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VIEWPOINT

Stem cells to restore insulin production and cure diabetes☆

V. Sordi [a], S. Pellegrini [a], M. Krampera [b], P. Marchetti [c], A. Pessina [d], G. Ciardelli [e], G. Fadini [f], C. Pintus [g], G. Pantè [h], L. Piemonti [a,*]

[a] Diabetes Research Institute (DRI) – IRCCS San Raffaele Scientific Institute, Milan, Italy
[b] Stem Cell Research Laboratory, Section of Hematology, Department of Medicine, University of Verona, Italy
[c] Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy
[d] CRC-StaMeTec (Mesenchymal Stem Cells for Cell Therapy), Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy
[e] DIMEAS – Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Turin, Italy
[f] Medicine Department (DIMED), University of Padua, Italy
[g] Italian National Transplant Center (CNT), Italy
[h] Italian Medicines Agency (AIFA), Italy

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KEYWORDS
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Abstract *Background:* The advancement of knowledge in the field of regenerative medicine is increasing the therapeutic expectations of patients and clinicians on cell therapy approaches. Within these, stem cell therapies are often evoked as a possible therapeutic option for diabetes, already ongoing or possible in the near future.
Aim: The purpose of this document is to make a point of the situation on existing knowledge and therapies with stem cells to treat patients with diabetes by focusing on some of the aspects that most frequently raise curiosity and discussion in clinical practice and in the interaction with the patient. In fact, at present there are no clinically approved treatments based on the use of stem cells for the treatment of diabetes, but several therapeutic approaches have already been evaluated or are being evaluated in clinical trials.
Data synthesis: It is possible to identify three large potential application fields: 1) the reconstruction of the β cell mass; 2) the immunomodulation in type 1 diabetes (T1D); 3) the treatment of complications. In this study we will limit the discussion to approaches that have the potential for clinical translation, deliberately omitting aspects of basic biology and preclinical data. Also, we intentionally omit the treatment of the complications that will be the subject of a future document. Finally, an overview of the Italian situation regarding the storage of cord blood cells for the therapy of diabetes will be given.

Introduction

There are currently no proven treatments for diabetes using stem cells. Nevertheless, a significant amount of experiences report the potential of stem cells to contribute to diabetes therapy. Pluripotent and multipotent stem cells deriving from embryos, fetal and adult tissues are under investigation for potential application in the treatment of diabetes and its complications. In particular, the field of differentiation of pluripotent stem cells into insulin producing cells has made great steps forward and landed in a clinical trial in patients with diabetes. Moreover, extensive studies are ongoing regarding the use of bone marrow-

☆ Study Group SID "Medicina rigenerativa in ambito diabetologico".
* Corresponding author.
E-mail address: piemonti.lorenzo@hsr.it (L. Piemonti).

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and cord blood-derived stem cells for immunomodulation in diabetes. We here give an overview of the current situation of these studies on stem cells, focusing on clinical applications in type 1 and type 2 diabetes.

Endogenous cells for β cell replacement

The first cell source to be considered for functional replacement is the endogenous β cell. Unfortunately endocrine pancreas is a tissue with a very slow turnover of cells, with a proliferative ratio of 0.1–0.3%/day in 1-year-old mice [1] and even lower proportions in humans. The potential use of endogenous endocrine cell as a source of new β cell for transplantation resides in the intrinsic capacities of proliferation, neogenesis and trans-differentiation (Fig. 1).

β cell proliferation. Several studies have shown that β cells mass is regulated dynamically and the relation between replication and apoptosis can determine the final mass [2,3]. In human, normal expansion of the β cell mass occurs during the neonatal period, but fades early in childhood [4]; in adult, β cell replication results increased in some physiological or pathological states, such as pregnancy [5] or an obesity-induced insulin-resistant state [6]. Thus, the use of external agents to expand β cells *ex vivo* for transplantation purpose or to stimulate endogenous cell proliferation *in vivo* in order to increase the β cell mass in diabetic patients may be an attractive approach for β cells supplementation. In fact, β cell regeneration has been observed also in T1D patients after onset [7] or even many years after diagnosis [8,9]. Transfection of many cell cycle regulators like cdks (cyclin dependent kinases) and cyclins into rodent and human islets *ex vivo*, leads to an increase in the replication rate of β cells [10,11], but the prolonged expression of these molecules would increase also the risk of oncogenesis. A safer option is represented by the addition in culture of growth factors, such as growth hormone (GH), glucagon-like peptide-1 (GLP-1) or hepatocyte growth factor (HGF), but in human the elevated proliferation is associated with a loss of β cell features, like Pdx-1 or insulin expression [12]. An *in vivo* therapy with long-acting GLP-1 analogues (exenatide or liraglutide) has been considered to have a potential for the stimulation of β cell replication in diabetic patients after proof-of-concept studies performed in patients treated with GLP-1 [13,14], but long-term data of the evidence of such increase in patients have yet to be provided. In the field of β cell proliferation, a gene therapy aimed at the reversible inclusion of genes capable of immortalizing β cells has been tried as well. During the past 30 years, a number of β cell lines have been established in rodent [15,16] and many attempts have been made to generate human β cell lines from many pancreatic sources, but insulin production by these cells was extremely low or limited at few passages [17,18]. In 2011 a human β cell line was established transducing human fetal pancreases with a lentiviral vector that expressed SV40LT and human telomerase reverse transcriptase (hTERT). One of the cell lines generated with this strategy, the EndoC-βH1, was further characterized and resulted able to secrete insulin in response to glucose stimulation, was stable at least for 80 passages and expressed many specific β cell markers, without any substantial expression of markers of other pancreatic cell types [19]. In view of clinical use, new generations of these cell lines have been recently developed and in particular a novel human β cell line called EndoC-βH3 that contains

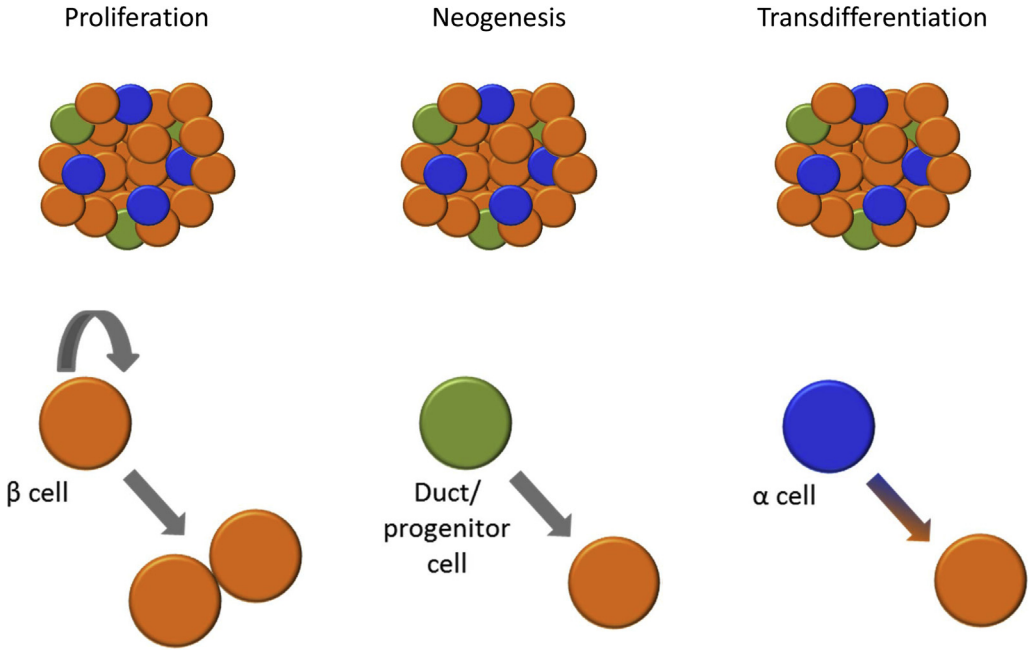

Figure 1 Mechanisms of β cell expansion: proliferation, neogenesis, transdifferentiation.

