

# Bone Marrow-Derived Stem Cell Transplantation for the Treatment of Insulin-Dependent Diabetes

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## ■ Abstract

The bone marrow is an invaluable source of adult pluripotent stem cells, as it gives rise to hematopoietic stem cells, endothelial progenitor cells, and mesenchymal cells, amongst others. The use of bone marrow-derived stem cell (BMSC) transplantation (BMT) may assist in achieving tissue repair and regeneration, and in modulating immune responses in the context of autoimmunity and transplantation. Ongoing clinical trials are evaluating the effects of BMSC to preserve functional beta-cell mass in subjects with type 1 and type 2 diabetes, and to favor engraftment and survival of trans-

planted islets. Additional trials are evaluating the impact of BMT (i.e., mesenchymal stem cells) on the progression of diabetes complications. This article reviews the progress in the field of BMSC for the treatment of subjects with insulin-dependent diabetes, by combining allogeneic islet transplantation with donor-specific BMSC. Clinical data is summarized from pilot studies performed at our research center over the last two decades.

**Keywords:** bone marrow-derived stem cell · diabetes · mesenchymal stem cell · transplant · islet transplantation · beta-cell replacement · chimerism · clinical trial · tolerance

## Introduction

 Cellular therapies for the treatment of diabetes may enable the restoration of glucose-sensing and -secreting machinery to attain physiologic metabolic control. Restoration of beta-cell function is an important therapeutic goal for the treatment of patients with insulin-dependent diabetes [1]. Pancreatic islets are highly specialized glucose sensors that finely regulate glucose metabolism in normal conditions. Functional islet mass becomes lost in an autoimmune process that selectively targets insulin-producing cells in type 1 diabetes (T1D). In type 2 diabetes (T2D), the loss is due to metabolic exhaustion. In these patients,

metabolic control throughout the day is very difficult to achieve by current medical therapy using exogenous insulin supply.

Encouraging results from clinical trials have demonstrated the ability of allogeneic islet transplants to impact positively on glycemic control in T1D patients. Transplantation benefits include a significant reduction of mean glycemic aberrances, normalization of glycosylated hemoglobin A1c (HbA1c) values, and the abrogation of severe hypoglycemia. These advances are retained even when exogenous insulin is required after transplantation of an inadequate islet mass, or after graft dysfunction [2]. The metabolic effects are paralleled by a significant improvement in the pa-



tients' quality of life [3-8]. Also, a positive impact of islet transplantation on the progression of diabetes complications has been reported. Although, most of the studies showing reduced complications were small-scale and nonrandomized [9-16].

#### Abbreviations:

ALG - anti-lymphocyte globulin  
 ATG - anti-thymocyte globulin  
 AZA - azathioprine  
 BID - twice daily  
 BM - bone marrow  
 BMSC - bone marrow-derived stem cell  
 BMT - bone marrow-derived stem cell transplantation  
 C1H - Campath-1H  
 CD3 - cluster of differentiation 3 (surface glycoproteins associated with the T cell receptor to activate T cells)  
 CD34 - cluster of differentiation 34 (surface glycoprotein, cell-cell adhesion factor, mediates attachment of stem cells to bone marrow)  
 CyA - cyclosporine A  
 DAC - daclizumab  
 EPC - endothelial progenitor cell  
 ETC - etanercept  
 GAD - glutamic acid decarboxylase  
 G-CSF - granulocyte colony-stimulating factor  
 GM-CSF - granulocyte-macrophage colony-stimulating factor  
 GvHD - graft-versus-host disease  
 HbA1c - glycosylated hemoglobin A1c  
 HOMA-B - homeostasis model assessment beta-cell (score for assessment of beta-cell function)  
 HOMA-IR - homeostasis model assessment insulin resistance (score for assessment of insulin resistance)  
 HOT - hyperbaric oxygen therapy  
 hrG-CSF - human recombinant granulocyte colony-stimulating factor  
 HSC - hematopoietic stem cell  
 IAK - islet after kidney  
 IEQ - islet equivalent  
 IFN - interferon  
 INF - infliximab  
 IRDM - insulin-requiring diabetes mellitus  
 ITA - islet transplantation alone  
 MHC - major histocompatibility complex  
 MMF - mycophenolate mofetil  
 MP - methylprednisolone  
 MSC - mesenchymal stem cell  
 mTOR - mammalian target of rapamycin (regulates cell growth, proliferation, motility, and survival)  
 NCT - national clinical trial  
 NIDDK - National Institute of Diabetes and Digestive and Kidney Diseases  
 OKT3 - muromonab-CD3 (trade name orthoclone OKT3; monoclonal antibody targeted against the CD3 receptor)  
 SIK - simultaneous islet-kidney (transplantation)  
 SIL - simultaneous islet-liver (transplantation)  
 SIR - sirolimus  
 TAC - tacrolimus  
 T1D - type 1 diabetes  
 T2D - type 2 diabetes  
 TNF - tumor necrosis factor

Current limitations of islet transplantation include the limited number of available cadaveric pancreata, which is too small for the number of potential recipients who could benefit from the treatment. Also, after islet infusion in the hepatic portal system of the recipients, a conspicuous mass of functional islets is lost due to poor engraftment. Therefore, a relatively large islet mass (usually more than one donor pancreas) is necessary to attain adequate metabolic control after transplantation. Finally, the need for life-long immunosuppression currently limits the indication of islet transplantation to adults with a brittle form of diabetes associated with recurrent severe hypoglycemia, and hypoglycemia unawareness.

