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Preliminary Communication

# Autologous Nonmyeloablative Hematopoietic Stem Cell Transplantation in Newly Diagnosed Type 1 Diabetes Mellitus

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## ABSTRACT

**Context** Type 1 diabetes mellitus (DM) results from a cell-mediated autoimmune attack against pancreatic beta cells. Previous animal and clinical studies suggest that moderate immunosuppression in newly diagnosed type 1 DM can prevent further loss of insulin production and can reduce insulin needs.

**Objective** To determine the safety and metabolic effects of high-dose immunosuppression followed by autologous nonmyeloablative hematopoietic stem cell transplantation (AHST) in newly diagnosed type 1 DM.

**Design, Setting, and Participants** A prospective phase 1/2 study of 15 patients with type 1 DM (aged 14-31 years) diagnosed within the previous 6 weeks by clinical findings and hyperglycemia and confirmed with positive antibodies against glutamic acid decarboxylase. Enrollment was November 2003-July 2006 with observation until February 2007 at the Bone Marrow Transplantation Unit of the School of Medicine of Ribeirão Preto, Ribeirão Preto, Brazil. Patients with previous diabetic ketoacidosis were excluded after the first patient with diabetic ketoacidosis failed to benefit from AHST. Hematopoietic stem cells were mobilized with cyclophosphamide (2.0 g/m$^2$) and granulocyte colony-stimulating factor (10 µg/kg per day) and then collected from peripheral blood by leukapheresis and cryopreserved. The cells were injected intravenously after conditioning with cyclophosphamide (200 mg/kg) and rabbit antithymocyte globulin (4.5 mg/kg).

**Main Outcome Measures** Morbidity and mortality from transplantation and temporal changes in exogenous insulin requirements (daily dose and duration of usage). Secondary end points: serum levels of hemoglobin $A_{1c}$, C-peptide levels during the mixed-meal tolerance test, and anti–glutamic acid decarboxylase antibody titers measured before and at different times following AHST.

**Results** During a 7- to 36-month follow-up (mean 18.8), 14 patients became insulin-free (1 for 35 months, 4 for at least 21 months, 7 for at least 6 months; and 2 with late response were insulin-free for 1 and 5 months, respectively). Among those, 1 patient resumed insulin use 1 year after AHST. At 6 months after AHST, mean total area under the C-peptide response curve was significantly greater than the pretreatment values, and at 12 and 24 months it did not change. Anti–glutamic acid decarboxylase antibody levels decreased after 6 months and stabilized at 12 and 24 months. Serum levels of hemoglobin $A_{1c}$ were maintained at less than 7% in 13 of 14 patients. The only acute severe adverse effect was culture-negative bilateral pneumonia in 1 patient and late endocrine dysfunction (hypothyroidism or hypogonadism) in 2 others. There was no mortality.

**Conclusions** High-dose immunosuppression and AHST were performed with acceptable toxicity in a small number of patients with newly diagnosed type 1 DM. With AHST, beta cell function was increased in all but 1 patient and induced prolonged insulin independence in the majority of the patients.

**Trial Registration** clinicaltrials.gov Identifier: NCT00315133

## INTRODUCTION

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Type 1 diabetes mellitus (DM) results from a cell-mediated autoimmune attack against pancreatic beta cells.[1] The course of autodestruction is subclinical until the amount of beta-cell mass is insufficient to maintain glucose homeostasis. Thus, at the time of clinical diagnosis, approximately 60% to 80% of the beta-cell mass has been destroyed.[2]

Type 1 DM comprises only 5% to 10% of all diabetic etiologies but is associated with a high frequency of vascular complications and compromises quality and expectancy of life.[3-4] Patients with type 1 DM depend on exogenous insulin administration for survival and for control of long-term complications. The best-established treatment is tight control of blood glucose achieved by frequent daily injections or continuous subcutaneous infusion of insulin, ie, intensive insulin therapy. This treatment reduces the risk of retinopathy, nephropathy, and neuropathy by 35% to 90% when compared with conventional therapy with 1 to 2 injections per day.[5]

