Autologous Hematopoietic Stem Cell Transplantation for Type 1 Diabetes

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In this review, we present (1) the scientific basis for the use of high-dose immunosuppression followed by autologous peripheral blood hematopoietic stem cell transplantation for newly diagnosed type 1 diabetes (T1D); (2) an update of the clinical and laboratory outcome of 20 patients transplanted at the University Hospital of the Ribeirão Preto Medical School, University of São Paulo, Brazil, and followed up to January/2008, including 4 relapses among 19 patients without previous ketoacidosis; (3) a commentary on criticisms to our article that appeared in four articles from the scientific literature; and (4) a discussion of the prospectives for cellular therapy for T1D.

Key words: **type 1 diabetes; stem cell transplantation; high-dose immunosuppression**

Introduction

The first convincing evidence that intense immunosuppressive therapy may cure a life-threatening autoimmune disease (AID) was obtained in the 1970s by Simon Slavin in a patient with mixed cryoglobulinemia and end-stage renal failure with a cryocrit level of 60%. This patient with monoclonal IgM and polyclonal IgG cryoglobulins was treated with a combination of cyclophosphamide and azathioprine. Treatment was complicated by lymphocytopenia and neutropenia followed by sepsis, but the patient recovered without stem cell support. After recovery, renal function normalized in parallel with elimination of the cryoglobulins, and the patient is alive and has remained disease-free for more than 25 years.[1] This case represents the longest observation of a patient with chemotherapy-induced self-tolerance after elimination of self-reactive lymphocytes and reestablishment of tolerance from uncommitted stem cells. Brodsky *et al*. extended this approach with encouraging results by treating a variety of autoimmune diseases (AIDs) with high-dose cyclophosphamide (200 mg/kg) without hematopoietic stem cell (HSC) infusion.[2]

Compared to the Brodsky approach, addition of infused peripheral blood HSCs shortens the duration of neutropenia by 4–5 days, theoretically decreasing the risk of serious infections. In addition, infused HSCs may have a positive effect in reconstituting a naïve immune system, and for these two reasons, autologous HSC infusion after high-dose immunosuppression became the preferred method in subsequent HSC transplantation (HSCT) studies for

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AIDs (see below). However, the clinical studies performed by Brodsky's group using high-dose immunosuppression alone were not accompanied by immunoreconstitution studies and have not been compared in randomized trials to high-dose immunosuppression with autologous HSC infusion, precluding a comparison between lymphoablative chemotherapy with and without autologous HSC reinfusion. This comparison would give clues about the role of the infused HSCs in the clinical and immunologic effects of autologous HSCT for severe AIDs. The lymphoablative but nonmyeloablative approach with reinfusion of HSCs described above has been further dose-escalated to use myeloablative regimens that were originally designed for cancer and that irreversibly destroy the bone marrow's ability to recover if HSCs are not reinfused.[3] Comparative trials of non-myeloablative versus myeloablative HSCT regimens for patients with AIDs have not been performed.

On the basis of (1) animal models of autoimmune diseases successfully treated with high-dose immunosuppression plus infusion of hematopoietic stem cells (autologous or allogeneic) and (2) remission of coincidental autoimmune diseases in patients treated for hematologic disorders (reviewed by Burt *et al.*[4] and Moore *et al.*[5]), in 1996 the first patients with isolated AIDs were treated with HSCT. To date, more than 1,000 patients with severe and refractory AIDs have been treated,[6] most with autologous HSCT, because of the lower risk of complications compared to allogeneic HSCT. Between one- and two-thirds of patients experienced sustained remission of disease. Relapses and treatment-related mortality rates after HSCT vary with type and status of disease, and type of HSCT regimen (myeloablative vs. nonmyeloablative conditioning regimens). In terms of risk benefit from HSCT, it is important to clarify and realize that the risk of mortality after HSCT in autoimmune diseases is $<1\%$ for nonmyeloablative regimens, $<2\%$ for reduced-intensity myeloablative regimens, and $>10\%$ for myeloablative regimens.[7] Patients with diseases without vital organ damage or prior history of chronic high-dose immune suppression are also at lower risk of HSCT treatment-related mortality independent of transplant regimen.