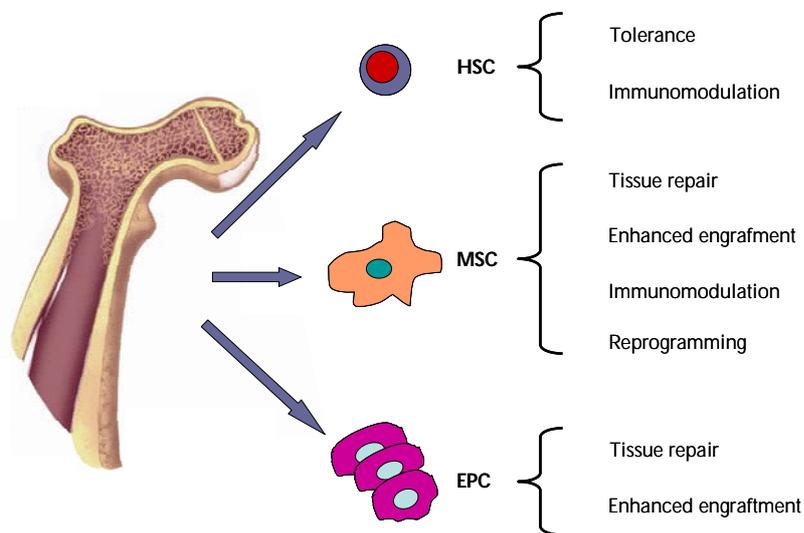
The bone marrow is an invaluable source of adult, pluripotent stem cells. Among others, it gives rise to hematopoietic stem cells (HSC), endothelial progenitor cells (EPC), and mesenchymal stem cells (MSC). Bone marrow cell-derived stem cell (BMSC) transplantation (BMT) can assist in achieving tissue repair and regeneration. Also, this therapy can modulate the immune response in the context of autoimmunity and transplantation (Figure 1).

Bone marrow-derived HSC contribute to the maintenance of hematopoietic cell homeostasis. Transplantation of autologous HSC has been attempted in combination with lymphodepleting protocols, as a means to restore self-tolerance, and halt the progression of autoimmunity in T1D subjects. It is also a means of providing precursor cells for tissue repair in chronic complications of diabetes (discussed in detail below). The use of allogeneic HSC obtained from the same donor of islet cells has been attempted to enhance engraftment, and possibly induce donor-specific acceptance of transplanted tissues. The great plasticity of BMSC includes tissue repair and increases the immune modulation potential of the MSC and EPC components. These advantages offer benefits towards the development of cellular therapies for diabetic complications, and for the preservation of functional beta-cell mass.

An exponential rise in recent publications and clinical trials, reflects the growing interest within the scientific community, in cellular therapies utilizing bone marrow-derived cell products for the treatment of diabetes and its complications. Currently, more than twenty clinical trials on the use of bone BMSC for the treatment of subjects with diabetes are listed in the ClinicalTrials.gov registry (Tables 1-3). This list includes clinical aimed at treating T1D (Table 1), T2D (Table 2), and some of the complications associated with diabetes (Table



3), in the United States and around the world. This manuscript reviews some of the recent research and clinical applications of bone marrow-derived stem cells used for the treatment of insulin-dependent diabetes.



**Figure 1. Schematics of the different cell subsets present in the bone marrow and their potential therapeutic application in the context of diabetes.** Hematopoietic stem cells (HSC) give rise to hematopoietic lineages. Autologous HSC may be used in combination with lymphodepletion preconditioning to restore self-tolerance in the context of autoimmune diabetes. Whereas, allogeneic HSC may be used to achieve mixed chimerism in recipients of donor-specific insulin-producing cells. The high plasticity of mesenchymal stem cells (MSC) may be exploited to induce tissue repair and modulation of inflammation, leading to the recovery of functional islet cell competence, and to the improvement of diabetes complications. MSC may also contribute to the engraftment of insulin-producing cells by providing trophic factors and/or stimuli in the local microenvironment. Also, it may be possible to reprogram MSC into insulin-producing cells *ex vivo* from a patient's own cells or from allogeneic donors. Endothelial progenitor cells (EPC) are present in the bone marrow and may be mobilized, or obtained from aspirates, and used to improve tissue repair in islets or diabetic complications. They may also be used to enhance the engraftment of insulin-producing cells.

### Bone marrow stem cell transplantation to induce hematopoietic chimerism in islet transplant recipients

Clinical and experimental data on the use of donor BMT following myelo- or lymphoablative conditioning, have shown the ability to induce hematopoietic chimerism and graft tolerance in

recipients of solid organ transplantation. This enabled the reduction of and complete weaning from immunosuppression [17-20]. Recent trials using high doses of donor CD34<sup>+</sup> HSC with minimal or non-ablative recipient conditioning showed successful engraftment, reduced adverse events, immunomodulation, and increased allograft survival [17, 18, 21-26]. During the last two decades, our research center has actively explored the possibility of long-term acceptance of solid organs (multi-visceral, liver, intestine, kidney, and heart) [27-34], and islet grafts, by combining BM-derived HSCs in the clinical setting [2, 35].

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Achieving immune tolerance in islet allograft recipients is an appealing prospect, as it may enable durable function of transplanted islets, without life-long adverse side effects from immunosuppression. Currently, almost all classical anti-rejection drugs used in the transplant setting (including, calcineurin-inhibitors, mTOR inhibitors, and steroids) are toxic to islet cells [36-40]. Their continuous use can lead to loss of islet graft function; post-transplant diabetes is a common outcome, even in subjects not prone to diabetes [41]. The induction of hematopoietic chimerism has additional advantages in patients with T1D, as it can enable restoration of self-tolerance, thereby

treating the underlying autoimmunity [20].

Several clinical pilot trials using allogeneic islets with donor-specific BMT have been performed at our center over the recent years (Table 4). The BMSC inoculum consisted of non-fractionated or enriched CD34<sup>+</sup> BMSC obtained from the vertebral bodies of the same islet donor. The protocols included non-myeloablative immunosuppression.

