 patients for at least 3 years, 3 patients for at least 2 years, and

4 patients for at least 1 year. In the patients in the continuously insulin-free group, mean value of HbA_{1c} was 8.0% at pretreatment and 5.4%, 5.7%, 5.7%, 5.5%, and 6.0%, respectively, at 3, 12, 24, 36, and 48 months after transplantation ($P < .001$ between pretreatment and all the follow-up values). Mean (SE) AUC of C-peptide levels before transplantation (225.0 [75.2] ng/mL per 2 hours) showed a significant increase at 24 months after transplantation (785.4 [90.3] ng/mL per 2 hours, $P < .001$) and at 36 months after transplantation (728.1 [144.4] ng/mL per 2 hours, $P = .001$) (FIGURE).

Eight patients became transiently free from exogenous insulin for a mean 17.7 months (range, 6-47 months). Of the 8 patients that restarted insulin, 4 resumed insulin after an upper respiratory tract infection. Daily insulin doses after insulin resumed were small, ranging from 0.1 to 0.3 IU/kg, to maintain good glucose control. In spite of the insulin use, mean (SE) AUC of C-peptide levels before transplantation (148.9 [75.2] ng/mL per 2 hours) also showed a significant increase at 36 months after transplantation (546.8 [96.9] ng/mL per 2 hours, $P = .001$) that was sustained at 48 months following transplantation (413.9 [124.7] ng/mL per 2 hours, $P = .22$, between 36 and 48 months after transplantation) (Figure).

Two patients (patient 2 and patient 4) became transiently insulin free for 47 and 43 months, respectively. After 4 and 2 months of resuming insulin and using daily insulin doses of 0.25 and 0.20 IU/kg, respectively, we opted to add sitagliptin (a dipeptidyl peptidase 4 inhibitor) 100 mg/d orally. Both became completely insulin free again after 2 months for patient 2 and 1 month for patient 4, and both remained free from exogenous insulin for 5 and 6 months, respectively. In patient 2, AUC of C-peptide levels presented increasing values from initial diagnosis (53.6 ng/mL per 2 hours) until 36 months following transplantation (470.4 ng/mL per 2 hours). Soon before insulin resumption, the AUC decreased to 135.9 ng/mL per 2 hours. Seven months after sitagliptin use, AUC of C-peptide

levels reached levels of 530.2 ng/mL per 2 hours. In patient 4, the same occurred with AUC of C-peptide levels (ie, increasing from initial diagnosis [134.6 ng/mL per 2 hours] until 36 months following transplantation [812.5 ng/mL per 2 hours]). At 43 months after transplantation, before insulin resumption, the AUC of C-peptide levels was 153.2 ng/mL per 2 hours and 6 months after sitagliptin use, the AUC of C-peptide levels was 1069.6 ng/mL per 2 hours.

Table 1. Pretreatment and Follow-up Characteristics of Patients With Type 1 Diabetes Mellitus Undergoing Autologous Nonmyeloablative Hematopoietic Stem Cell Transplantation