TABLE 1. Scientific Evidence for the Possible Benefit of High-Dose Immunosuppression Followed by Hematopoietic Stem Cell Transplantation (HSCT) in Newly Diagnosed Type 1 Diabetes

Evidence	Reference
Beneficial effects of HSCT for human severe autoimmune diseases	3–8
Results of HSCT in experimental models of T1D	10–12
Beneficial effects of immunosuppression in human T1D	13–17
Lack of benefit of HSCT in long-term T1D	18
Transfer of T1D during HSCT in humans	19

Mechanistic studies have been performed after autologous HSCT for AIDs and suggest that immune regeneration occurs and that the regenerated immune system is more self-tolerant with a regulatory phenotype, marked by increased numbers of naïve and regulatory T cells and greater T cell receptor repertoire diversity.[8,9]

Rationale for Using HSCT in Human Type 1 Diabetes

After more than 10 years of clinical use of HSCT for severe and refractory AIDs, what is the evidence that this approach could be beneficial for human type 1 diabetes (T1D)? The evidence is derived from experimental studies with animal models of T1D and from clinical studies using immunosuppression for early-onset T1D or in hematopoietic stem cell transplantation for hematologic diseases where donor or recipient had T1D (Table 1).

Experimental Studies

There are, in general, two types of experimental models of animal autoimmune diseases,

one genetically predetermined and the other environmentally induced. This also holds true for animal models of T1D. Nonobese diabetic (NOD) mice develop autoimmune diabetes spontaneously, with onset of symptoms around 90 days of age. Alternatively, diabetes may be induced in non-diabetic-prone mice with repeated injections of small doses of streptozocin, a drug that causes β cell injury. Streptozocin-induced T1D arises from subtotal or incomplete β cell death, which primes diabetogenic T cells that subsequently perpetuate islet cell injury.[10]

In NOD mice, development of clinically overt T1D is easily prevented by allogeneic stem cell transplantation, but not by autologous HSCT, a result that can be anticipated by the genetic nature of the disease in this model.[11] On the other hand, overt T1D in NOD mice cannot be reversed by allogeneic HSCT alone, requiring a source of pancreatic β cells.[12] These findings indicate that allogeneic HSCs can reinduce tolerance to pancreatic β cells in T1D, but cannot restore the pool of those cells once it was completely destroyed by the autoimmune process.

In contrast to spontaneous-onset T1D in NOD mice, streptozocin-induced T1D can be cured early after disease onset by a syngeneic (the animal equivalent of autologous) HSCT. Reversal of diabetes occurs regardless of whether the syngeneic donor is normal or also has streptozocin-induced T1D. The prevention or reversal of streptozocin-induced T1D early after disease onset is associated with HSCT regeneration of autoregulatory cells.[32] It is unknown whether T1D in humans is predominately genetic, akin to NOD mice, and would require an allogeneic stem cell transplant for cure, or environmentally induced, akin to streptozocin-induced T1D, and cured by autologous HSCT with regeneration of auto-regulatory cells. Whether using autologous or allogeneic HSCs, transplant must be performed early after disease onset before complete destruction of the β cell compartment to reverse diabetes.

Immunosuppression for Early-Onset Disease

Immune-mediated islet cell destruction is not complete until some time after clinical onset of T1D. This has lead, beginning in the 1980s, to immunosuppression trials for new-onset T1D (reviewed by Staeva-Vieira *et al.*[13] and Couri *et al.*[14]). Early-diagnosed T1D patients were treated with prednisone, and cyclosporine and/or azathioprine. Several trials, including French, Canadian–European, Australian, and American, indicated that cyclosporine and/or azathioprine preserved insulin secretion and/or increased the duration of insulin independence. The best results seemed to occur for patients within 8 weeks of T1D onset. Despite preserving insulin secretion, long-term immunosuppression was impractical on account of chronic side effects. These studies indicate that islet cells persist, at least for a short time interval of weeks to months after T1D onset. Measurements of C-peptide, a marker for endogenous insulin, indicate persistence of islet cells with low normal C-peptide levels for 1 year after T1D onset.