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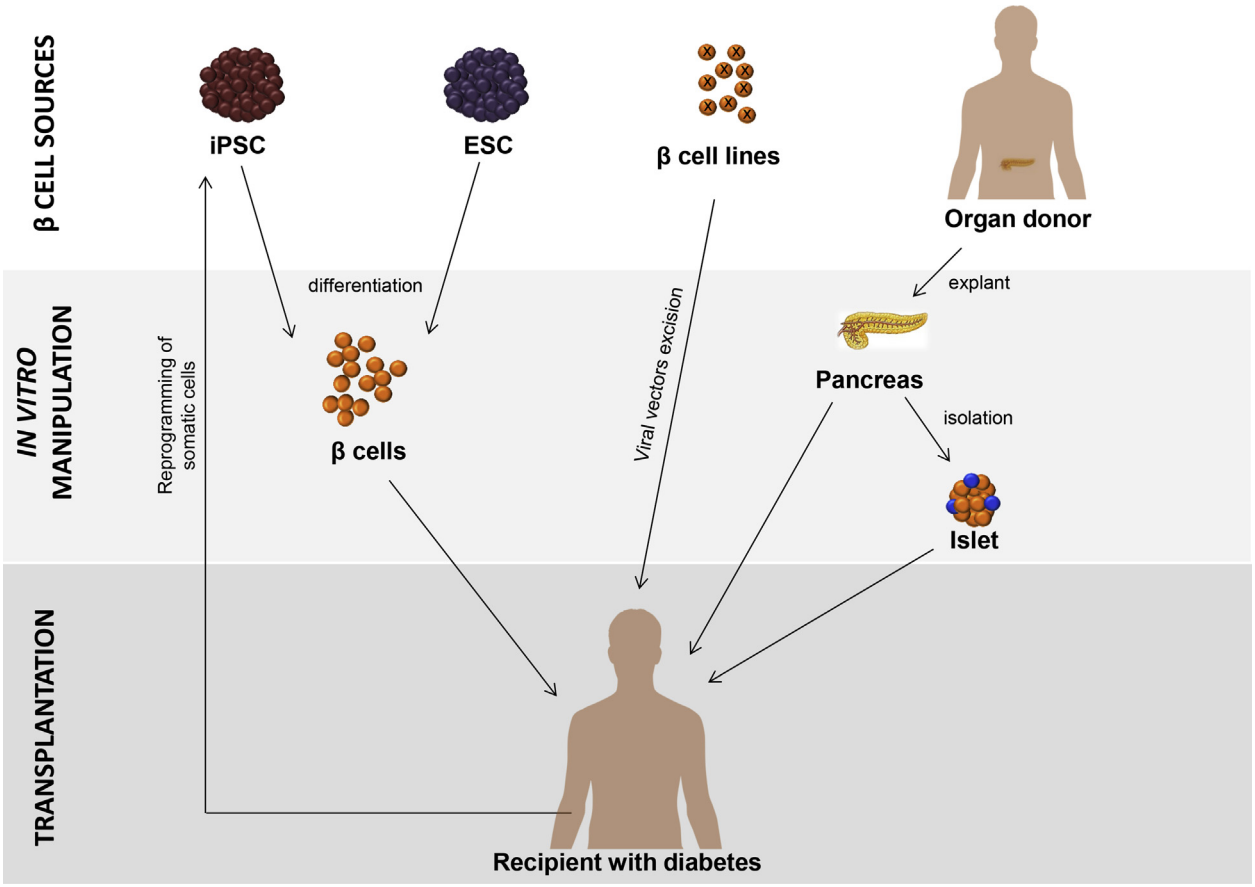

Figure 2 Potential sources of β cell replacement in type 1 diabetes: pluripotent stem cells, immortalized β cell lines and pancreatic islets from cadaveric donor. iPSC = induced Pluripotent Stem Cells, ESC = Embryonic Stem Cells.

floxed immortalizing transgenes and an integrated tamoxifen (TAM)-inducible form of CRE recombinase has been created [20]; such lines can be massively amplified and then have the immortalizing transgenes removed by simple addition of TAM, giving rise to non-proliferating functional human β-cells. These newly produced cells potentially represent a preclinical model for cell replacement therapy in diabetes, but further studies are required to determine their actual safety (see Fig. 2).

β cell neogenesis. Another completely different point of view is the theory that neogenesis is the mechanism responsible for β cell mass expansion in conditions like pregnancy or obesity. An autopsy study on human pancreata during or after pregnancy supports this hypothesis: Butler et al. observed the presence of more new small islets rather than an increase in β cell replication, islet size or change in apoptosis [21] (Butler et al., 2010). They also observed an increased number of insulin positive cells within ducts, indicating that duct cells can differentiate in β cells in certain conditions or that pancreatic stem/progenitor cells are localized in pancreatic ducts. Experiments of 90% pancreatectomy in rats show the substantial regenerative capacity of the adult pancreas [22] and in a recent work it was demonstrated that this regeneration follows a dedifferentiation–redifferentiation paradigm, in which mature duct cells dedifferentiate to a progenitor-like state and then differentiate to form all pancreatic cell types, including β cells [23]. Also in this work an increased proliferation rate of the remaining β cells was observed, indicating that replication and neogenesis are not mutually exclusive and they both contribute to maintain an adequate β cell mass after birth, but there are important differences in the balance of these two pathways depending on species and age [24].

Transdifferentiation into β cell. The potential of α cells as possible source of insulin-producing cells has also been explored, since these cells are preserved in diabetic patients [25] and are the most abundant endocrine cells in islets other than β cells. Collombat et al. [26] have shown that the ectopic expression of Pax4 could force mature α cell conversion to β cells, reversing chemically-induced diabetes in mice. In addition, Thorel et al. [27] confirmed the differentiation potential of α cells reporting their spontaneous conversion to new functional β cells using a selective diphtheria toxin-mediated β cells ablation model. Recently it was shown that GABA and the antimalarial drug artemether, which act on GABAergic pathways, can drive pancreatic cells with an α-cell phenotype toward a β-cell-like phenotype. As reported in two papers [28,29], these drugs can stimulate the production of sufficient numbers of new β-like cells to reverse severe diabetes in mice. These data suggest a therapeutic potential of GABA pathways to restore the β cell mass in diabetes.