Subgroup analysis of the Diabetes Control and Complications Trial showed that patients with a larger beta cell reserve demonstrable by serum C-peptide levels presented a slower decline of these levels during the study and experienced fewer microvascular complications than patients with low or undetectable C-peptide concentrations. Therefore, beta cell preservation is another important target in the management of type 1 DM and in the prevention of its related complications.[6]

Many clinical trials have evaluated the role of immunointervention in preventing residual beta cell loss by blocking the autoimmune response with prednisone,[7] azathioprine,[8-9] prednisone plus azathioprine,[10] cyclosporine,[11] antibodies against CD3,[12-13] heat shock protein,[14] and rabbit antithymocyte globulin.[15] These therapies were shown to induce a slower decline or some improvement in C-peptide levels when compared with placebo groups. However, almost all patients required exogenous insulin use.

Since 1996, organ-threatening systemic lupus erythematosus[16] and other autoimmune diseases[17] have been successfully treated with high-dose immunosuppression followed by autologous nonmyeloablative hematopoietic stem cell transplantation (AHST). Organ function was salvaged and in many cases improved following AHST. In animal models, allogeneic bone marrow transplantation prevents both insulitis and the development of type 1 DM in susceptible strains of mice.[18]

On the basis of these observations, we initiated a phase 1/2 study in November 2003 analyzing the safety, metabolic effects, and ability of AHST to preserve beta cell function in patients with newly diagnosed type 1 DM. Here we report the first prospective trial, to our knowledge, of stem cell therapy in human DM. We describe 15 patients with type 1 DM, submitted to AHST, and observed from 7 to 36 months (mean 18.8 months) after treatment.

## METHODS

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### Patients

Inclusion criteria were patients of both sexes, aged 12 to 35 years, with a diagnosis of type 1 DM during the previous 6 weeks confirmed by measurement of serum levels of anti–glutamic acid decarboxylase (anti-GAD) antibodies. From September 2003 to February 2007, more than 100 patients were offered screening for enrollment (most by e-mail or telephone interviews). Of those patients, 52 fulfilled the inclusion criteria and were personally interviewed, 15 patients opted to participate, and all 15 were subsequently enrolled between November 2003 and July 2006 and observed until February 2007 at the Bone Marrow Transplantation Unit of the School of Medicine of Ribeirão Preto, Ribeirão Preto, Brazil.

The main reasons for not fitting the inclusion criteria were the duration of type 1 DM longer than 6 weeks or previous episodes of diabetic ketoacidosis. Concerns about the probable adverse effects related to the immunosuppression were the main cause of refusing study participation. The first patient enrolled was diagnosed with diabetic ketoacidosis and received hydrocortisone (200 mg) and methylprednisolone (125 mg) to prevent rabbit antithymocyte globulin reactions. This patient's continued insulin dependence after AHST (see Results section) resulted in modification of the protocol to exclude patients with diabetic ketoacidosis-onset diabetes and to remove glucocorticoids from the immunosuppression regimen. Other exclusion criteria were positive serology for human immunodeficiency virus, hepatitis B or C, and underlying hematologic, nephrologic, cardiac, psychiatric or hepatic disease. Serum levels of $\beta$-human chorionic gonadotropin were determined in all women to exclude pregnancy.

Participants were initially treated by their own physicians until admission to the present study. Race/ethnicity was self-reported and was assessed because of the diversity of the Brazilian population along with its prevalence of black/white biracialiy. HLA class II typing was performed at low/medium resolution using reverse sequence-specific oligonucleotide probes (RSSOP-One Lambda, Canoga Park, Calif), and at high resolution using sequence–specific primers (SSP, One Lambda). The study protocol was approved by the research ethics committees of both the University Hospital of the School of Medicine of Ribeirão Preto and the Brazilian Ministry of Health. An informed consent according to the Declaration of Helsinki was signed by patients or their parents.