The most encouraging results have been observed after short-term courses of engineered anti-CD3 monoclonal antibodies. These studies were pioneered by Eisenbarth *et al.*,[15] who induced transient remission (up to 8 months) in a small group of patients with T1D treated with prednisone plus antithymocyte globulin (ATGAM). In one recent study, 12 patients treated with the antibody showed better β cell function and lower insulin dosage after 1 year compared to the placebo group.[16] In a subsequent study with larger number of patients and extended follow-up, the metabolic (increase in C-peptide levels) and clinical (decrease in insulin usage) benefits were maintained up to 2 years after diagnosis.[17] However, in neither study did a significant number of patients became insulin-free after immunointervention. In those studies, the long-term increase of regulatory T cells (Tregs) could be implicated in prolonged protection

(18–24 months) of pancreatic β cells from autoimmune aggression. Currently, several trials of immunosuppression for early-onset T1D are being conducted, using polyclonal ATG, anti-IL2 receptor monoclonal antibody, mycophenolate mofetil, sirolimus, tacrolimus, anti-CD52 (Campath-1H) or anti-CD20 (rituximab) monoclonal antibodies.[13]

HSCT for Hematologic Diseases When Donor or Recipient Had T1D

Only one retrospective report, from Seattle, investigated the effect of high-dose immunosuppression and HSCT on the metabolic control of three patients with long-established T1D who recived the transplant for hematological diseases (Fanconi's anemia, T cell acute lymphoblastic leukemia, or acute myelomonocytic leukemia).[18] Two patients received HLA-identical bone marrow transplantation from family relatives (mother or sister) and one patient received syngeneic HSCs from an identical twin. In this study with 3–7 years of follow-up, diabetes was not changed by HSCT, as evaluated by continuous use of insulin after transplantation. This result is consistent with data from animal models in that allogeneic HSCT in NOD mice or autologous HSCT in streptozocin-induced T1D must be performed before the β cell compartment is completely destroyed. On the other hand, there are few reports of transference of T1D from donor to recipient of allogeneic HSCs for hematologic diseases,[19] indicating that adoptive transfer of diabetogenic immune cells may, in the allogeneic transplantation setting, transfer disease.

Autologous HSCT for Newly Diagnosed T1D

On the basis of the foregoing evidence, HSCT for early-onset T1D was proposed in review articles in the literature in 2001[20] and 2002,[21] and a cooperative protocol between Northwestern University in Chicago, USA (Richard Burt) and the University of São Paulo in Ribeirão Preto, Brazil (Júlio Voltarelli and others) was started in Brazil in the end of 2003 after approval by local and national institutional review boards.

The object of the treatment was to stop autoimmune destruction of β cells with high-dose immunosuppressive drugs (cyclophosphamide and rabbit antithymocyte globulin) and to "reset" the deleterious immunologic system with a reconstituted one originated from autologous HSCs.[22] The rationale was to preserve residual β cell mass and facilitate endogenous mechanisms of β cell regeneration. Hematopoietic stem cells probably do not have the capacity to differentiate *in vivo* into large numbers of β cells, and therefore HSCs are used to regenerate a new immune system with reestablishment of β cell tolerance through chemotherapeutic depletion of diabetogenic effector cells and HSC regeneration of tolerizing regulatory cells (see below). The exact mechanism of action operating in this treatment is still unclear. However, it has been suggested that AHSCT may shift the balance from destructive immunity to immune tolerance through clonal exhaustion, regulatory cells, cytokine alterations, and changes in T- or B cell repertoires.[4–6]

AHSCT comprises several steps from patient selection through long-term follow-up (Table 2). Most patients interested in the study were excluded for not fulfilling protocol criteria, especially the short time period (6 weeks) from diagnosis, positivity for anti-GAD antibodies, or fully understanding and complying with the study protocol. Apart from the diabetic status, all treated patients were in good health without prior immune suppression before transplantation, which explains in part the low frequency and severity of adverse effects (see below). This result is also explained by the rapid engraftment of neutrophils (mean of 9 days) and platelets (mean of 11.4 days)[22] and relative lack of infections.