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Stem cells to generate β cells: pluripotent stem cells

Several publications have reported the ability to differentiate or transdifferentiate into insulin producing cells of stem cells of different origin or precursors isolated from pancreas or other tissues. For many of these approaches, the results have not been confirmed in other laboratories or in clinical studies and are therefore controversial. At the moment the only consistent results in terms of quantity and quality are those achieved with the use of pluripotent stem cells (embryonic stem cells or pluripotent stem cells obtained by the reprogramming of somatic cells) (see Fig. 2). As short-term clinical perspectives, the most advanced approach (as clinical phase 1–2 study already started) refers to the possibility of using pancreatic progenitor cells derived from pluripotent stem cells implanted subcutaneously within a macro-device where cells can differentiate *in vivo* into insulin-producing cells [30–33]. The "product" in question is called VC-01™ [34] and consists of pancreatic progenitor cells (referred to as PEC-01™), derived from an embryonic stem cell line [35] encapsulated in a macro-device called Encaptra™. The US Food and Drug Administration ("FDA") has approved the Investigational New Drug Application ("IND") for the use of VC-01™ in the treatment of T1D in August 2014. VC-01™ was developed by ViaCyte, a Californian company supported by both the California Institute for Regenerative Medicine (CIRM) and the Juvenile Diabetes Research Foundation (JDRF). The clinical study (called STEP ONE, "A Safety, Tolerability, and Efficacy Study of VC-01™ Combination Product in Subjects With Type One Diabetes Mellitus; NCT 02239354, ClinicalTrials.gov) is a prospective, multicenter open-label study which provides the VC-01™ system to patients with T1D in the absence of immunosuppression, since the macro-device should be able to protect cells from the immune response. The study involves the recruitment of forty subjects and the first patient has been transplanted on October 29, 2014. At the moment there is no information on the preliminary results. The study will soon be replicated in Canada, at the University of Alberta. In the short-to-medium term it is likely that similar approaches will also be developed by other research groups, since in recent years at least two other protocols to differentiate insulin-producing cells with a seven steps protocol with high efficiency have been described. In fact, researchers at βLogics Venture, a subsidiary of Johnson & Johnson, in collaboration with the University of British Columbia, developed a highly efficient differentiation protocol able to generate mature insulin-secreting cells *in vitro* starting from pluripotent stem cells [36–41]. Similarly, the Harvard Stem Cell Institute described a third protocol to generate *in vitro* insulin secreting mature cells from pluripotent stem cells with high efficiency [42].

Stem cells as feeder or immunomodulatory cells for the treatment of diabetes

In recent years, the well-established clinical experience in the field of hematology has encouraged the use of stem cells derived from bone marrow (BM) or cord blood in non-hematological diseases. Several clinical studies have been initiated for the treatment of type 1 and type 2 diabetes, involving hematopoietic stem cells and stromal/mesenchymal stem cells (MSC) derived from BM and cord blood (or from the extra-embryonic annexes), thanks to the availability of simple protocols for the collection, expansion and storage of these stem cells. Many groups have studied their potential role in the induction and/or restoration of tolerance and in the remodeling of pancreatic tissue as "feeder" cells while their direct differentiation into insulin-producing cells turned out to be less and less likely (Fig. 3).

Intra-pancreatic infusion of autologous BM

In the past the possibility for BM cells to differentiate into cells capable of producing insulin in response to glucose was suggested [43–45] but such results are extremely controversial and have not been confirmed by other studies [46–48]. More recently it was assumed that BM cells could have a different role, thanks to the evidence that, in some models of BM transplantation, cells can initiate regeneration of the endocrine pancreas by stimulating both β cell proliferation and islet neogenesis [49,50]. Based on these assumptions, some clinical trials for treatment of diabetes have been conducted with unpurified mononuclear cells derived from autologous BM infused intra-arterially in the pancreas (see Table 1). Among these experiences there are those of the hospitals in India [51,52], Argentina [53], China [54,55] and Spain [56]. In relation to T1D, the clinical study conducted at the University Hospital Clinic of Barcelona has reported no effects on the serum levels of C-peptide (basal and stimulated), no changes in insulin requirements or metabolic control in the treated patients. Due to lack of efficacy the study, initially designed to recruit ten subjects, was stopped after the third patient. In relation to type 2 diabetes (T2D), published results are difficult to interpret. Twenty-five patients with T2D received autologous mononuclear BM cells injected through the dorsal artery of the pancreas in combination with hyperbaric oxygen treatment in Buenos Aires [53]: all metabolic parameters measured (blood glucose and fasting c-peptide, HbAlc, insulin) were better than the baseline in the first year of follow-up. Improved glycemic control and decreased insulin requirements or use of oral hypoglycemic agents have also been reported in 31 patients with T2D recruited in China [54] and treated in a similar manner. Moreover, Hu et al. [55] have described the effectiveness at 3 years of the administration of autologous mononuclear BM cells, in comparison with the conventional therapy, in 118 patients with T2D, reporting a significant improvement of the glucose control and a decrease in insulin requirements or in the use of oral hypoglycemic drugs in transplanted patients. A similar experience was also described in India. Here a study was started involving the use of hematopoietic stem cells and, as a site of injection, of the pancreaticoduodenal artery, that vascularizes preferentially

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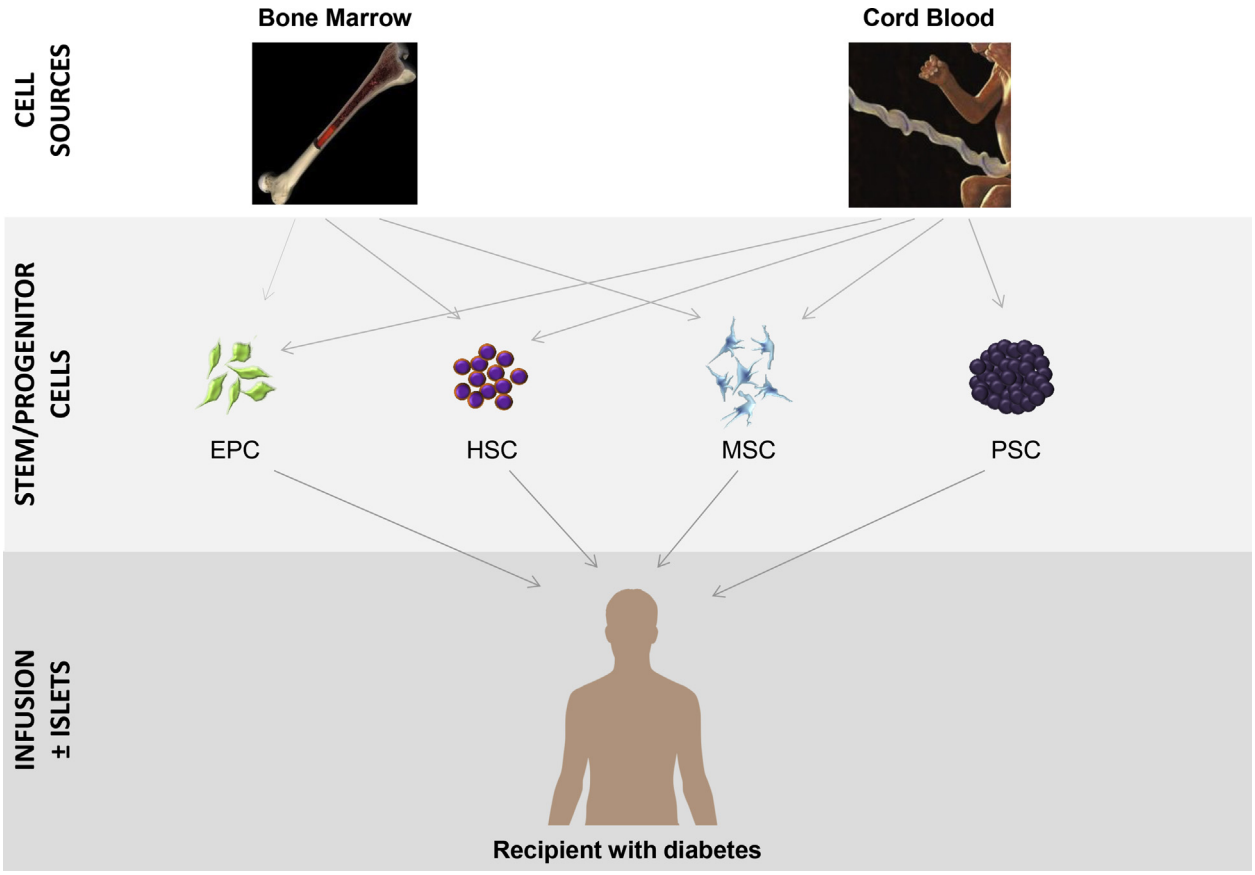