**Table 1.** Clinical trials using bone marrow-derived stem cells for the treatment of T1D

Trial ID	Title	Location	Condition	Intervention	Study design
NCT00021801	Islet Cell Transplantation Alone and CD34 <sup>+</sup> -Enriched Bone Marrow Cell Infusion in Patients With Diabetes Mellitus: Steroid-Free Regimen	University of Miami, USA; NIDDK	T1D	Islet cell transplantation	Phase II Masking: open label Primary purpose: treatment
NCT00315133	Safety and Efficacy Study of Autologous Stem Cell Transplantation for Early Onset T1D	University of Sao Paulo Ribeirão Preto, Brazil; Northwestern University, Chicago, USA; Genzyme Corporation	T1D	Immunosuppression and autologous stem cell transplantation	Phase I/II Allocation: non-randomized Control: active control Endpoint classification: safety/efficacy study Intervention model: parallel assignment Masking: open label Primary purpose: treatment
NCT00315614	Islet Cell Transplantation Alone and CD34 <sup>+</sup> -Enriched Donor Bone Marrow Cell Infusion in Patients With T1D; Steroid-Free Regimen	University of Miami, USA; NIDDK	T1D	Islet transplantation	Phase II Allocation: non-randomized Control: uncontrolled Endpoint classification: safety/efficacy study Intervention model: single group assignment Masking: open label Primary purpose: treatment
NCT00821899	Bone Marrow Autotransplantation in T1D	Hospital Clinic of Barcelona, Spain	T1D	Administration of autologous bone marrow blood	Control: uncontrolled Endpoint classification: safety/efficacy study Intervention model: single group assignment Masking: open label Primary purpose: treatment
NCT00971503	Safety and Efficacy of Arterial Delivery of Autologous Bone Marrow Cells in the Treatment of Insulin-Dependent Diabetes	University of Moron, Argentina; Stematix, Inc - Houston, Texas, USA	T1D	Autologous bone marrow implantation filgrastim  Other: saline injection	Phase II Allocation: randomized Control: placebo-controlled Endpoint classification: safety/efficacy study Intervention model: parallel assignment Masking: single blind (outcomes assessor) Primary purpose: treatment
NCT01143168	Stem Cell Therapy for T1D	Armed Police General Hospital, Beijing, China; Cellonis Biotechnology Co. Ltd.	T1D	Autologous bone marrow mononuclear cells and umbilical cord mesenchymal stem cells	Phase I Allocation: non-randomized Control: uncontrolled Endpoint classification: safety/efficacy study Intervention model: single group assignment Masking: open label Primary purpose: treatment
NCT01157403	Autologous Transplantation of Mesenchymal Stem Cells for Treatment of Patients With Onset of T1D	Third Military Medical University, Chongqing, Chongqing, China	T1D	Autologous transplantation	Phase II/III Allocation: randomized Control: placebo-controlled Endpoint classification: safety/efficacy study Intervention model: parallel assignment Masking: double blind (subject, investigator) Primary purpose: treatment
NCT00465478	Autologous Bone Marrow Mononuclear Cells Transplantation in Treating Diabetes Patients	Shandong University, China	T1D T2D	Autologous bone marrow mononuclear cell transplantation	Phase I/II Allocation: non-randomized Control: active control Endpoint classification: safety/efficacy study Intervention model: parallel assignment Masking: open label Primary purpose: treatment
NCT01102699	Bone Marrow Progenitor Cell Mobilization in Diabetes	University of Padova, Italy	Diabetes	Drugs: filgrastim, hrG-CSF	Phase IV Allocation: non-randomized Control: uncontrolled Endpoint classification: efficacy study Intervention model: parallel assignment Masking: open label Primary purpose: diagnostic

**Legend:** Source: ClinicalTrials.gov (updated August 2010).

**Table 2.** Clinical trials using bone marrow-derived stem cells for the treatment of T2D

Trial ID	Title	Location	Condition	Intervention	Study design
NCT00465478	Autologous Bone Marrow Mononuclear Cells Transplantation in Treating Diabetes Patients	Shandong University, China	T1D T2D	Autologous bone marrow mononuclear cell transplantation	Phase I/II Allocation: non-randomized Control: active control Endpoint classification: safety/efficacy study Intervention model: parallel assignment Masking: open label Primary purpose: treatment
NCT01102699	Bone Marrow Progenitor Cell Mobilization in Diabetes	University of Padova, Italy	Diabetes	Drugs: filgrastim, hrG-CSF	Phase IV Allocation: non-randomized Control: uncontrolled Endpoint classification: efficacy study Intervention model: parallel assignment Masking: open label Primary purpose: diagnostic
NCT00644241	Efficacy Of Autologous Bone Marrow Derived Stem Cell Transplantation In Patients With Type 2 Diabetes Mellitus (SCT)	Postgraduate Institute of Medical Education and Research, Pgimer, Chandigarh, India	T2D	Stem cell harvest. Angiographic transplantation of stem cells	Phase II Endpoint classification: safety/efficacy study Intervention model: single group assignment Masking: open label Primary purpose: treatment
NCT01065298	Efficacy Of Autologous Bone Marrow Derived Stem Cell Transplantation In Patients With Type 2 Diabetes Mellitus	Postgraduate Institute of Medical Education and Research, Chandigarh, India	T2D	Stem cell transplantation	Phase II/III Observational model: case control Time perspective: prospective
NCT00767260	Autologous Mesenchymal Stem Cell and Bone Marrow Stem Cell Infusion Combined With Hyperbaric Oxygen Therapy in Type 2 Diabetes Mellitus	Fuzhou General Hospital, Fuzhou, Fujian, China	T2D	MSC, BMSC, HOT BMSC, HOT BMSC HOT	Phase I/II Allocation: randomized Control: active control Endpoint classification: safety/efficacy study Intervention model: factorial assignment Masking: open label Primary purpose: treatment
NCT01142050	Stem Cell Therapy for Type 2 Diabetes Mellitus	Cellonis Biotechnology Co. Ltd.; Armed Police General Hospital, Beijing, China	T2D	Mesenchymal stem cells	Phase I Control: uncontrolled Endpoint classification: safety/efficacy study Intervention model: single group assignment Masking: open label Primary purpose: treatment

**Legend:** Source: ClinicalTrials.gov (updated August 2010).

Primary objectives of these trials were to induce hematopoietic chimerism and to promote acceptance of islet allografts.