The first patient, who was enrolled in December 2003 and received a transplant in

TABLE 2. Steps of Autologous Hematopoietic Stem Cell Transplantation (AHSCT) for Patients with Type 1 Diabetes

1. Patient selection: age 12–35 years old, typical clinical onset, insulin dependence, hyperglycemia <6 weeks, positive anti-GAD antibodies
2. Pretransplant evaluation: C-peptide levels during mixed-meal tolerance test, Hb A1c, infectious and malignancy screening, pregnancy test for women, assessment of cardiac, renal, hepatic, pulmonary, and hematologic function
3. Mobilization of HSCs: cyclophosphamide (1 g/m^2, i.v., in 24 hours divided in 2 doses 12 hours apart) + G-CSF (10 μg/kg/day, s.c., until counts of $CD34^+$ cells in the peripheral blood > 10/μL)
4. Collection of peripheral blood HSCs: by leukoapheresis (one or more sessions, minimum of 3×10^6 $CD34^+$ cells/kg) without *in vitro* manipulation.
5. Cryopreservation of HSCs: in liquid nitrogen (−195°C) or mechanical freezer (−85°C)
6. Conditioning regimen: cyclophosphamide (50 mg/kg/day from day −4 to −1) plus rabbit ATG (Thymoglobulin) 0.5 mg/kg/day on day −5 followed by 1.0 mg/kg/day on days −4 to −1 (the day of HSCT is considered day 0).
7. Infusion of HSCs: through central venous catheter, minimum 2×10^6 $CD34^+$ cells/kg
8. Patient care: from conditioning to engraftment, patients are maintained in reverse isolation (Hepa-filtered rooms), received antimicrobial prophylaxis (acyclovir, low-dose amphotericin B or fluconazole, and ciprofloxacin) and low-microbe diet
9. Short-term follow-up: blood glucose monitoring at least 4 times a day, serum electrolytes, cell blood counts, creatinine, urea, bilirrubin, liver enzymes daily from conditioning through engraftment, chest X-ray weekly
10. Long-term follow-up: same tests as item 9 above plus cytomegalovirus antigenemia weekly through day +60, anti-GAD antibodies, C-peptide levels during mixed-meal tolerance test, self-monitoring blood glucose at least 2 times a day, Hb A1c every 6 months and then indefinitely. At the end of 2007 we included in the study protocol a 3-day continuous glucose monitoring (CGMS) performed every 6–12 months.

January 2004, presented a discouraging response. His insulin requirements increased progressively for 12 months after transplantation (when he abandoned follow-up), reaching a dose 250% higher than his initial requirement. His hemoglobin A1c level was 11.1% at 12 months and his C-peptide concentration did not increase. The possible cause for his poor clinical response is the very low β cell reserve, predicted by the previous diagnosis of diabetic ketoacidosis, and further jeopardized by the β cell apoptotic effect of glucocorticoids used to prevent rabbit antithymocyte globulin reactions. Considering these possibilities, we decided to delete glucocorticoids from the conditioning regimen in subsequent patients and to exclude those with previous diabetic ketoacidosis from eligibilty.

At January 2008, after a mean follow-up of 23.3 months (range between 1 to 47 months), all but one of the subsequent 19 patients became insulin-free, most of them shortly after starting high-dose immunosuppression, even before stem cell infusion. Four out of 19 patients resumed insulin use after transient periods free from insulin ranging from 7 to 12 months (mean 9.2 months). Three of them are receiving 30–40% of insulin doses compared to the doses used before transplantation, and one patient is using higher doses than were required pre-transplantation. Two of these patients resumed insulin use after an upper respiratory tract infection. The other 14 patients are continuously insulin-free since insulin suspension: three patients for at least 3 years, four patients for at least 2 years, three patients for at least 1 year, and four patients for at least 3 months (Fig. 1). The 20th patient, who had no period free from insulin, had inadvertently received steroids (300 mg hydrocortisone) along with stem cell infusion to prevent reactions to DMSO.