Patient No./Sex	Age, y	Race	HLA Type	At Diagnosis			Hemoglobin A _{1c} at Pretransplantation, %	Duration of Symptoms of Hyperglycemia, d	Insulin Dose Premobilization, IU/kg/d	Time Free From Insulin, mo	Follow-up, mo ^b
				Body Mass Index ^a	Blood Glucose, mg/dL	Anti-GAD, U/mL					
1/M ^{c,d}	24	Biracial ^e	DRB1*03,*04/ DQB1*0201,*0302	22.6	477	36.0	7.6	35	0.51	NS	12
2/M	27	Black	DRB1*03,*04/ DQB1*0201,*0302	22.9	589	49.0	7.5	2	0.34	47 (T) ^f	58
3/M	21	Biracial ^e	DRB1*03,*04/ DQB1*0201,*0302	19.0	381	1.1	9.3	5	0.27	52 (C)	56
4/M	15	White	DRB1*01,*07/ DQB1*0201,*0501	23.0	321	22.0	8.0	10	0.23	43 (T) ^g	56
5/M	16	White	DRB1*04,*10/ DQB1*0302,*0501	17.5	404	51.0	7.7	21	0.38	46 (C)	47
6/M	14	White	DRB1*01,*03/ DQB1*0201,*0501	23.4	504	17.0	7.3	7	0.42	43 (C)	46
7/F	20	White	DRB1*04,*12/ DQB1*0302,*0301	16.8	391	4.0	10.0	20	0.44	7 (T)	43
8/M	16	Biracial ^e	DRB1*03,*04/ DQB1*0201,*0302	17.6	314	48.0	5.4	50	0.55	39 (C)	40
9/F	18	White	DRB1*03,*13/ DQB1*0201,*0602	19.1	330	102.0	6.7	14	0.35	39 (C)	40
10/F	17	White	DRB1*01/ DQB1*0501	20.1	612	44.0	8.9	30	0.29	9 (T)	39
11/M	16	Biracial ^e	DRB1*03,*04/ DQB1*0201,*0302	17.8	130	11.0	5.4	5	0.13	12 (T)	15
12/F	14	Biracial ^e	DRB1*01,*04/ DQB1*0302,*0501	19.8	581	11.0	8.1	7	0.45	31 (C)	32
13/M	24	White	DRB1*03/ DQB1*0201	18.4	269	24.0	8.1	14	0.58	30 (C)	31
14/M	31	White	DRB1*04,*04/ DQB1*0302,*0402	22.1	273	37.0	7.8	14	0.37	29 (C)	30
15/M	16	White	DRB1*01,*03/ DQB1*0201,*0501	16.6	291	21.1	10.1	30	0.44	9 (T)	29
16/M	16	White	DRB1*03,*04/ DQB1*0201,*0302	18.3	384	5.3	8.4	21	0.56	17 (C)	18
17/M	17	White	DRB1*04,*08/ DQB1*0302,*0402	20.7	236	12.0	9.0	7	0.21	16 (C)	17
18/M	21	White	DRB1*04/ DQB1*0302	17.8	324	7.0	9.1	14	0.59	16 (C)	17
19/M	15	White	DRB1*09/ DQB1*0302	18.4	793	1.1	9.1	7	0.50	14 (C)	15
20/M ^d	13	White	DRB1*03,*04/ DQB1*0201,*0302	18.3	485	29.0	7.6	7	0.49	NS	13
21/F ^c	16	White	DRB1*01,*04/ DQB1*0302,*0501	21.1	439	16.0	9.5	60	0.49	NS	15
22/F	15	Asian American	DRB1*04,*12/ DQB1*0302,*0302	22.6	390	10.0	11.6	20	0.33	9 (T)	9
23/M	22	White	DRB1*03/ DQB1*0201	20.0	250	14	10.0	30	0.54	6 (T)	7
Mean (SD)	18.4 (4.6)			19.7 (2.2)	398.6 (148.6)	24.9 (23.1)	8.4 (1.5)	18.7 (14.8)	0.41 (0.13)	^h	29.8 (16.3)

Abbreviations: C, continuously; GAD, glutamic acid decarboxylase; NS, not suspended; T, transiently.

SI conversion factor: To convert glucose to mmol/L, multiply by 0.0555.

^aBody mass index was calculated as weight in kilograms divided by height in meters squared.

^bMonths since mobilization regimen.

^cPatients 1 and 21 presented with diabetic ketoacidosis.

^dPatients 1 and 20 used corticosteroids in the conditioning regimen.

^eDenotes patients who self-identified as having both black and white racial parentage.

^fPatient 2 became insulin free for 47 months after transplantation when he resumed insulin use. Four months after resuming insulin, oral sitagliptin (100 mg/d) was prescribed and patient became insulin free again 2 months later and is still insulin free for 5 months.

^gPatient 4 became insulin free for 43 months after transplantation when he resumed insulin use. Two months after resuming insulin, oral sitagliptin (100 mg/d) was prescribed and patient became insulin free again 1 month later and is still insulin free for 6 months.

^hFor the continuously insulin-free group, mean (SD) was 31 (13.1) months and for the transiently insulin-free group, mean (SD) was 17.7 (16.9) months.

Only 3 patients did not experience any time free from insulin. One patient presented with diabetic ketoacidosis at diagnosis and received glucocorticoids to prevent rabbit antithymocyte globulin reactions, 1 developed diabetic ketoacidosis before enrollment, and 1 had inadvertently received corticosteroids (300-mg hydrocortisone) along with stem cell infusion. None of the patients achieved HbA_{1c} levels of less than 7%, in spite of progressive increase in daily insulin doses (>0.8 IU/kg/d).