Figure 3 Potential sources of stem cells for immunomodulation and tissue remodeling, deriving from bone marrow and cord blood. EPC = Endothelial Progenitor Cells, HSC = Hematopoietic Stem Cells, MSC = Mesenchymal Stem Cells, PSC = Pluripotent Stem Cells.

the head of the pancreas and part of the body. Six out of ten treated patients showed a significant reduction in the need for insulin from baseline (74%, with a patient who achieved and maintained insulin independence for fifteen months) [51,52] (see Table 1).

In general the results of these studies are difficult to interpret: the experimental design is often inadequate and studies lack a control arm, have a high percentage of drop-outs and heterogeneous populations with different hypoglycemic treatments and poor glycemic control at baseline. Even when a control group was included in the experimental design [54] the study was not randomized, and indeed the opportunity to choose the treatment arm has been left to patients. A benefit, usually transient, is generally reported, but it is unclear whether the effect is induced by the best treatment due to the entry in the clinical trial or to real benefits determined by the infusion of BM cells. In conclusion, at the present time there is no clear evidence to support the use of intra-pancreatic autologous BM cell infusion. According to the Reflection paper on classification (http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2015/06/WC500187744.pdf) of the Committee for Advanced Therapies (CAT) of European Medicines Agency (EMA), a product whose active substance is made up of BM mononuclear cells infused in the pancreas intra-arterially with the aim to restore or modify plasma insulin levels, and thus treat diabetes, has to be classified as advanced therapy medicinal product (ATMP). Such a medicinal product must then be considered an investigational medicinal product and should therefore be offered to patients only within controlled clinical trials, adequately assessed by the ethics committee and the competent regulatory authorities.

Transplantation of BM hematopoietic stem cells

Hematopoietic stem cell (HSC) transplantation is now widely recognized as a curative therapy for many hematologic diseases. Over the past two decades, the transplantation of autologous HSC has also been studied as a treatment option for patients with severe autoimmune diseases considered refractory to conventional therapy [57]. The rationale behind these studies lies in the conviction that it is possible to replace the defective immune system, which recognizes the self-proteins as antigens, with a healthy immune system, regenerated starting from autologous HSC in the absence of the accidental environmental circumstances that led to the development of the autoimmune response. In clinical routine, the recipients of HSC transplantation are subjected to a powerful immunosuppressive therapy after HSC mobilization from the BM to the peripheral blood using different protocols

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Table 1 Studies on transplantation of autologous bone marrow mononuclear cells for the treatment of diabetes present in ClinicalTrial.gov.

ClinicalTrial.gov	Place	Cells	Infusion site	Diabetes	Status	Ref
NCT00821899	Hospital Clinic Universitari, Barcelona, Spain	BM mononuclear cells	Intrapancreatic, intraarterial	Type 1	Completed	[56]
NCT00644241 NCT01065298	Postgraduate Institute of Medical Education and Research, Pgimer, Chandigarh, India	BM mononuclear cells	Intrapancreatic, intraarterial	Type 2	Unknown	[51,52]
NCT00767260	Fuzhou General Hospital Fuzhou, Fujian, China	BM mononuclear cells + hyperbaric therapy	Intrapancreatic, intraarterial	Type 2	Active, not recruiting	–
NCT01677013	Peking University Aerospace Centre Hospital, Beijing, China	BM mononuclear cells + hyperbaric therapy	Intrapancreatic, intraarterial	Type 2	Active, recruiting	–
NCT00465478	Qilu Hospital of Shandong University, China	BM mononuclear cells	Intrapancreatic, intraarterial	Type 1 and 2	Unknown	–
NCT00971503	Pontificia Universidad Catolica de Chile, Santiago de Chile, Chile	Total BM	Intrapancreatic, intraarterial	Type 1	Suspended	–
NCT01143168 NCT01142050	Armed Police General Hospital, P. R. Beijing, China	BM mononuclear cells + cord blood	Intrapancreatic, intraarterial, intravenous systemic	Type 1 and 2	Unknown	–
NCT01832441	Chaitanya Hospital, Pune, Maharashtra, India	BM mononuclear cells	Not clearly stated	?	Active, recruiting	–
NCT01786707	University of Miami, USA	BM mononuclear cells + hyperbaric therapy	Intrapancreatic, intraarterial	Type 2	Completed	