### Study Design

Key end points of the study were morbidity and mortality from transplantation and temporal changes in exogenous insulin requirements (daily dose and duration of usage). Secondary end points were serum levels of hemoglobin $A_{1c}$, C-peptide levels during the mixed-meal tolerance test, and anti-GAD antibody titers measured before and at different times following transplantation.

Blood samples for hemoglobin $A_{1c}$ determination were collected after an 8-hour fast at pretreatment and every 3 months thereafter. Blood samples for the determination of C-peptide, an indirect measure of endogenous insulin secretion, 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 at pretreatment, 6 months, 1 year and then yearly following AHST. Serum anti-GAD antibodies were titrated at the same intervals.

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) between mobilization and the conditioning phase and then indefinitely after discharge from the hospital. During hospitalization, blood glucose monitoring was performed before meals and at bedtime. Insulin titration was based on fasting before meals and 2 hours after meals with target blood glucose levels of less than 120 mg/dL (6.7 mmol/L) and less than 140 mg/dL (7.7 mmol/L), respectively. The dose of insulin was reduced by 1-2 IU/mL if patients presented clinical findings of hypoglycemia and/or blood glucose levels less than 4.9 mmol/L (90 mg/dL).

Standard recommendations for lifestyle modification (performing physical activities and a low-sugar diet) after AHST 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, SP, Brazil). Cyclophosphamide (2 g/$m^2$) 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/$m^2$, was infused over 24 hours to bind toxic cyclophosphamide metabolites in the bladder. Granulocyte colony-stimulating factor (10 µg/kg per day) 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. Apheresis was continued daily until the number of harvested progenitor cells reached a minimum of 3.0 x $10^6$ $CD34^+$ cells/kg 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 given intravenously in divided doses of 50 mg/kg per day over 1 hour on days 5, 4, 3, and 2 before stem cell infusion. Rabbit antithymocyte globulin (thymoglobulin, IMTIX Sangstat, Lyon, France) was administered at a dose of 0.5 mg/kg per day on day 5 before, and at a dose of 1 mg/kg per day on days 4, 3, 2, and 1 before stem cell infusion. Except for the first patient, prophylaxis of antithymocyte globulin reactions was done with dexchlorpheniramine (6 mg by mouth) avoiding the use of glucocorticoids. Stem cell infusion was performed on day 0 and granulocyte colony-stimulating factor (5 µg/kg per day) was administered subcutaneously from day 5 after stem cell infusion until neutrophil count was greater than 1000/µL.

**Supportive Care**

Patients were isolated in rooms equipped with high-efficiency particulate air filters. After hospital admittance for conditioning, antimicrobial prophylaxis was started with ciprofloxacin (500 mg every 12 hours intravenously), acyclovir (250 mg/$m^2$ every 8 hours by mouth until day 35), amphotericin B (0.2 mg/kg per day intravenously and 10 mg/d aerosolized). Ciprofloxacin was replaced by cefepime (2 g every 12 hours intravenously) during febrile episodes. After engraftment, antifungal prophylaxis was changed to fluconazole (400 mg/d by mouth until day 60) and sulfamethoxazole/trimethoprim (800/160 mg every 12 hours by mouth 2 times per week) or dapsone (100 mg 3 times per week) was given through day 60 for prevention of *Pneumocystis jiroveci* pneumonia. Weekly monitoring of cytomegalovirus antigenemia in circulating neutrophils was performed until day 60.

During pretreatment evaluation, semen samples were collected and frozen in liquid nitrogen. Leuprolide acetate depot (3.75 mg by intramuscular injection) was given to female patients to prevent menstrual bleeding and to protect ovarian function. All women opted to use oral contraceptive methods after AHST.