The first trial (1994-1996) enrolled eight patients who received simultaneous islet-kidney (SIK,  $n = 7$ ), or islet after kidney (IAK,  $n = 1$ ) transplantation. The mean islet mass transplanted was  $14,800 \pm 7,152$  IEQ/kg. Two patients received a combined islet preparation from 2 donors. Additionally,  $10^9$  non-fractionated donor-specific BMSC per kg body weight were injected on days 5 and 11 after islet transplantation. The immunosuppression protocol consisted of an induction with either the anti-CD3 T cell antibody OKT3 ( $n = 5$ ), or anti-lymphocyte globulin (ALG,  $n$

$= 3$ ), followed by maintenance treatment with triple immunosuppression (tacrolimus (TAC), methylprednisolone (MP), and azathioprine (AZA) or mycophenolate mofetil (MMF)). Chimerism levels were monitored in peripheral blood by polymerase chain reaction and flow cytometry [42]. It showed that  $7.1 \pm 1.4\%$  of donor cells at one month, and  $\sim 1\%$  at 6 and 12 months, persisted in the recipients. Overall loss of islet graft function was observed at a mean time of  $142 \pm 53$  days after transplantation (Figure 2). In one patient, rejection of both kidney and islet grafts occurred after treatment with interferon (IFN)- $\alpha$  for acute hepatitis C virus infection. Subsequently, the patient died of complications secondary to the viral

**Table 3.** Clinical trials using bone marrow-derived stem cells for the treatment of diabetes complications

Trial ID	Title	Location	Condition	Intervention	Study design
NCT00292357	Local Application of Autologous Bone Marrow Cells for Treatment of Chronic Diabetic Ulcers	University of Heidelberg, Germany	Diabetic foot; Diabetes complications	Application of autologous bone marrow	Phase I Allocation: non-randomized Control: active control Intervention model: parallel assignment Masking: open label Primary purpose: treatment
NCT00872326	Autologous Bone Marrow Derived Mononuclear Cells in Treating Diabetic Patients With Critical Limb Ischemia	University Hospital Virgen Macarena, Seville, Spain; Fundacion Progreso y Salud, Spain	Peripheral vascular diseases; Diabetic foot	Autologous bone marrow mononuclear cells	Phase I/II Endpoint classification: safety/efficacy study Intervention model: single group assignment Masking: open label Primary purpose: treatment
NCT01065337	Induced Wound Healing by Application of Expanded Bone Marrow Stem Cells in Diabetic Patients With Critical Limb Ischemia	Ruhr University of Bochum; Herz- und Diabeteszentrum NRW, Bad Oeynhausen, Germany	Diabetic foot	Tissue repair cells (TRC); Bone marrow stem cells (BMSC)	Phase II Allocation: randomized Endpoint classification: safety/efficacy study Intervention model: parallel assignment Masking: open label Primary purpose: treatment
NCT00987363	Intraarterial Infusion of Autologous Marrow Derived Mononuclear Cells in Diabetic Patients With Chronic Critical Limb Ischemia	Fundacion Progreso y Salud, Spain; Multicenter Trial (Cordoba, Granada, Murcia, Sevilla), Spain	Arterial occlusive disease; Diabetic foot	Intraarterial infusion of autologous bone marrow cells	Phase I/II Allocation: randomized Endpoint classification: safety/efficacy study Intervention model: parallel assignment Masking: open label Primary purpose: treatment
NCT00955669	Autologous Transplantation of Bone Marrow Mesenchymal Stem Cells on Diabetic Foot	Third Military Medical University, Chongqing, Chongqing, China	Diabetic foot	Autologous transplantation of mesenchymal stem cells	Phase II/III Allocation: randomized Control: placebo control Endpoint classification: safety/efficacy study Intervention model: parallel assignment Masking: double blind (subject, caregiver, investigator, outcomes assessor) Primary purpose: treatment
NCT00282685	Safety and Feasibility Study of Autologous Progenitor Cell Transplantation in Diabetic Neuropathy	Johann Wolfgang Goethe University Hospitals, Frankfurt, Germany	Diabetic neuropathies	Intraarterial bone marrow progenitor cell transplantation	Phase I Allocation: non-randomized Control: uncontrolled Endpoint classification: safety/efficacy study Intervention model: single group assignment Masking: open label Primary purpose: treatment

**Legend:** Source: ClinicalTrials.gov (updated August 2010).

infection. Also, an IAK recipient experienced a kidney rejection episode (creatinine 2.5 mg/dl), 3 months after islet transplantation (4 years after kidney transplant). The remaining patients maintained kidney function without rejection episodes during the follow-up period. Development of cytotoxic anti-donor MHC antibodies occurred in six patients, but it was not associated with loss of kidney graft function [43].