There was a statistically significant reduction of mean hemoglobin A1c concentrations after transplantation. In all but two patients (the first and the 11th) all measurements were below 7% (upper limit of good glucose control) during follow-up. As noted above, soon after inclusion, the first patient did not achieve good glucose control, whereas the 11th patient

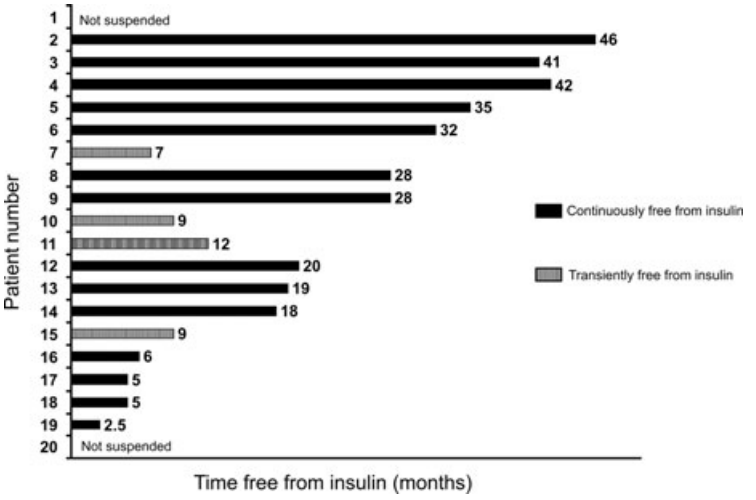

Figure 1. Time free from insulin in 20 patients who underwent autologous hematopoietic stem cell transplantation at the University Hospital of the Ribeirão Preto Medical School from January 2004 through December 2007 and followed through January 2008. The first patient had previous diabetic ketoacidosis (DKA) and received corticosteroids during the conditioning regimen and the 20th patient had no previous DKA, but accidentally received steroids previous to stem cell infusion. All the other 18 patients became insulin-free after the procedure for variable periods of time (from 2.5 to 46 months).

presented A1c levels <7% until 12 months after transplantation, when insulin use was restarted and hemoglobin A1c began to increase.

Regarding the time course of β cell function, of the first 14 patients who had C-peptide levels partially analyzed, the majority ($n = 11$) had increased values in comparison with pretreatment levels, indicating preservation and even improvement of β cell function. Analyzing C-peptide levels during a stimulus with mixed-meal tolerance test, we see a statistically significant increase in mean area under the curve 6 months after transplantation, an increase that was maintained until 24 months after stem cell transplantation.[22]

In the face of the good metabolic results presented, the adverse effects were acceptable. With respect to acute complications, most patients had febrile neutropenia, nausea, vomiting and alopecia due to the immunosuppressive agents used in the mobilization and conditioning phases of the protocol. Bilateral pneumonia of unidentified etiology that required supplementary oxygen therapy and responded completely to broad-spectrum antibiotics occurred in one patient and it was the only severe acute complication of the transplantation procedure. During long-term follow-up, patient 2 developed Graves disease 3.5 years after transplantation, patient 3 developed autoimmune hypothyroidism and transient renal dysfunction associated with rhabdomyolysis, a complication that was successfully treated with levothyroxine. Patient 10 presented mild transient hypergonadotropic hypogonadism 12 months after transplantation. These late-onset endocrine dysfunctions in these 3 patients may be related either to the transplant procedure itself or to an autoimmune polyendocrine syndrome frequently associated with T1D. There was no mortality.

In July 2007 we initiated a similar study of nonmyeloablative autologous hematopoietic stem cell transplantation in newly diagnosed individuals with T1D who had previous diabetic ketoacidosis. By January 2008 only one patient had been enrolled in the study and insulin independence was not achieved, but insulin doses decreased by less than 40% of the initial requirements.