**Laboratory Assessment of Diabetic Status**

Serum C-peptide levels were measured by radioimmunoassay using commercial kits (Diagnostic Systems Laboratories Inc, Webster, Tex). The lower limit of detection was 0.1 ng/mL and undetected values were reported as 0.1 ng/mL. Serum levels of anti-GAD antibodies were measured by radioimmunoassay using commercial kits (RSR Limited, Cardiff, UK) and the results were considered positive if greater than 1 U/mL. Hemoglobin $A_{1c}$ was measured by low-pressure liquid chromatography.

**Statistical Analysis**

Multiple comparisons of total area under the curve of serum C-peptide measured during the mixed-meal tolerance test (during fasting and at 30, 60, 90, and 120 minutes) were made using a model of multiple regression of mixed effects for periods 0, 6, 12, and 24 months posttransplantation. The same model was used to test anti-GAD titers. To present the mean variation of hemoglobin $A_{1c}$ levels with time, a model of linear regression of random effects was constructed 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 using PROC MIXED, SAS statistical software, version 8 (SAS Institute Inc, Cary, NC).

## RESULTS

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Fifteen patients aged 14 to 31 years (mean 19.2 years) were enrolled in the study between November 2003 and July 2006. Individual demographic characteristics and follow-up variables are listed in Table 1 and Table 2. Mean body mass index (calculated as weight in kilograms divided by height in meters squared) at diagnosis was 19.8 (range, 16.6-23.4) and mean plasma glucose was 391 mg/dL (21.7 mmol/L) (range, 130-612 mg/dL [7.2-33.9 mmol/L]). All patients presented symptoms of hyperglycemia (polyuria, polydipsia, and weight loss) at diagnosis. Six patients presented both HLA haplotypes characteristic of high risk for type 1 DM, 7 patients presented 1 of those haplotypes and 2 patients presented 0.

| View this table: [in this window] [in a new window] [as a PowerPoint slide] | **Table 1.** Pretreatment and Follow-up Variables of Patients With Type 1 Diabetic Mellitus Undergoing Autologous Nonmyeloablative Hematopoietic Stem Cell Transplantation (Patient Demographics, HLA Type, Blood Glucose, Hemoglobin $A_{1c}$, Weight Loss, Hyperglycemia Symptoms, Body Mass Index) |
|---|---|

| View this table: [in this window] [in a new window] [as a PowerPoint slide] | **Table 2.** Pretreatment and Follow-up Variables of Type 1 Diabetic Patients Undergoing Autologous Nonmyeloablative Hematopoietic Stem Cell Transplantation (Anti–Glutamic Acid Decarboxylase, C-Peptide, Insulin Dose, Insulin-Discontinuation Time, Insulin-Free Time) |
|---|---|

Time from diagnosis to mobilization ranged from 25 to 56 days (mean, 38.4) and mean duration of hospital stay for transplantation (from conditioning to discharge) was 19.2 days (range, 15-24). Mean number of infused $CD34^+$ cells was 11.0 x $10^6$/kg (range, 5.8-23.1 x $10^6$/kg). Neutrophil engraftment (>500/μL) occurred between days 8 and 10 after transplantation (mean 9.1 days) and platelet engraftment (>20 000/μL) was detected between day 0 and day 15 after transplantation (mean 11.4 days).

Most patients had febrile neutropenia, nausea, vomiting, alopecia, and other common transplantation-related complications due to the drugs used in the mobilization and conditioning (Table 3). Bilateral pneumonia of unidentified etiology that required supplementary oxygen therapy and responded completely to broad-spectrum antibiotics occurred in patient 2 and was the only severe acute complication of AHST. During long-term follow-up, patient 3 developed autoimmune hypothyroidism and transient renal dysfunction associated with rhabdomyolysis, a complication that was treated successfully with levothyroxine. Measurements of gonadal function (follicle-stimulating hormone and lutenizing hormone in both sexes, testosterone in men, and estradiol in women) were in the normal range in 14 of 15 patients. Patient 2 fathered a child 2 years after transplantation (by natural means) and patient 10 presented mild hypergonadotropic hypogonadism at 12 months following transplantation. There was no mortality.