A subsequent ITA trial (year 1998) in patients with T1D (n = 5) involved intra-hepatic transplantation of allogeneic islets, combined with intravenous infusion of enriched CD34<sup>+</sup> BMSC on days 5 and 11 after islet transplantation. In three pa-

tients, immunosuppression treatment consisted initially of daclizumab (biweekly for the first 5 doses and monthly for the first year). Maintenance therapy was carried out with TAC, MMF, and MP. Stable islet graft function for over one year was achieved in one patient who received 6,922 IEQ/kg. This patient had measurable C-peptide ( $0.63 \pm 0.14$  at one year vs. 0.2 ng/ml pre-transplantation), ~40% reduction of insulin requirements, and improved HbA1c levels ( $6.9 \pm 0.6\%$  post-transplantation vs.  $8.1 \pm 0.6\%$  pre-transplantation). Chimerism levels declined over time in this patient (7.7%, 3.6%, and 1% at 1, 6, and 12 months, respectively). By protocol design,

**Table 4.** Clinical trials combining allogeneic islets and donor bone marrow stem cell transplant performed at the Diabetes Research Institute, University of Miami

Year	No.	Indication	Type of transplant	Immunosuppression	
				Induction	Maintenance
1994-96	1	IRDM	IAK + BMSC	ALG	TAC, AZA or MMF, MP
1994-96	7	IRDM	SIK + BMSC	OKT3	
1994-97	5	T1D	SIL + BMSC	None	TAC, MMF, MP
1996-98	1	T1D	SIK + BMSC	OKT3	TAC, AZA or MMF, MP
1998-99	5	T1D	ITA + CD34+ BMSC	DAC or ATG (n = 1)	TAC, MMF, MP, CYA (n = 1)
2000-02	6	T1D	ITA + CD34+ BMSC	DAC ± INF	SIR, TAC
2005-07	3	T1D	ITA + CD34+ BMSC	C1H + ETC	SIR, TAC → MMF

**Legend:** ALG: anti-lymphocyte globulins. ATG: anti-thymocyte globulin. AZA: azathioprine. BMSC: bone marrow-derived stem cells. C1H: campath-1H. CYA: cyclosporin A. DAC: daclizumab (humanized monoclonal antibody to the alpha subunit of the IL-2 receptor of T cells). ETC: etanercept (TNF inhibitor, functioning as a decoy receptor that binds to TNF). INF: infliximab (monoclonal antibody against TNF $\alpha$ ). IAK: islet after kidney. IRDM: insulin-requiring diabetes mellitus. ITA: islet transplantation alone. MMF: mycophenolate mofetil. MP: methylprednisolone. SIR: sirolimus. OKT3: muronab (murine monoclonal IgG2a antibody directed to the human CD3). SLI: simultaneous islet-liver transplantation. T1D: type 1 diabetes. TAC: tacrolimus.

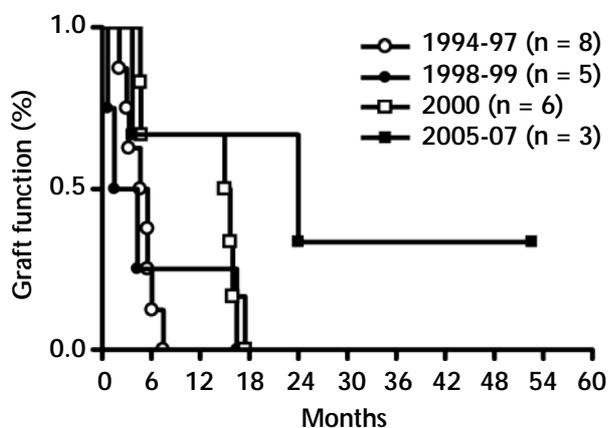
this subject was weaned from immunosuppression, and subsequently lost graft function (Figure 2). Another patient in this group received 10,536 IEQ/kg, and showed good function (C-peptide:  $0.72 \pm 0.21$  ng/ml). Due to poor tolerance to immunosuppressive drugs, the subject developed graft loss by day 45 post-transplant. A third patient showed primary non-function with no measurable C-peptide after transplantation of 5,774 IEQ/kg. Notably, this subject displayed high basal serum TNF- $\alpha$  levels, which may have contributed to the failure of the islets to engraft. Two additional patients received induction with thymoglobulin (rabbit anti-human thymocyte globulin, ATG), and maintenance with cyclosporine A (CyA) and MMF. One of them showed normalization of HbA1c to 6.4%, and good islet graft function after transplantation of 10,669 IEQ/kg (C-peptide:  $0.92 \pm 0.26$  ng/ml) for approximately 130 days. Then, loss of graft function occurred, possibly due to failure to achieve adequate trough levels of the immunosuppressive drugs. The other patient showed rapid reduction of insulin requirements and good graft function (C-peptide:  $0.52 \pm 0.46$  ng/ml) after receiving 7,981 IEQ/kg, but developed serum sickness syndrome secondary to ATG treatment following the second BMSC infusion. This event required hospitalization. Immunosuppression was stopped, resulting in full recovery from symptoms, but loss of graft function by day 21. Microchimerism was detected in all patients in this study. No

episodes of graft-versus-host disease (GvHD) were observed. All patients returned to pre-transplant insulin requirements after stopping immunosuppression.

In the year 2000, we performed an ITA trial (NCT00315614) with Edmonton style immunosuppression and donor-specific BMSC, in five patients with brittle T1D and hypoglycemia unawareness. All subjects received a single intrahepatic islet infusion on day 0, followed by two intravenous donor-specific CD34<sup>+</sup> BMSC treatments on days 5 and 11, post-transplant. One patient received a single infusion of non-fractionated BMSC. Induction treatment consisted of daclizumab (DAC), and a single dose of the anti-tumor necrosis factor (TNF)- $\alpha$  monoclonal antibody infliximab (INF), followed by tacrolimus and sirolimus maintenance [2]. Endpoints of the study included (i) induction of hematopoietic chimerism, and (ii) acceptance of donor-specific islets after stopping immunosuppressive drugs. The latter were tapered gradually in patients with a functional islet graft at one year, as per protocol design. Measurable islet graft function was observed in all study subjects who received  $8,629 \pm 2,102$  IEQ/kg. Loss of graft function was observed in two patients (at 141 and 143 days post-transplantation, respectively), possibly due to failure to achieve therapeutic trough levels of sirolimus. Four patients who received  $9,029 \pm 2,589$  IEQ/kg sustained islet graft function for up to one year, showing maximal