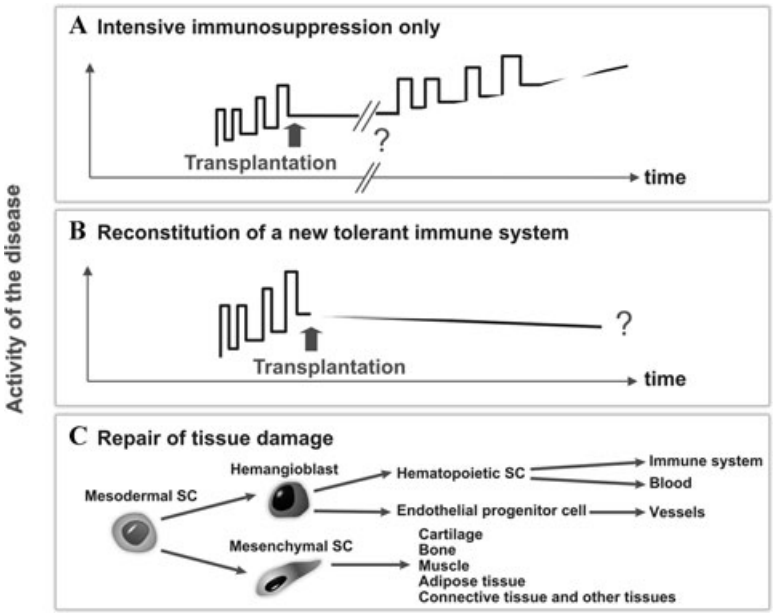

Figure 2. Possible mechanisms of action of autologous hematopoietic stem cell transplantation in autoimmune diseases. From the reports in the literature and our own unpublished work, mechanism B (reinduction of tolerance by autologous stem cells after lymphoablative conditioning) seems more probable at present.

We are currently performing exhaustive studies of immunoreconstitution (phenotypic and functional) in the patients undergoing transplantation to determine the mechanisms by which AHSCT produces clinical benefit in T1D patients. Preliminary results show that after transplantation, there is an increase in the numbers of regulatory $CD4^+CD25^+Foxp3^+$ T cells and Th2 cytokine-producing cells, compared to the pre-transplant status. In addition, we found after AHSCT, profound qualitative and quantitative changes in TCR repertoire, as well as alterations in the expression of pro- and anti-apoptotic genes.[23,24] Anti-GAD autoantibodies decreased in most patients but did not correlate with clinical response. Our immune reconstitution results are under review and preliminary results resemble those observed in other autoimmune diseases after AHSCT.[8,9] The results support the suggested hypothesis that a new and more tolerant immune system is generated after the treatment, explaining the reduction of autoimmune destruction and clinical improvement (mechanism B, Fig. 2). However, in the presence of anti-GAD autoantibodies and in the absence of specific immunologic reactivity tests to β cell antigens, we cannot be sure that our treatment blocks autoimmune attack to endocrine pancreas more efficiently and for a longer time than other immunosuppressive interventions, particularly current ongoing anti–T cell therapies.

Comments on Our Study and Reply

Among dozens of reports and comments about our study in *JAMA*[22] that subsequently appeared in the lay and scientific media, we selected three comments that appeared in peer-reviewed scientific journals and one that appeared in a society publication, *The Hematologist: American Society of Hematology News and Reports*. The editorial that accompanied the paper in *JAMA*[25] raised the possibility that anti-GAD-positive type 2 diabetes patients could have been enrolled in the study, but dismissed the suggestion based on other characteristics

of the patients (age, HLA typing, weight loss, and significant hyperglycemia). Then the editorial pointed to four limitations of the study: (1) absence of randomized control groups that received either no intervention or only immunointervention without stem cells; (2) short follow-up, insufficient to determine whether improvement in C-peptide levels was sustained; (3) mechanism of action of AHSCT (immune reconstitution, interference with immune-mediated β cell destruction and/or β cell regeneration) is not clear; and (4) naturally occurring honeymoon period of relative remission after the onset of T1D complicates the interpretation of the results. We agree to the limitations of our preliminary study, but, in regards to issue (4), after almost 4 years of follow-up of the earliest patient undergoing successful transplant and of 18 of 19 nonketoacidotic patients discontinuing insulin after HSCT, it is hard to ascribe these results to the honeymoon period of T1D.

A letter send to *JAMA*[26] raised ethical concerns about our study based on the two following factors: (1) Inclusion of legally incompetent minors violated the Declaration of Helsinki, a research ethics standard recognized internationally and subscribed to by Brazil. (2) Age-matched controls were needed to determine the extent of benefits and of adverse effects; the possibility was also raised of spontaneous or immunosuppression-induced honeymoon. We replied[27] stating that: (1) All minor patients and one of their parents signed the informed consent of the study and minor patients (14–18 years or older) were included only after three adult patients had undergone transplantation, two of them of them successfully; and (2) It is standard for clinical trials to progress in a sequential order of phases 1, 2, and 3 studies in which a control group is usually not implemented until the phase 3 design. In addition, we stated that it is very unlikely that our results could be explained by spontaneous T1D honeymoon (see above).