typically based on the use of Granulocyte-Colony Stimulating Factor (G-CSF) and/or cyclophosphamide. Despite the well-documented clinical success of autologous HSC transplantation in correcting certain autoimmune diseases [58], an accurate explanation of the mechanisms of action of this treatment is still lacking. Clearly, transplantation is accompanied by a large debulking of the recipient's immune system with powerful immunosuppressive conditioning protocols such as total body irradiation (TBI), cyclophosphamide, the use of depleting monoclonal antibodies (anti-CD2, anti-CD52), fludarabine or anti-thymocyte globulin (ATG); these treatments determine a strong lymphopenia of long duration associated with reduced levels of plasma cells capable of producing autoantibodies [59] and the use of such lympho-ablative therapies, even in the absence of hematopoietic cell transplantation, is able to stop or slow the progression of autoimmune diseases [60]. Associated with the effect of non-specific immunosuppressive induction protocols, it has been also demonstrated that the transplant of HSC can restore immune tolerance by modulating regulatory T cells and reactivating thymic function [61–63]. Unfortunately, due to the persistence of autoreactive immune cells (T cells and B memory, long-lived plasma cells), autoimmunity may recur after an autologous stem cell infusion and further studies are needed to identify the optimal induction protocols to achieve stable and lasting remission of autoimmunity. Allogeneic HSC transplantation is potentially more effective in preventing the recurrence of autoimmunity. In fact, a moderate conditioning not involving the complete ablation of autologous HSC, associated with an allogeneic hematopoietic cell transplantation, is able to induce a condition called "mixed hematopoietic chimerism" in which hematopoietic cells of donor and recipient coexist to form a mixed immune system. In this condition, T lymphocytes with high affinity for the autologous proteins can be eliminated by ensuring the development of a new tolerance towards the self [64,65]. Being T1D an autoimmune disease, the possibility of using transplantation of HSC has been evaluated in recent years. The use of allogeneic BM transplantation to prevent the development of T1D has been proposed for the first time in 1985 in the NOD mouse model [66] and more recently the transplantation of allogeneic stem cells and the development of "mixed hematopoietic chimerism" have received a lot of attention for the treatment of T1D. Numerous preclinical studies have demonstrated the efficacy of allogeneic HSC transplantation both in prevention and in remission of T1D [67–69] but, despite preclinical results, autologous transplantation was preferred compared to allogeneic transplantation in early clinical trials, given the lower risk of severe toxicity. The first attempt to evaluate the safety and efficacy of a non-myeloablative immunosuppressive regimen followed by autologous HSC transplant in patients at onset of T1D has been proposed by Voltarelli et al. in Brazil [70,71] (ClinicalTrials.gov Identifier: NCT00315133). In this Phase I/II study, 23 patients aged between 13 and 31 years with T1D onset within the past six weeks have been subjected to the mobilization of HSC (and later recovery and cryopreservation) with G-CSF and cyclophosphamide. Before the re-infusion of autologous HSC, patients received an immunosuppressive conditioning therapy with ATG and cyclophosphamide. In the following months (mean follow-up 29.8) 20 out of 23 patients had remission of the disease and acquired insulin independence. Twelve of these 20 have maintained a state of insulin independence at last follow-up (mean 31 months, range 15–52 months), while

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8 had a recurrence of diabetes requiring insulin therapy, even at low doses (0.1–0.3 IU/kg). It has not been reported mortality associated with medication, although two patients developed bilateral nosocomial pneumonia, 3 patients late endocrine dysfunction, and 9 oligospermia. In 2009–2011 similar findings were replicated in 8 patients treated in Poland using the same protocol [72,73]. All patients achieved insulin independence with a significant improvement in metabolic control (HbA1c from 12.3% to 6.2% at 6 months after autologous transplantation) and one patient presented recurrence of diabetes at 7 months. Li et al. [74] reported the results obtained in 13 patients with the same mobilization and conditioning scheme, extending the indications within 12 months from onset of diabetes: 1) in 11 patients out of 13 there has been a significant reduction in insulin requirement, 2) in 3 of 11 patients insulin independence was achieved in the 3 months following stem cell transplantation and it was maintained for 7 months, more than 3 and more than 4 years respectively, 3) normal HbA1c levels were maintained in 7 of the 8 patients who achieved a mean follow-up of 2 years. Furthermore, the same group published a case report in which it is shown that insulin independence can be achieved even in patients who have had an onset of diabetes with ketoacidosis, a condition that had been excluded from previous trials [75]. In spite of these results, Gu et al. have shown in a prospective phase 2 study that autologous HSC may be more effective if the population didn't have ketoacidosis at onset [76,77]. More recently, however, less encouraging data about the effectiveness of this approach have been published: a Chinese team reported the results of a study designed to evaluate the safety and efficacy of autologous HSC infusion in comparison with conventional insulin therapy in 42 children (aged 1.5–12.5 years) at the onset of T1D: 14 patients underwent transplantation within 3 months from onset while 28 control patients were treated with insulin therapy in the same period. The reported results of follow-up (3–5 years) showed that auto-transplantation determined: 1) insulin independence in 3 of 14 patients for 2, 3 and 11 months, respectively, 2) the absence of episodes of ketoacidosis, 3) no significant differences in insulin requirements and C-peptide values, 4) HbA1c significantly higher than controls. The conclusion of the study is that there is no real benefit in favor of autologous HSC transplantation [78]. Summarizing these experiences [79]: in total about sixty-five patients were treated, 59% achieved insulin independence within 6 months after transplantation, and 32% have maintained it at the last follow-up. Also in all the patients a decrease in HbA1c and an increase in C peptide values were reported. In spite of the promising results, 52% of patients experienced adverse events and one death has been reported, highlighting the difficulty to justify a treatment so potentially dangerous at diabetes onset. Some clinical studies based on this approach are still active or pending longer follow-up (NCT01121029, NCT01285934) and, when published, will help to complete the picture. At the moment it is difficult to express a final judgment. Also in this case it is necessary to emphasize that, in spite of the numerous clinical experiences, the majority of the studies did not include a group of randomized control with traditional insulin therapy or with only immunosuppression, and when it was done, it is not evident if a real benefit with the autologous transplantation of HSC was obtained [78]. Only the long-term monitoring of patients treated so far can help to clarify the risk/benefit ratio of this approach in T1D therapy. It is difficult to imagine that this will be a justifiable approach in diabetes, considering morbidity and mortality associated to autologous hematopoietic stem cell transplant in the field of autoimmune diseases [80–82] (see Table 2).

Transplantation of BM stromal/mesenchymal stem cells (MSC)

MSC are another cellular component of the BM and are essential for maintaining the niche of hematopoietic stem cells. MSC have been the subject of extensive research for decades. More than thirty thousand scientific papers on these cells have been published in peer reviewed journals describing their ability to differentiate into multiple cell lineages, to support hematopoiesis, to exert immune regulation and to secrete growth factors and cytokines. This field of study has grown especially in the last 20 years with the discovery of new functionality of these cells [83–85]. In fact, early MSC were isolated from BM and classified as multipotent stem cells only for the mesenchymal lineage (bone, fat, cartilage), in a second period instead these cells began to be isolated from virtually all postnatal tissues (adipose tissue, Wharton's jelly, dental pulp, pancreas, amniotic fluid, liver) and their ability to differentiate *in vitro* also along the ectodermal and endodermal lineage was reported. In a third and final phase, the interest in MSC has moved from their plasticity to their ability to modulate tissue function; a large number of studies have in fact shown that these cells have immunomodulatory and "feeder" cell functions that are exerted both by direct cell–cell contact or by secretion of cytokines and/or other soluble factors [86]. The assumption that they can contribute to the regeneration of tissues by modulating inflammation has opened a new interest in their use as a therapeutic tool to suppress inflammation and inhibit immune responses in graft versus host disease (GVHD), in Crohn's disease and in autoimmune diseases such as diabetes, multiple sclerosis, rheumatoid arthritis and, as recently demonstrated, under extremely severe conditions such as Acute Respiratory Distress Syndrome or ARDS [87]. The immunomodulatory and anti-inflammatory properties are not always constitutively expressed by MSC, but are rapidly induced by inflammatory cytokines such as IFN-γ and TNF-α, a process that occurs both *in vitro* and *in vivo*, and configures as a requirement for their therapeutic efficacy [88] (see Table 3).

In relation to the immunomodulatory properties and potential use of MSC in clinical protocols some key elements have now been defined, as well reviewed recently by Wang and [86]. In short:

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Table 2 Studies on transplantation of autologous hematopoietic stem cells for the treatment of type 1 diabetes present in ClinicalTrial.gov.