| View this table: [in this window] [in a new window] [as a PowerPoint slide] | **Table 3.** Transplantation Complications and Gonadal Function Tests* |
|---|---|

The first patient enrolled in the study presented few minor complications of transplantation (Table 3). However, this patient's insulin requirements increased progressively and at 12 months following transplantation when he abandoned follow-up, he was using a dose 250% higher than his initial requirement (1.7 IU/kg per day). His hemoglobin $A_{1c}$ levels were 7.6%, 8.2%, 8.9%, 9.7%, and 11.1% at 0, 3, 6, 9, and 12 months following transplantation, respectively, and his C-peptide levels were low at study entry (basal level, 0.4 ng/mL; peak stimulated level, not available) and did not increase after 1 year (basal, 0.3 ng/mL; peak stimulated level, 0.4 ng/mL) (Table 1 and Table 2). Anti-GAD antibody levels were 36.0, 9.9, and 7.7 U/mL at 0, 6, and

12 months following transplantation, respectively. Since the protocol was changed after treating this patient, his data were not included in the statistical analysis. Thus, hemoglobin $A_{1c}$ (Figure 1) and results of C-peptide levels (Figure 2) refers to 14 patients fulfilling the same selection criteria and receiving the same conditioning regimen.

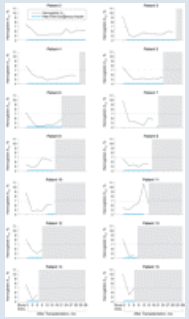

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**Figure 1.** Hemoglobin $A_{1c}$ Levels and Periods Free From Exogenous Insulin Requirement

Data from patient 1 were not included. Mean hemoglobin $A_{1c}$ values were adjusted with a model of linear regression of random effects based on the following equation: $y = 7.8185 - 2.4237 \times \log(\text{time}) + 0.5512 \times [\log(\text{time})]^2$. Differences between pretransplantation and all posttransplantation levels were statistically significant ($P<.05$). Horizontal dotted lines indicate hemoglobin $A_{1c}$ treatment goal < 7%. Gray tint indicates end of follow-up.

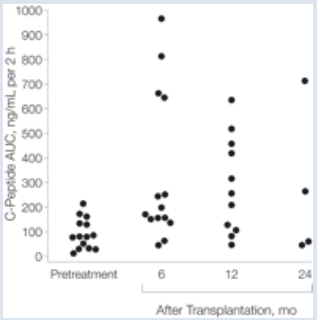


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**Figure 2.** Time Course of Total Area Under the Curve of C-Peptide Levels During Mixed-Meal Tolerance Test

Data from patient 1 were not included. Statistical analysis was performed using a model of multiple regression of mixed effects. $P<.001$ between pretreatment and 6 months; $P = .85$ between 6 and 12 months; $P = .18$ between 12 and 24 months following transplantation. SI conversion factor: to convert C-peptide to nmol/L, multiply by 0.331.

Before the mobilization regimen, all patients required exogenous insulin (mean, 0.38 IU/kg per day, range, 0.13-0.58). By February 2007, 13 patients were free from exogenous insulin for 1 to 35 months (mean, 16.2) (Table 2). Patient 7 used a fraction of the initial insulin dose for 20 months and discontinued insulin use in January 2007. Patient 10 discontinued insulin transiently during transplantation (from 2 days before to 7 days after), then resumed insulin use (0.34 IU/kg per day) and after progressive reduction in its dose discontinued insulin again 1 year after transplantation. Patient 11 was free from insulin from 3 days before transplantation until 360 days after,when insulin use was resumed (0.43 IU/kg per day) after an upper respiratory tract viral infection. The time course of individual insulin doses in different phases is presented in Table 2.