COMMENT

Many study protocols have been developed to try to interdict the autoimmune process involved in type 1 DM disease process. Although some prom-

ising studies have suggested improvement in beta-cell function, no intervention has resulted in a definitive increase of beta-cell function.^{3,9-18} Moreover, although some studies showed sustained beta-cell function, cases of exogenous insulin suspension were rare.

In 2007,⁵ we reported intriguing findings of a group of 15 patients with newly diagnosed type 1 DM, with a mean follow-up of 18.8 months. Thirteen of 15 patients experienced continuous time free from insulin and 1 patient became transiently insulin free. However, one of the limitations of our previous study was the short period that the patients were followed up as well as lack of convincing C-peptide data to confirm a treatment effect rather than a prolonged hon-

eymoon effect from close medical follow-up and physician-directed changes in lifestyle. In our current study, we showed that 20 of 23 patients became insulin free (12 continuously and 8 transiently) for periods as long as 4 years associated with good glucose control. To confirm that this is a treatment effect that improves beta-cell mass rather than a prolonged honeymoon phase, we now report the long-term outcome of C-peptide levels.

In the group of continuous insulin-free individuals, the increment of C-peptide levels occurred up to 24 months after transplantation and were maintained until at least 36 months. In the group of transiently insulin-free individuals, the increments still reached sig-

Table 2. Transplantation Complications

Patient No.	Mobilization Complications	Minor Conditioning Complications ^a	Major Conditioning Complications	Late Complications
1	Nausea, vomiting, pyoderma	Anorexia, fever, catheter infection	None	None
2	Dysuria		Bilateral pneumonia (from day -2 to day +14)	Graves disease (≥ 2.8 y) ^b
3	None	Diarrhea, sinusitis, rash, fever	None	Rhabdomyolysis, hypothyroidism (≥ 1 y)
4	Nausea, vomiting	Fever, catheter infection, herpes simplex, right cephalic vein thrombosis	None	Leukopenia, oligospermia
5	None	Anorexia, fever, urticaria	None	Oligospermia
6	None	Anorexia, fever, rash, hypokalemia, mucositis	None	None
7	None	Rash, diarrhea, fluid overload	None	None
8	None	Rash, anorexia, diarrhea	None	Oligospermia
9	None	Diarrhea, anorexia, fever	None	None
10	None	Rash	None	Transient hypogonadism (≥ 1 y)
11	Fever	Anorexia, fever	None	None
12	None	Epistaxis	None	None
13	Fever	Diarrhea, rash	None	Oligospermia ^c
14	Sialorrhea	Rash, fever, fluid overload	None	Oligospermia
15	Nausea, vomiting, anorexia	Rash, fever	None	Oligospermia
16	Purulent amigdalitis	Anorexia	None	Oligospermia
17	Fever	Fever, rash	None	Oligospermia
18	None	Rash	None	Oligospermia
19	None	Headache, bradycardia	None	None
20	None	Urticaria, fever	None	None
21	None	Urticaria	None	None
22	Folliculitis	Rash, epistaxis	Bilateral pneumonia (from day +1 to day +11)	None
23	None	Urticaria, fever	None	None

^aAll patients except 4, 5, 7, and 8 presented with nausea; vomiting presented in all patients except 4 and 6; and all presented with alopecia.

^bPatient 2 fathered a child 2 years after transplantation.

^cPatient 13 fathered a child 2 years after transplantation.

nificance at 36 months with sustained increase until at least 48 months. The C-peptide data confirm increased endogenous insulin production as the predominate mechanism of euglycemic control in both patients who remained insulin free as well as those who needed to restart insulin at reduced doses. Because late complications of diabetes on the microvascular compartment are inversely related to C-peptide levels,¹ it is probable that even those patients who resumed insulin are at lower risk for long-term diabetes complications.

Recent studies using antibodies against CD3,^{14,15} heat shock protein,¹⁶ rabbit anti-lymphocyte globulin,¹⁷ and alum-formulated glutamic acid decarboxylase¹⁸ showed only transient increase followed by slower declines of C-peptide levels along the follow-up. Moreover, almost all patients required exogenous insulin use.