In his comment for *The Hematologist*,[28] Dr. Chao repeated the argument that our results could be explained by spontaneous remission associated with the honeymoon period shortly after diagnosis and raised the possibility that relapses of the disease, which we are now observing, could be related to the reinfusion of autoimmune lymphocytes with unmanipulated autologous graft. However, a controlled study in HSCT for rheumatoid arthritis did not show any influence of *ex vivo* selection of stem cells in the outcome of transplantation,[29] and manipulation of the graft has not been used in many recent trials of HSCT for AIDs. Moreover, infusion of mature autoimmune lymphocytes with the graft would produce more acute relapses than the ones we are observing in our patients.

Finally, in a "Priority Paper Evaluation" which appeared in the journal *Regenerative Medicine*, Fousteri *et al.*[30] listed the following issues as the strengths and weaknesses of our study. The strengths included (1) selection of truly T1D patients (with positive anti-GAD antibodies and requiring insulin from the onset of the disease), (2) use of nonmyeloablative conditioning, which lowers morbidity and mortality of AHSCT, and (3) achievement of an extended period of insulin independence, which has never been observed in previously published studies. As the weaknesses they noted the following: (1) Twenty-six percent of the patients undergoing transplant developed minor, major or late complications after HSCT, and that intolerable complications, including mortality, could be expected if a larger number of patients were to be treated; and (2) Three additional arms of the trial are required in order to determine whether insulin independence was due to immunosuppression, stem cell infusion, or both: a group with no intervention, another receiving immunosuppression without stem cells, and a third one receiving stem cells without immunosuppression.

We also expect the occurrence of severe toxicity, even mortality, in a few patients if a large number of them are treated with HSCT, but, from the results of hundreds of patients treated

with the same regimen for aplastic anemia,[31] early and long-term complications of HSCT will be less severe than those of T1D itself and, as recently summarized by Burt *et al.*,[7] nonmyeloablative HSCT in autoimmune diseases has a mortality less than 1%. We have already addressed the need for control groups in future studies, but the use of high-dose immunosuppression without hematopoietic stem cell support certainly increases the risk for severe toxicities and mortality, as was recognized in the comments made by Fousteri *et al.*[30]

Conclusions and Prospectives

Our preliminary study of autologous HSCT in a subset of nonketoacidotic newly diagnosed patients with T1D yields unexpected positive results: 18 of 19 patients could stop insulin use after initiation of high-dose immunosuppression, and 14 patients have maintained this status after a median follow-up of almost 2 years (maximum of 4 years). Four patients relapsed after stopping insulin use and one patient never discontinued insulin. Posttransplant immunotherapy may reinduce remission in these relapsing patients. Longer follow-up and controlled studies are certainly needed to evaluate the full potential of the procedure in the reversal of T1D.

The underlying mechanism of action of the various components of HSCT (cyclophosphamide, ATG, and stem cells) cannot be studied by direct methods in humans, but our immune reconstitution study, which is currently under way, and similar studies in other AIDs, as well as in animal models, suggest that the immune system is reset towards a tolerant phenotype by increased regulatory T cell numbers and by regeneration of a different and more diverse TCR repertoire. We hypothesize that the combination of high-dose immunosuppression and HSC infusion act synergistically to downregulate the autoreactive cells, to renew the immune system, and to improve the immune regulatory networks.

While our approach provides the proof of principle that high-dose immunosuppression coupled with autologous hematopoietic stem cell boosting can reverse clinical T1D in humans, it will hardly solve the problem of the disease. Firstly, only a small subset of patients was successfully treated with AHSCT, whereas millions of patients with long-standing T1D need another source of stem cells to regenerate pancreatic β cells and other damaged tissues. Secondly, HSCT is an expensive, cumbersome, and complex procedure performed in specialized bone marrow transplantation facilities and has the potential for life-threatening short- and long-term complications. In the future, simpler approaches such as chemical, biological, or cellular immunoregulatory interventions may accomplish the same therapeutic goal and may be applied to millions of patients with T1D who need a definitive treatment. In the meanwhile, HSCT remains the only treatment to reverse the disease in humans and has to be tested in other groups of patients (those with previous ketoacidosis, those with longer duration of the disease, and young children).

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Conflicts of Interest

The authors declare no conflicts of interest.

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