All 14 patients treated according to the same protocol (patients 2-15) complied with blood glucose self-monitoring and scheduled medical appointments. The time course of hemoglobin $A_{1c}$ concentrations of those patients is presented in Figure 1. There was a statistically significant reduction of hemoglobin $A_{1c}$ levels after transplantation. At entry into the study, 11 of 14 patients presented values above 7% and within 3 months after AHST, hemoglobin $A_{1c}$ values were below this level and were maintained during follow-up (except for the relapsing patient 11).

The time course of fasting and peak stimulated C-peptide levels and of the area under the curve response curve during mixed-meal tolerance test are shown in Table 2 and Figure 2. Compared with pretreatment levels, peak stimulated C-peptide levels following transplantation increased in 11 of 13 patients studied at 6 months, in 8 of 10 patients studied at 12 months, in

4 of 4 patients studied at 24 months, and in 1 patient studied at 36 months. Mean peak stimulated C-peptide levels were 1.3 ng/mL at pretreatment and following transplantation 4.0 ng/mL at 6 months, 3.7 ng/mL at 12 months, and 4.5 ng/mL at 24 months. The increase at 24 months following transplantation was statistically significant compared with all other time points (Table 2). Mean area under the curve of C-peptide levels before transplantation (92.0 ng/mL per 2 hours) showed a statistically significant increase at 6 months following transplantation (332.7 ng/mL per 2 hours), which was not different from 12 months (289.2 ng/mL per 2 hours) and 24 months (270.3 ng/mL per 2 hours) (Figure 2).

Mean values of anti-GAD antibodies at diagnosis and at 6, 12, and 24 months after treatment were 31.8 U/mL, 17.3 U/mL, 12.5 U/mL, and 18.7 U/mL, respectively (Table 2). Statistical differences were observed between pre- and and post-6-month titers but not among posttreatment times. Anti-GAD titers showed as negative in only 1 patient (patient 3) at 6 months posttreatment, and continued to show as negative at the 2-year-follow-up.

## COMMENT

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Many clinical trials have analyzed the effect of various immunointervention regimens in blocking autoimmune response and preserving beta-cell function. Short chronic use (≤12 months) of prednisone,[7] azathioprine,[8-9] azathioprine plus prednisone,[10] and cyclosporine[11] in randomized controlled trials produced variable degrees of improvement in C-peptide levels at the end of follow-up compared with pretreatment values. However, these effects were not maintained after immunosuppression was discontinued.[7-11]

Recent studies using short-term treatment with anti-CD3 monoclonal antibodies or heat-shock protein showed long-lasting improvements on C-peptide levels (up to 18 months), however with only partial improvement in insulin usage.[12-14] Control groups in the recent studies of immunointervention (treated with intensive insulin therapy) experienced progressive declines of C-peptide levels after study entry or after transient increase in its levels and a parallel increase in insulin needs.[12-15]

In our study, the increase of C-peptide levels and reduction of hemoglobin $A_{1c}$ were maintained 2 years after insulin discontinuation, excluding the acute effect of insulin therapy on C-peptide concentrations and metabolic control. The natural history of type 1 DM was more altered in our study than in other immunosuppression interventions because, different from those studies, 14 of 15 or 93% of our patients experienced variable periods of insulin independence and most of them maintained this status throughout the follow-up.