Two patients who resumed insulin administration had an increase in C-peptide levels after the use of oral dipeptidil peptidase 4 (sitagliptin), a novel

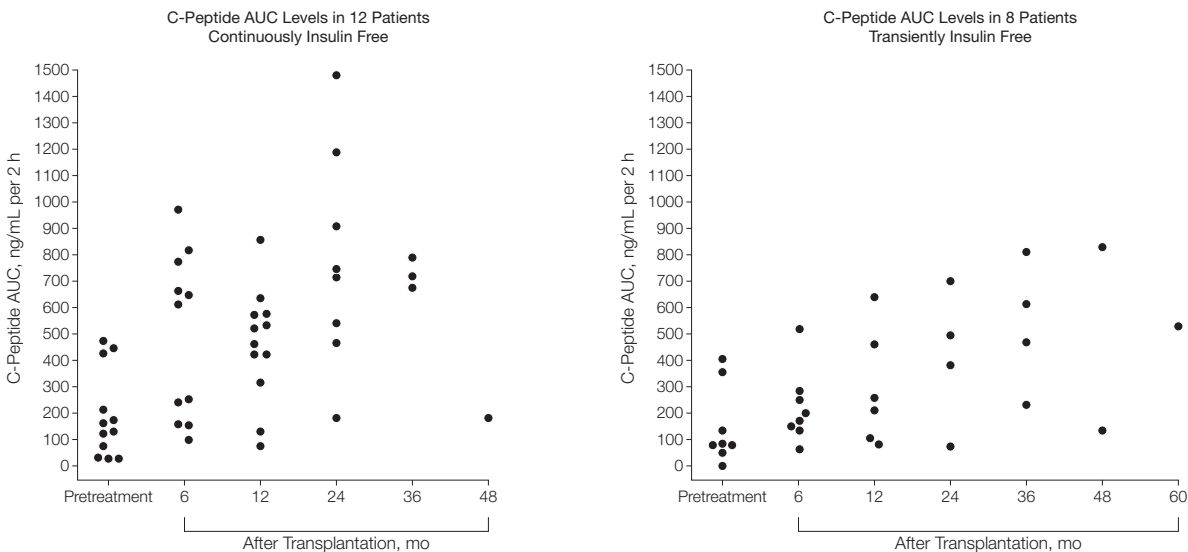
class of oral antihyperglycemic agents.¹⁹ Dipeptidil peptidase 4 inhibitors enhance meal-stimulated active glucagon-like peptide 1 and glucose-dependent insulinotropic polypeptide levels, improve measures of beta-cell function, suppress glucagon levels produced by alpha cells, and increase beta-cell mass in rodent models.²⁰⁻²² The increase of C-peptide levels in those patients may not completely explain the metabolic improvement observed since the period of sitagliptin prescription to insulin suspension was too short. One alternative explanation is that sitagliptin promoted a rapid suppression of glucagon levels in parallel with further increase in insulin production. In addition, sitagliptin may also have immunoregulatory function in autoimmune insulinitis.²³ All these results must be confirmed in a larger group of patients with a longer follow-up.

With regard to late adverse effects, 3 patients developed late endocrine dysfunctions that could be caused by autoimmune dysregulation associated with

the transplant procedure²⁴ or by autoimmune polyendocrine syndrome frequently associated with type 1 DM.²⁵ Nine patients developed cyclophosphamide-related oligospermia after transplantation. Regarding the early adverse effects, 2 patients had bilateral pneumonia and most patients presented fever, rash, or urticaria. Those adverse effects are not negligible but may outweigh long-term risks of type 1 DM.

In conclusion, autologous nonmyeloablative HSCT was able to induce prolonged and significant increases of C-peptide levels associated with absence of or reduction of daily insulin doses in a small group of patients with type 1 DM. We showed that 20 of 23 patients became insulin free (12 continuously and 8 transiently) for periods as long as 4 years associated with good glucose control and acceptable incidence of adverse effects. At the present time, autologous nonmyeloablative HSCT remains the only treatment capable of reversing type 1 DM in humans. Randomized controlled trials and

Figure. Time Course of Total Area Under the Curve (AUC) of C-Peptide Levels During Mixed-Meal Tolerance Test in 12 Patients Continuously Insulin Free and in 8 Patients Transiently Insulin Free