mean C-peptide levels at 3 months ( $1.06 \pm 0.23$  ng/ml), and  $68 \pm 26\%$  reduction in insulin requirements from baseline. Transient insulin independence was achieved in three patients. Hematopoietic chimerism in peripheral blood was transient in these patients ( $4.6 \pm 0.5\%$  at one month,  $0.14 \pm 0.05\%$  at one year). Patients with sustained graft function at one year underwent weaning from immunosuppression, as per protocol design. After immunosuppression was stopped, loss of islet graft function invariably occurred at a mean time of  $95 \pm 25$  days (range, 74-131 days) (Figure 2).

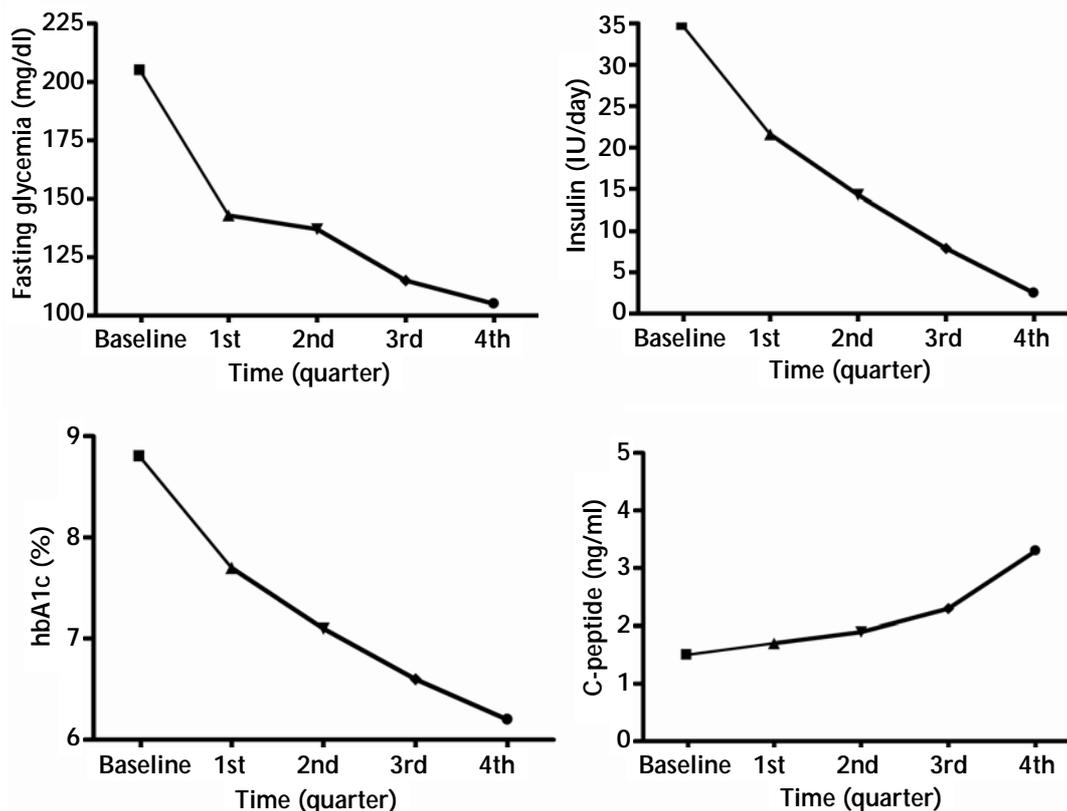


**Figure 2.** Cumulative graft survival of allogeneic islet grafts combined with donor-specific BMSC transplantation in single, center pilot clinical trials performed at the Diabetes Research Institute of Miami, USA. Graft function was defined as measurable C-peptide.

The most recent trial (NCT00021801) performed in the years 2005-2007, consisted of ITA in three patients with brittle T1D and donor-specific CD34<sup>+</sup> BMSC transplantation on days 5 and 11 after islet transplantation. Lymphodepletion at induction was achieved with Campath-1H (C1H; 20 mg on days -1 and 0). Anti-inflammatory treatment peri-transplant was based on TNF-inhibitor etanercept (ETC) given intravenously before islet infusion (50 mg). Thereafter, it was given subcutaneously, twice weekly for 2 weeks (25 mg). TAC was given continuously from the day before ITA,

and adjusted to achieve trough levels of 4-6 ng/ml for three months. Then, patients were switched to MMF maintenance (250 mg a maximum of 1 g BID, calcineurin-sparing protocol). Sirolimus (Rapamune) was given orally from the day before ITA to achieve trough levels of 12-15 ng/ml for 4 months and 7-10 ng/ml thereafter. The mean islet mass transplanted in this small cohort of patients was  $6,553 \pm 1,272$  IEQ/kg. All subjects displayed graft function, with measurable levels of C-peptide, and decreased insulin requirements after transplantation. One patient achieved insulin independence that was sustained for approximately 1.7 years. Then, the patient developed graft dysfunction with complete loss of C-peptide at 2 years. The other two patients displayed sustained graft function for 0.3 and 4.3 years, respectively (Figure 2). Chimerism levels were not detectable at any assessed time point. No major adverse side effects or GvHD were observed in the study subjects.