ClinicalTrial.gov	Place	Mobilization	Conditioning	Diabetes	Status	Ref
NCT00315133	University of São Paulo, School of Medicine of Ribeirão Preto, Brasil	cyclophosphamide (2.0 g/m^2), G-CSF (10 μg/kg/die)	cyclophosphamide (200 mg/kg), ATG (4.5 mg/kg).	<6 weeks from onset	Unknown	[70,71]
NCT01341899	Hospital of Nanjing University, Jiangsu, China	cyclophosphamide (2.0 g/m^2), G-CSF (10 μg/kg per day)	cyclophosphamide (200 mg/kg), ATG (4.5 mg/kg).	<12 weeks from onset	Active, recruiting	[74,75]
NCT01121029	Hospital Universitario Dr. José Eleuterio González; Monterrey, Nuevo Leon, Mexico	cyclophosphamide (1.5 g/m^2), G-CSF (10 μg/kg per day)	cyclophosphamide (500 mg/kg); fludarabine (30 mg/m^2)	<4 weeks from onset	Completed	–
NCT00807651	Shanghai Jiao Tong University School of Medicine, Shanghai, China	cyclophosphamide (2.0 g/m^2), G-CSF (10 μg/kg per day)	cyclophosphamide (200 mg/kg), ATG (4.5 mg/kg).	<6 months from onset	Active, not recruiting	[76,77]
NCT01285934	Northwestern University, Chicago, Illinois, United States	cyclophosphamide (2.0 g/m^2), G-CSF (10 μg/kg per day)	cyclophosphamide (200 mg/kg), ATG (4.5 mg/kg), rituxan (500 mg)	<5 months from onset	Active, recruiting	–

Table 3 Studies on transplantation of mesenchymal stromal/stem cells for the treatment of diabetes present in ClinicalTrial.gov.

ClinicalTrial.gov	Place	Mechanism	Cells	Infusion site	Diabetes	Status	Ref
NCT01068951	Uppsala University Hospital, Sweden	Immunomodulation	Autologous, from BM	Systemic infusion	Type 1, onset	Completed	[113]
NCT02057211	Uppsala University Hospital, Sweden	Immunomodulation	Autologous, from BM	Systemic infusion	Type 1, onset	Recruited	[113]
NCT00690066	Mesoblast International Srl in partnership con JDRF	Immunomodulation	Mesenchymal stem cell line, from BM (Prochymal)	Systemic infusion	Type 1, onset	Completed	–
NCT01322789	University of São Paulo, School of Medicine of Ribeirão Preto, Brasile	Immunomodulation	Allogeneic, from BM of first-degree relative	Systemic infusion	Type 1, onset	Recruiting	–
NCT01219465	Qingdao University, Qingdao, Cina	Immunomodulation	Alogeneic, from umbilical cord blood	Systemic infusion	Type 1, onset	Unknown	–
NCT01157403	Hospital of the Third Military Medical University, Chongqing, China	Immunomodulation	Autologous, from BM	Systemic infusion	Type 1, onset	Unknown	–
NCT01374854	Fuzhou General Hospital Fuzhou, Fujian, China	Tissue repair	Alogeneic, from umbilical cord blood	Intrapancreatic, intraarterial	Type 1	Unknown	–
NCT01496339	First Affiliated Hospital of Zhejiang University, Hangzhou, Zhejiang, China	Tissue repair	Allogeneic, from menstrual blood	Intrapancreatic, intraarterial	Type 1	Unknown	–
NCT02302599	Chinese PLA General Hospital, Beijing, Beijing, China	Tissue repair	Alogeneic, from umbilical cord blood	Systemic infusion	Type 2	Recruiting	–
NCT01759823	Postgraduate Institute of Medical Education and Research, Pgimer, Chandigarh, India	Tissue repair	Autologous, from BM	Intrapancreatic, intraarterial	Type 2	Recruiting	–
NCT01576328	Mesoblast International Srl	Tissue repair	Mesenchymal stem cell line, from BM (Prochymal)	Systemic infusion	Type 2	Active, not recruiting	–
NCT01954147	Diabetes Care Center of Nanjing Military Command, Fuzhou, Fujian, China	Tissue repair	Alogeneic, from umbilical cord blood + liraglutide	Systemic infusion	Type 2	Active, not recruiting	–
NCT01453751	Ageless Institute, Miami, Florida, United States	Tissue repair	Autologous, from adipose tissue	Intrapancreatic, intraarterial systemic intravenous	Type 2	Recruiting	–
NCT01413035	Department of Hematology of the 2nd Hospital of Shandong University Jinan, Shandong, China	Tissue repair	Allogeneic, from umbilical cord blood and placenta	Systemic infusion	Type 2	Unknown	–

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- MSC, when injected intravenously, remain mostly trapped in the lungs, and another significant part is the subject of an immune system attack (instant blood-mediated inflammatory reaction (IBMIR) [89,90] but some cells, in case of tissue damage, are able to migrate into the site of injury and participate in the process of repair [91].
- The percentage of MSC that persist in the homing site is low and the permanence is generally of short duration, suggesting a "hit-and-run" effect on damaged tissue [85].
- In response to inflammatory mediators, MSC can produce a large number of soluble factors (cytokines, chemokines, growth factors) capable of regulating inflammation and tissue remodeling. Among the factors that have been described there are: TNF-α, IL-1, IL-6, IFN-γ, TGF-β, HGF, EGF, IGF, FGF, PDGF, KGF, angiopoietin-1, PGE2, VEGF, SDF-1, IDO, NO and iNOS [92].
- MSC have the ability to modulate the immune response either as suppressors or amplifiers, depending on the type and intensity of the signals that they receive from the microenvironment [93]. Once activated by inflammatory stimuli, they are able to exert an effect on the immune system cells in both innate and adaptive reaction, and, in particular, they are able to suppress the function of T and B lymphocytes, NK cells, dendritic cells, macrophages and neutrophils [88].
- In the process of tissue repair MSC are also able to exert an action on the endogenous cells of the damaged tissue, for example by protecting them from apoptosis or stimulating their proliferation [94].

However, it remains to be determined what are the functional tests *in vitro* that can best predict the therapeutic efficacy of MSC as immunomodulatory agents, thus functioning as release criteria of the cells to be used in the patient, as well as setting the basis for a comparison of the results of various clinical protocols based on MSC. In this regard, a big effort in scientific societies such as the International Society for Cellular Therapy (ISCT) is devoted to share a platform of functional tests that can quickly lead to guidelines for therapeutic use of MSC in inflammatory and autoimmune diseases [95,96].

Similarly to the general process of knowledge on MSC, also for their application in the field of diabetes we have witnessed a first phase focused on differentiation into insulin-producing cells, with the objective of providing an autologous source of tissue for transplantation, and a second phase, in which the use of MSC has been finalized to tissue remodeling and modulation of the immune response.