Statistical analysis was performed using a model of multiple regression of mixed effects. For the C-peptide AUC levels in 12 patients continuously insulin free, data from 1 patient at 6 months and from 2 patients at 36 months after transplantation were not available ($P < .001$ between pretreatment and 6 months; $P = .001$ between pretreatment and 12 months; $P < .001$ between pretreatment and 24 months; $P < .001$ between pretreatment and 36 months; $P = .10$ between 12 and 24 months after transplantation; $P = .58$ between 24 and 36 months after transplantation). For the C-peptide AUC levels in 8 patients transiently insulin free, data from 1 patient at 24 months after transplantation were not available ($P < .001$ between pretreatment and 36 months; $P = .22$ between 36 and 48 months after transplantation). To convert C-peptide to nmol/L, multiply by 0.331.

further biological studies are necessary to confirm the role of this treatment in changing the natural history of type 1 DM.

Author Affiliations: Departments of Clinical Medicine (Drs Couri, Oliveira, Stracieri, Moraes, Pieroni, Barros, Madeira, Malmegrim, Foss-Freitas, Simões, Foss, and Voltarelli) and Social Medicine (Dr Martinez), School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil; and Division of Immunotherapy, Northwestern University Feinberg School of Medicine, Chicago, Illinois (Dr Burt).

Author Contributions: Dr Voltarelli had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Couri, Oliveira, Foss-Freitas, Burt, Voltarelli.

Acquisition of data: Couri, Oliveira, Moraes, Pieroni, Madeira, Simões, Foss, Voltarelli.

Analysis and interpretation of data: Couri, Stracieri, Barros, Malmegrim, Martinez, Foss, Voltarelli.

Drafting of the manuscript: Couri.

Critical revision of the manuscript for important intellectual content: Couri, Oliveira, Stracieri, Moraes, Pieroni, Barros, Madeira, Malmegrim, Foss-Freitas, Simões, Martinez, Foss, Burt, Voltarelli.

Statistical analysis: Couri, Martinez.

Obtained funding: Couri.

Administrative, technical, or material support: Couri, Simões.

Study supervision: Couri, Simões, Foss, Burt, Voltarelli.

Financial Disclosures: None reported.

Funding/Support: This work was supported by the Bra-

zilian Ministry of Health, FAEPA-HCRP, FUNDHERP, CAPES, FAPESP, CNPq, FINEP, Genzyme Corporation, and Johnson & Johnson-LifeScan-Brazil.

Role of the Sponsors: The funding organizations did not participate in the design and conduct of the study, in the collection, management, analysis, and interpretation of the data, or in the preparation, review, or approval of the manuscript.

Additional Contributions: We thank Sebastião L. Brandão Filho, technician from the Endocrinology and Metabolism Laboratory, for performing C-peptide tests; the multiprofessional team of the Bone Marrow Transplantation Unit; and the Regional Blood Center of the Hospital das Clínicas of Ribeirão Preto, University of São Paulo, São Paulo, Brazil. No individuals named received any compensation from a funding sponsor for their contribution to this article.