Collectively, our experience suggests that the use of BMSC with nonmyeloablative immunosuppression is feasible. It is not associated with adverse side effects or with dreadful GvHD (a frequent clinical issue in patients who receive BMT for hematological conditions). Therefore, it may be speculated that lack of harsh treatment to favor stable engraftment of the BMSC inoculum may have contributed to preventing the occurrence of GvHD. The most recent trials showed that overall better outcomes may be ascribed to the steady improvements recorded in the islet transplantation field over the years i.e., islet quality, immunosuppression, and patient management. Possible limitations in our studies include the use of conventional immunosuppression and/or the lack of myeloablation. Our protocols might have precluded adequate engraftment and/or survival of the BMSC inoculum. Indeed, it has been proposed that it is necessary to 'make space' in the recipient's marrow to allow for donor bone marrow cells to engraft [24]. In the two trials performed in 1998 and 2000, immunosuppression was weaned one year after islet transplant, by protocol design. Subsequently, all subjects lost graft function. At that time, there were no sufficiently sensitive immune monitoring methods, nor objective criteria, to guide the decision to stop, or to continue, immunosuppression in the study subjects. More recent studies are trying to identify specific markers and/or molecular signatures of a tolerogenic status in organ transplant recipients [44-48]. Such knowledge may assist the design of stringent rules for future trials.



**Figure 3.** Metabolic control and insulin requirements in a clinical trial of autologous bone marrow stem cell transplantation and hyperbaric oxygen therapy in 25 subjects with T2D. (adapted from [59]).

### Potential of bone marrow-derived stem cells to improve islet transplant outcomes

Increasing the number of BMSC in the peripheral blood of intrahepatic islet graft recipients may benefit islet engraftment. In a rodent experimental model of intrahepatic islet transplantation, mobilization of BMSC by administration of granulocyte-macrophage colony-stimulating factor (GM-CSF) resulted in enhanced vascularization and improved function [49]. This phenomenon was associated with increased peripheral blood angioblasts and higher intra-insular vascular density [49].

Tissue engineering approaches may enable manipulation of islet grafts prior to transplantation. The use of marrow-derived MSC and endothelial cells in culture, to coat pancreatic islets, has been proposed as a means to improve islet

graft neovascularization and engraftment. This strategy was tested *in vitro*. It showed that a significantly higher neovasculation in a three-dimensional fibrin gel assay can be achieved, with preservation of islet functional potency [50]. Transfer of this approach to *in vivo* transplantation settings may contribute to tissue remodeling in the early post-transplant period, and enhance islet engraft and survival.

Another approach to enhance engraftment and survival of allogeneic islet cells is the use of BM-derived MSC. Several properties have been attributed to MSC, including tissue repair and immune modulation. Recently, a trial was performed in a nonhuman primate model of allogeneic islet transplantation. Donor MSC were co-transplanted intraportally with islets, and subsequently, intravenously with donor marrow on postoperative days 5 and 11. It showed that islet engraftment and function assessed at 1 month post-transplant were significantly enhanced compared with control



animals that received islets without MSC [51]. Interestingly, additional infusions of donor-specific, or third-party, MSC resulted in reversal of rejection episodes and prolonged islet function in some animals. Stable islet allograft function was associated with increased numbers of T regulatory cells in peripheral blood. These data suggest that MSC may assist in enhancing islet engraftment, and that MSC may represent a viable adjuvant anti-rejection therapy.

Recently, another approach of combined treatment with islets and BMSC has been proposed. It consists of islet graft implantation directly into the recipient's bone marrow [52]. This approach has proven effective in a murine model of syngeneic islet transplantation. Long-term reversal of diabetes and sustained euglycemia were achieved.

### **Bone marrow-derived stem cell transplantation to preserve/restore beta-cell function in type 1 diabetes**

T1D is the consequence of beta-cell destruction by an autoimmune process. Restoration of self-tolerance may preserve functional beta-cell mass, and improve long-term clinical outcome in patients with T1D. In experimental models of T1D, allogeneic BMT has been shown to contribute to the prevention of islet destruction, and restoration of self-tolerance [20]. This has been achieved by induction of lymphodepletion with physical or chemical means, followed by BMT. The use of cellular therapies for the treatment of T1D is gaining momentum, as demonstrated by the increasing number of clinical trials using BMSC that have been registered at ClinicalTrials.gov in recent years (Table 1).

An increasing amount of data documents significant improvements in clinical outcomes following BMT for severe autoimmune diseases [53-55]. A recent trial of autologous BMT in recent onset T1D patients showed encouraging results, in terms of preservation of beta-cell function [56, 57]. Twenty three study subjects with new onset T1D received mobilization of BMSC by cyclophosphamide and granulocyte colony-stimulating factor (G-CSF). Mobilized BMSC were collected by leukapheresis and cryopreserved. The patients underwent a nonmyeloablative, lymphodepleting conditioning protocol consisting of rabbit anti-thymocyte globulin (ATG) and cyclophosphamide, followed by intravenous stem cell infusion ( $\geq 3 \times 10^6$  CD34<sup>+</sup> cells/kg body weight), and subcutaneous treatment with G-CSF. Twenty of the 23 study subjects achieved insulin independence. Twelve

subjects were insulin-free for continuous periods (in one case up to 4 years), while 8 patients showed transient insulin independence. Insulin requirements were reduced in most of the subjects, who also showed increased C-peptide production during follow-up. Two subjects developed major complications, consisting of bilateral pneumonia. Several subjects developed fever, urticaria, and/or rash. Three subjects developed endocrine dysfunction (i.e., Graves disease, hypothyroidism, and transient hypogonadism, respectively).

Collectively, this trial is developing quite encouraging. It demonstrates that autoimmune diabetes can be halted. However, the need for harsh preconditioning of the recipients increases the risk of severe morbidity. Therefore, the approach raises important concerns regarding its application in young T1D patients. Also, the long-term effects of this protocol need to be ascertained before it can be considered for large-scale clinical use. Nonetheless, the information gathered from this trial may assist in developing safe immune protocols to restore self-tolerance in T1D.