Many attempts have been made to differentiate the MSC *in vitro* into cells that produce insulin. Several studies have reported the appearance of insulin mRNA in cell cultures treated with defined combinations of growth factors [97–99]. An example for the many studies made in this area is a published study in which a 18-day differentiation protocol with the use of FGF-β, EGF, activin and β-cellulin was applied [100]. Differentiated cells formed aggregates, some of which are very similar to pancreatic islets, capable of producing C-peptide. The limits of this and of many previously published studies are that, on closer inspection, none of these differentiated cells show the characteristics necessary to be defined as β cells, in particular the secretion of insulin in response to glucose stimuli and the ability to normalize glycemia in diabetic animal models. Furthermore, in a recent study, some aspects of safety were highlighted, because, in experiences in which MSC have been forcedly converted into another type of cell, the differentiated cells obtained were able to decrease glycemia in diabetic mice but also had a tumorigenic potential [101]. So far, though aware that the risk of neoplastic transformation may be even greater, the most convincing data on the reprogramming of MSC into functional β cells derive from studies that use genetic modification. This approach is based primarily on the forced expression of transcription factors involved in the embryonic development of the pancreas such as Pdx1 and/or Ngn3 [102–107], but this strategy must be improved in order to increase its effectiveness before it can generate a good candidate for β cell replacement in clinical applications, although it is clear that the risk of tumorigenesis strongly limits this approach.

The ability of MSC to modulate the immune response and favor tissue repair has been tested and validated in several preclinical models of diabetes [108–111]. The experiments *in vitro* and in animal models, together with the growing number of data with regard to the clinical applications of MSC in other diseases [112], have led to the development of clinical trials in the field of diabetes. Among these clinical studies, to date only one has been completed and the data have been published [113]. This study (ClinicalTrials.gov Identifier: NCT01068951) was performed at the University of Uppsala (Sweden) and was aimed to evaluate the safety and efficacy of the administration of autologous MSC derived from BM in patients with recent onset of T1D. The starting hypothesis is that an increase in the number of circulating MSC would provide immunomodulation, and then would be able to interrupt the immune process that causes β cell death in T1D. Twenty patients were randomized to cell infusion or to the control group. The safety of the treatment has been demonstrated, since treatment with autologous MSC was well tolerated and no side effects were observed. The primary efficacy endpoint was centered, as was shown by improved secretory response of C-peptide to a mixed meal test during the first year post treatment in patients treated with MSC compared to controls. These encouraging results have led to a larger, randomized, double-blind study, with a longer follow-up, to validate the obtained results. This new study (ClinicalTrials.gov Identifier: NCT02057211) is ongoing, even if participant recruitment has recently been suspended for updated regulations of cell culture

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procedure. Another important clinical study was carried out by Mesoblast International Srl, in collaboration with JDRF. This Phase II, multicenter, randomized, double-blind, placebo-controlled trial (ClinicalTrials.gov Identifier: NCT00690066) is designed to test the safety and efficacy of Prochymal®, a product consisting of human MSC derived from BM, in patients with newly diagnosed T1D. The interim evaluation at one year showed that the systemic infusion of Prochymal® is well tolerated and there were no differences in rates of adverse events in the treated and placebo groups. In terms of efficacy, there was no evidence of benefits with regard to preservation of secretory function measured as release of C-peptide under stimulus, although a trend towards fewer hypoglycemic events for patients treated with Prochymal compared to controls was highlighted. This study is now completed and a full analysis of data is expected. Among the other active studies, the only one who apparently is active and recruiting patients is ongoing in Brazil (ClinicalTrials.gov Identifier: NCT01322789) in which the intravenous infusion of autologous MSC obtained from the BM of first-degree relatives is tested in patients with newly diagnosed T1D.

The potential of MSC to counteract hyperglycemia in diabetic animals through the release of trophic factors (able to protect existing β cells, stimulate the generation of endogenous β cells from pancreatic precursors and reduce peripheral resistance to insulin) has prompted research on their use in type 2 and long lasting T1D [114–116]. Many clinical trials have been initiated, and, among these, data from a study about the use of Mesenchymal Precursor Cells (MPC, rexlemestrocel-L) in T2D were recently published [117]. The study was conducted by Mesoblast (ClinicalTrials.gov Identifier: NCT01576328), the same company that has tested Prochymal® in T1D, and it is a randomized, placebo-controlled, dose-escalation study, which aims to evaluate the safety and tolerability of a single intravenous infusion of allogeneic MSC in patients with suboptimal control with metformin. The study demonstrated the safety and feasibility of the infusion of allogeneic mesenchymal precursors derived from adult BM (maximum dose 2×10^6 cells/kg) and also described a modest metabolic improvement in terms of HbA1c associated with the treatment, although the follow up to 12 weeks and the low number of recruited subjects [91] do not allow to draw definitive conclusions. Another interesting experience, still ongoing, is a Chinese study (ClinicalTrials.gov Identifier: NCT01954147), which is experiencing a combination therapy of allogeneic MSC from umbilical cord and liraglutide in patients with T2D. Other studies, based on analogue rationale, provide systemic or intra-pancreatic infusion of MSC obtained from various districts including the umbilical cord, the menstrual blood and the placenta (NCT01759823, NCT01453751, NCT02302599, NCT01759823), but at the moment results have not been published. So at the present time there is no clear evidence to support the use of MSC as a standard therapy for diabetes although some clinical evidences have been encouraging. This procedure should only be proposed in controlled clinical trials and properly evaluated by the Ethics Committees and the competent regulatory authority.

Umbilical cord blood and extra-embryonic annexes

The umbilical cord is another possible source of stem cells with potential of differentiation and immunoregulatory capacity similar to those obtained from BM. In humans, the umbilical cord normally contains two umbilical arteries and a vein, hold within a connective tissue called Wharton's jelly [118]. Umbilical cord blood consists of the blood left in the umbilical cord and placenta after childbirth. After the first use of cord blood in 1988 for the treatment of Fanconi anemia [119], there has been considerable development of its application as a source of cells for the treatment of many hematological diseases [120]. In fact, stem cells derived from cord blood can be easily collected and cryopreserved for years without significant loss of vitality [121,122]. The umbilical cord contains 60–200 ml of blood and its collection allows the isolation of about 10×10^6 cells per ml [123]. Umbilical cord blood is composed of red blood cells, white blood cells, platelets, plasma, and it is also rich in multipotent or pluripotent stem cells that can differentiate into various tissues [124]. Among the isolated stem cells are embryonic stem cells, endothelial progenitor cells, hematopoietic stem cells and MSC [125,126]. Embryonic stem cells from the umbilical cord have recently been described as a population of cells, characterized by very small dimensions, that express the embryonic markers Oct4, Nanog and SSEA-4 and are considered virtually totipotent [127]. The endothelial precursor cells are $CD133^+$ $CD34^+$ $VEGFR2^+$ and are considered as the most promising source of stem cells for integration into vascular structures with the aim of regenerating the blood vessels [128]. MSC are identified as $CD44^+$ $CD73^+$ $CD90^+$ $CD105^+$ cells, with the potential to differentiate into various cell lineages such as chondrogenic, adipogenic and osteogenic. These cells can be easily harvested either from cord blood or from Wharton's jelly [118]. Finally, hematopoietic stem cells are the most solidly known and used. Unlike the hematopoietic stem cells obtained from adult BM, those obtained from umbilical cord blood have numerous benefits, including increased proliferative potential and increased telomere length [125] (see Table 4).