REFERENCES

1. The Diabetes Control and Complications Trial Research Group. Effect of intensive therapy on residual beta-cell function in patients with type 1 diabetes in the Diabetes Control and Complications Trial. *Ann Intern Med.* 1998;128(7):517-523.
2. Couri CEB, Foss MC, Voltarelli JC. Secondary prevention of type 1 diabetes mellitus: stopping immune destruction and promoting β -cell regeneration. *Braz J Med Biol Res.* 2006;39(10):1271-1280.
3. Staeva-Vieira T, Peakman M, von Herrath M. Translational mini-review series on type-1 diabetes: immune-based therapeutic approaches for type 1 diabetes. *Clin Exp Immunol.* 2007;148(1):17-31.
4. Burt RK, Slavin S, Burns WH, Marmont AM. Induction of tolerance in autoimmune diseases by hematopoietic stem cell transplantation: getting closer to a cure? *Blood.* 2002;99(3):768-784.
5. Voltarelli JC, Couri CE, Stracieri AB, et al. Autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus. *JAMA.* 2007;297(14):1568-1576.
6. Ross LF, Philipson LH. Ethics in hematopoietic stem cell transplantation in type 1 diabetes mellitus. *JAMA.* 2007;298(3):285-, author reply 285-286.
7. Skyler JS. Cellular therapy for type 1 diabetes: has the time come? *JAMA.* 2007;297(14):1599-1600.
8. Cerná M. Genetics of autoimmune diabetes mellitus. *Wien Med Wochenschr.* 2008;158(1-2):2-12.
9. Elliott RB, Crossley JR, Berryman CC, James AG. Partial preservation of pancreatic β -cell function in children with diabetes. *Lancet.* 1981;19:631-632.
10. Harrison LC, Colman PG, Dean B, Baxter R, Martin FI. Increased in remission rate in newly diagnosed type 1 diabetic subjects treated with azathioprine. *Diabetes.* 1985;34(12):1306-1308.
11. Cook JJ, Hudson I, Harrison LC, et al. Double-blind controlled trial of azathioprine in children with newly diagnosed type 1 diabetes. *Diabetes.* 1989;38(6):779-783.
12. Silverstein J, Maclaren N, Riley W, Spillar R, Radjenovic D, Johnson S. Immunosuppression with azathioprine and prednisone in recent-onset insulin-dependent diabetes mellitus. *N Engl J Med.* 1988;319(10):599-604.
13. Canadian-European Randomized Control Trial Group. Cyclosporin-induced remission of IDDM after early intervention: association of 1 yr of cyclosporin treatment with enhanced insulin secretion. *Diabetes.* 1988;37(11):1574-1582.
14. Herold KC, Hagopian W, Auger JA, et al. Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. *N Engl J Med.* 2002;346(22):1692-1698.
15. Keymeulen B, Vandemeulebroucke E, Ziegler AG, et al. Insulin needs after CD3-antibody therapy in new-onset type 1 diabetes. *N Engl J Med.* 2005;352(25):2598-2608.
16. Raz I, Elias D, Avron A, Tamir M, Metzger M, Cohen IR. β -cell function in newly-onset type 1 diabetes and immunomodulation with a heatshock protein peptide (DiaPep277): a randomised, double-blind, phase II trial. *Lancet.* 2001;358(9295):1749-1753.
17. Saudek F, Havrdova T, Boucek P, Karasova L, Novota P, Skibova J. Polyclonal anti-T-cell therapy for type 1 diabetes mellitus of recent onset. *Rev Diabet Stud.* 2004;1(2):80-88.
18. Ludvigsson J, Faresjö M, Hjorth M, et al. GAD treatment and insulin secretion in recent-onset type 1 diabetes. *N Engl J Med.* 2008;359(18):1909-1920.
19. Aschner P, Kipnes MS, Luncford JK, Sanchez M, Mickel C, Williams-Herman DE; Sitagliptin Study 021 Group. Effect of the dipeptidyl peptidase-4 inhibitor sitagliptin as monotherapy on glycemic control in patients with type 2 diabetes. *Diabetes Care.* 2006;29(12):2632-2637.
20. Mari A, Sallas WM, He YL, et al. Vildagliptin, a dipeptidyl peptidase-IV inhibitor, improves model-assessed beta-cell function in patients with type 2 diabetes. *J Clin Endocrinol Metab.* 2005;90(8):4888-4894.
21. Herman GA, Bergman A, Stevens C, et al. Effect of single oral doses of sitagliptin, a dipeptidyl peptidase-4 inhibitor, on incretin and plasma glucose levels after an oral glucose tolerance test in patients with type 2 diabetes. *J Clin Endocrinol Metab.* 2006;91(11):4612-4619.
22. Herman GA, Stevens C, Van Dyck K, et al. Pharmacokinetics and pharmacodynamics of sitagliptin, an inhibitor of dipeptidyl peptidase IV, in healthy subjects: results from two randomized, double-blind, placebo-controlled studies with single oral doses. *Clin Pharmacol Ther.* 2005;78(6):675-688.
23. Kim SJ, Nian C, Doudet DJ, McIntosh CH. Dipeptidyl peptidase IV inhibition with MK0431 improves islet graft survival in diabetic NOD mice partially via T-cell modulation. *Diabetes.* 2009;58(3):641-651.
24. Au WY, Lie AK, Kung AW, Liang R, Hawkins BR, Kwong YL. Autoimmune thyroid dysfunction after hematopoietic stem cell transplantation. *Bone Marrow Transplant.* 2005;35(4):383-388.
25. Eisenbarth GS, Gottlieb PA. Autoimmune polyendocrine syndromes. *N Engl J Med.* 2004;350(20):2068-2079.