Beta cell function in newly diagnosed type 1 DM is a measurable outcome that predicts long-term clinical status. Thus, preservation of beta-cell mass can be expected to provide long-term benefits.[6, 19] The first patient failed to show a clinical benefit probably because of a very low beta-cell reserve at study entry, predicted by previous ketoacidosis that was further jeopardized by the beta-cell apoptotic effect of glucocorticoids used during conditioning.[20] Most of the subsequent 14 patients treated without glucocorticoids in the conditioning regimen demonstrated increased beta-cell function measured by C-peptide levels and became insulin-independent for 1 to 35 months. Two patients (identified as 7 and 10) who initially remained on insulin use shortly after transplantation developed insulin independence 20 and 12 months after AHST, respectively, probably secondary to progressive elevations in C-peptide levels over time. The reverse was seen in patient 11, who presented a decline in C-peptide levels after 1 year and resumed insulin use after that time. With the exception of patient 1, irrespective of insulin use all others achieved and maintained peak stimulated C-peptide levels greater than 0.60 ng/mL, which is known to be associated with reduced prevalence of diabetic complications.[21] Area under the curve levels of C-peptide increased significantly after transplantation and remained high up to 24 months thereafter.

All patients experienced common transplantation-related complications of high-dose immunosuppression and only 1 patient presented a major infectious complication. The low frequency of severe acute complications after AHST is expected in a group of young patients with early-onset type 1 DM in contrast to other advanced autoimmune diseases.[16-17] On the other hand, 2 patients presented late endocrine dysfunctions that could be caused by autoimmune dysregulation associated with the transplant procedure[22] or by autoimmune polyendocrine syndrome frequently associated with type 1 DM.[23] We cannot exclude the occurrence of long-term complications related to high-dose cyclophosphamide use.

The exact mechanism of action of AHST in autoimmune disorders is not fully understood. Whether the mechanism is active or passive tolerance, ie, T-regulatory cell suppression or clonal deletion, is unknown. In multiple sclerosis, evidence supporting post-AHST immune resetting includes an increase in thymus-derived naive T cells, decreased central-memory T cells, increased output of recent thymic emigrants, and recovery of a diverse but distinct T-cell receptor repertoire following AHST.[24] Detailed studies of immune reconstitution are underway in these patients to better understand the mechanisms of action of AHST in new-onset diabetes. Preliminary data suggest a resetting of the immune system toward a tolerant phenotype beyond 1 year after transplantation, as observed in multiple sclerosis (K.C.R.M. and J.C.V., unpublished data, 2006). In the patients of this study, persistence of anti-GAD antibodies, even at low titers, shows that the conditioning regimen was not fully ablative for autoreactive B-cell clones and confirms that the magnitude of the humoral response is not predictive of beta cell reserve or clinical response.[19]

Improvement of beta-cell function after intensive immunosuppression could be explained by regeneration of beta cells from surviving beta cells or from pancreatic or bone marrow stem cells.[25-26] However, pancreatic stem cells have not been clearly demonstrated, and significant in vivo generation of islet cells from hematopoietic stem cells was not observed in animal models of type 1 DM[18] or in patients with long-term type 1 DM treated with allogeneic hematopoietic stem cell transplantation for concomitant blood disorders.[27]

This is, to our knowledge, the first report of high-dose immunosuppression followed by autologous nonmyeloablative hematopoietic stem cell transplantation for human type 1 DM. Very encouraging results were obtained in a small number of patients with early-onset disease. Ninety-three percent of patients achieved different periods of insulin independence and treatment-

related toxicity was low, with no mortality. Further follow-up is necessary to confirm the duration of insulin independence and the mechanisms of action of the procedure. In addition, randomized controlled trials and further biological studies are necessary to confirm the role of this treatment in changing the natural history of type 1 DM and to evaluate the contribution of hematopoietic stem cells to this change.

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**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.

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*Analysis and interpretation of data*: Voltarelli, Couri, Stracieri, Malmegrim, Foss-Freitas, Simões, Foss, Squiers, Burt.

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*Critical revision of the manuscript for important intellectual content*: Voltarelli, Couri, Oliveira, Moraes, Pieroni, Coutinho, Malmegrim, Foss-Freitas, Simões, Foss, Squiers, Burt.

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*Study supervision*: Voltarelli, Malmegrim, Foss, Squiers.

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