Recently, a study evaluated the impact of autologous, unfractionated BM-derived mononuclear cells obtained from iliac crest aspirate after mobilization with G-CSF, and injection via superselective cannulation of a pancreatic artery in three subjects who had T1D for more than 5 years [58]. The study showed no effect in terms of C-peptide levels after treatment (both basal and stimulated). The study aimed at enrolling 10 study subjects, but was halted by the local research ethic committee for lack of efficacy [58]. Possible limitations of this trial were lack of immune interventions to favor restoration of self-tolerance, and selection of a target population of diabetics who had undetectable C-peptide levels at baseline.

### **Bone marrow stem cell transplant in type 2 diabetes**

T2D is a metabolic disorder characterized by a combination of insulin resistance and pancreatic beta-cell dysfunction. The latter is the major defect, causing beta-cells to secrete reduced insulin amounts in response to increased glycemic values. The common denominator of beta-cell dysfunction in T1D and T2D is inflammation leading to loss of functional pancreatic endocrine mass and a need for exogenous insulin therapy. Recent data reported in the medical literature suggest that autologous BMT may assist in improving metabolic control in subjects with T2D. In recent years, several trials have been initiated to study the im-



pact of BMSC for the treatment of patients with T2D (Table 2).

A recent prospective phase 1 study of 25 patients, evaluated the impact of autologous BMT on the metabolic control of subjects with T2D [59]. The mononuclear fraction of a buffy coat, obtained from BMSC (from iliac crest aspirates) and peripheral blood, was injected via selective cannulation of the dorsal pancreatic artery, under angiographic guidance. The study subjects underwent a daily 1-h session of hyperbaric oxygen therapy (HOT, 100% at 2.3-2.5 atm), 5 days before and 5 days after BMT. Reductions in nonfasting and postprandial plasma glucose, and HbA1c levels, were observed in these subjects. This result was paralleled by an increase in C-peptide levels over the 1-year follow-up, and by a decrease in the number and dose of oral hypoglycemic drugs or insulin (Figure 3).

Another clinical trial of autologous BMT was performed in ten human subjects who were negative for glutamic acid decarboxylase (GAD) antibody [60]. The patients were treated with triple oral antidiabetic drugs and insulin ( $\geq 0.7$  U/kg/day) for  $\geq 1$  year. However, the patients failed to respond to this treatment. A mean of  $3.5 \pm 1.4 \times 10^8$  mononuclear cells obtained through posterior iliac spine aspirate were injected via the gastroduodenal artery by transfemoral cannulation. Seven subjects showed reduced insulin requirements ( $\sim 75\%$  of baseline) by 48 days. Three subjects achieved insulin independence for some time. Also, reduced HbA1c values were recorded in these subjects. In the three responders, this reduction was more pronounced ( $\sim 7\%$  from baseline). The improved metabolic control after BMT was paralleled by amelioration of fasting and glucagon-stimulated C-peptide levels, and increased HOMA-B. Whereas, no changes in HOMA-IR were observed. Importantly, no serious adverse effects were recorded in this trial.

Overall, both trials showed encouraging results. Numerous studies have shown the beneficial effects of BMT in preventing the progression of the autoimmune process and/or restoring self-tolerance in T1D, both in clinical and experimental settings (see above). However, the underlying mechanisms are currently unknown. It has been hypothesized that BMSC transplant might hamper inflammation and preserve functional beta-cell mass. Also, it might provide precursors and/or signals to favor tissue repair and regeneration. Prospective, controlled, randomized studies on large cohorts of subjects are needed in the clinical

setting to characterize this interesting phenomenon.

## Conclusions

Cellular therapies offer new opportunities for the treatment of human diseases. The research endeavor is developing rapidly in the areas of regenerative medicine and immunity. However, there are still many challenges ahead. One of which is the regulatory aspect of handling cellular products for transplantation. This regulation requires a great investment burden to implement cell processing methods within a current good manufacturing practice (cGMP) framework. Simultaneously, it is necessary to adhere to standard protocols, establish strict product release criteria, and secure dedicated infrastructures and personnel. In the case of cellular products derived by enrichment and/or by *in vitro* expansion, all reagents and their sources must be tested and certified for sterility, and for lack of potentially adventitious agents (i.e., endotoxin, etc.). Also, definition of potency, cellular identity, viability, and phenotypical characteristics should be part of the standard assessment performed on each cellular product before transplantation.

Monitoring of clinical trials is essential. This is achievable through institutional review boards (IRB), ethical committees, and independent clinical research organizations. Monitoring protocol implementation and appropriate stopping of trials is necessary to warrant the validity of data generated in clinical trials. Only data from clinical trials that adhere to international ethical standards for human research should be published in peer-reviewed journals. Development of a transplant registry for the collection of clinical trials data may help to interpret results and to assess safety and efficacy. Also, this action may accelerate progress in the field.

Present and future trials will help to assess and optimize safety and efficacy of clinical protocols. They will help to identify the optimal cell type or combination for a specific condition, the optimal cell markers for characterization and product release criteria, and the optimal treatment for a cellular product recipient. Also, further studies will help to find out which primary endpoints enable optimal assessment of efficacy, and how the results of different pilot clinical trials performed at different centers should be compared. The development of multi-center randomized trials may be desirable, but require standardization



of cellular processing, and protocols based on the results emerging from pilot clinical studies.

The presence of pluripotent stem cells in the bone marrow makes this compartment an appealing source of cell subsets. Their application in transplantation regimens will hopefully one day be useful in curing diabetes and its complications. Currently, an increasing body of evidence points to the invaluable potential of bone marrow-derived stem cells for diabetes treatment. Furthermore, the encouraging preliminary data from clinical studies recently reported justify cautious optimism for the near future.

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