Besides, because of the immunological immaturity of this tissue, in case of unrelated umbilical cord transplant, HLA disparity between donor and recipient is better tolerated and associated with a lower risk of severe acute GVHD [126,129,130]. Hematopoietic cells of the cord blood are now considered the most appropriate cells for transplantation procedures for the treatment of diseases, hematological and not, for patients in whom it is not possible to identify a compatible donor [120,131].

In recent years, the use of cells obtained from the umbilical cord for the modulation of the immune system in autoimmune diseases has acquired great interest [132–135]. In theory, umbilical cord may have a significant role in the treatment of diabetes because of the variety of

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Table 4 Studies on transplantation of cord blood cells for the treatment of diabetes present in ClinicalTrial.gov.

ClinicalTrial.gov	Place	mechanism	cells	Infusion site	Diabetes	status	Ref
NCT00305344	University of Florida, Gainesville, Florida, United States	Immunomodulation	Autologous, from umbilical cord blood	Systemic infusion	Type 1, onset	Completed	[143,144]
NCT00873925	University of Florida, Gainesville, Florida, United States	Immunomodulation	Autologous, from umbilical cord blood + vitamin D3 and Omega 3FA	Systemic infusion	Type 1, onset	Completed	[140]
NCT00989547	Technische Universität München	Immunomodulation	Autologous, from umbilical cord blood	Systemic infusion	Type 1, onset	Unknown	[145]
NCT01996228	The Second Xiangya Hospital, Changsha, Hunan, China,	Immunomodulation	Allogeneic, from umbilical cord blood (Stem Cell Educator therapy)	*Ex vivo* "contact" with autologous lymphocytes	Type 1	Recruiting	[149]
NCT01350219	The Second Xiangya Hospital, Changsha, Hunan, China,	Immunomodulation	Allogeneic, from umbilical cord blood (Stem Cell Educator therapy)	*Ex vivo* "contact" with autologous lymphocytes	Type 1	Recruiting	[149]
NCT01415726	General Hospital of Jinan Military Command, Jinan, Shandong, China	Immunomodulation	Allogeneic, from umbilical cord blood (Stem Cell Educator therapy)	*Ex vivo* "contact" with autologous lymphocytes	Type 2	Completed	[150]
NCT01219465	Qingdao University, Qingdao, Cina	Immunomodulation	Allogeneic, MSC from cord blood	Systemic infusion	Type 1, onset	Unknown	
NCT01954147	Diabetes Care Center of Nanjing Military Command, Fuzhou, Fujian, China	Tissue repair	Allogeneic, MSC from cord blood + liraglutide	Systemic infusion	Type 2	Active, not recruiting	
NCT01143168	Armed Police General Hospital, P. R. Beijing, China	Tissue repair	Allogeneic, MSC from cord blood	Intrapancreatic intraarterial,	Type 1	Unknown	
NCT02302599	Chinese PLA General Hospital, Beijing, Beijing, China	Tissue repair	Allogeneic, MSC from cord blood	Systemic infusion	Type 2	Recruiting	

stem cells available in this tissue; in fact, both the control of autoimmunity through the induction of chimerism and immune tolerance, and the opportunity to overcome the shortage of insulin-producing cells through processes of differentiation could be exploited using cells from the umbilical cord. Some experimental data showed a potential of the cells obtained from the umbilical cord to be transformed into β-like cells, as confirmed by the production of insulin and C-peptide, but their engraftment and survival *in vivo* has not been tested [136,137] or was unsatisfactory for proceeding to hypotheses of clinical use in humans [138,139].

The use of cord blood cells for the treatment of T1D in relation to their immunoregulatory potential seems more promising. Starting from the evidence that cord blood contains a large population of immature T regulatory lymphocytes ($CD4^+$, $CD25^+$, $FoxP3^+$), the possibility to infuse autologous cryopreserved cord blood cells at onset of T1D was explored in a clinical trial [140,141]. In fact, regulatory T cells have the ability to inhibit the inflammatory response and induce anergy in effector T lymphocytes that play a key role in β cell destruction [142]. In a first pilot study, fifteen children (mean age 5.5 years) newly diagnosed with T1D (mean 4.1 months from onset) received an infusion of autologous cord blood cells and metabolic and immunological responses were tracked over the time. At 6 months after infusion an increase in the population of regulatory T cells in peripheral blood, in the absence of significant adverse events, was observed [140,141]. However, one year after the transplant, there were no observed changes in insulin requirements, C-peptide levels, level of autoantibodies or number of regulatory T-lymphocytes, indicating that the procedure is feasible and safe, but did not show effectiveness [143]. The same negative results were seen at the end of the study (2 years of observation), leading to the conclusion that a single infusion of umbilical cord blood in children with T1D is not effective in reverting or treating the disease [144], even when the infusion was followed by one year of supplementation with vitamin D and Omega-3 [140]. One of the reasons for the failure of these studies could be that an insufficient number of cells with regenerative or immunoregulatory capacity was transferred in patients. In support of this hypothesis is another study, performed in seven children with newly diagnosed T1D, which has highlighted, at 6 months after infusion, a correlation between the number of $CD34^+$ hematopoietic cells in the cord blood and the residual β cell function, as assessed by measurement of C-peptide after stimulation [145].

A different approach has been proposed by Zhao et al., who have described *in vitro* and in preclinical models the immunomodulatory effect of umbilical cord derived stem cells on allogeneic T lymphocytes [146,147]. Based on the

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experimental results obtained, this group has developed a strategy called “Stem Cell Educator therapy”, and applied it in a clinical study in humans [148]: 15 subjects (mean age 29 years, range 15–41) with an average history of diabetes of 8 years (range 1–21) were re-infused with autologous lymphocytes derived from peripheral blood “re-educated” through contact with allogeneic umbilical cord stem cells. This therapy has apparently improved C-peptide levels, reduced HbA1c values and decreased insulin daily requirement in patients with or without a residual pancreatic function, leading the authors to conclude that the approach was able to control the immune response sufficiently to allow regeneration of native β cells [149]. The same approach was followed in a second open-label Phase I/II trial in 36 patients with T2D of long duration, showing again that the treated patients achieved a better metabolic control [150]. The efficacy and safety of this approach is currently being tested in a phase I/II clinical trial in children with T1D (ClinicalTrials.gov Identifier: NCT01996228, NCT01350219). Very recently a new clinical trial, based on the use of umbilical cord blood for the prevention of T1D, has started. In this world first clinical trial (CoRD Study, U1111-1139-5067), Australian researchers are investigating the potential to prevent or delay the onset of T1D in high risk autoantibody-positive children using autologous cord blood. The first cell infusion was performed in January 2017 and follow up will last for 3 years.

In conclusion, the use of autologous cord blood as a source of immunomodulatory cells for T1D therapy was ineffective or still has to be proven. Other approaches that use allogeneic cord blood cells are currently being tested and should be assessed with great attention in terms of efficacy before they can be applied to a greater number of patients.

Storage of stem cells derived from cord blood and extraembryonic annexes for the therapy of diabetes

One of the recurring questions in clinical practice, most frequently by expectant parents with T1D, is if collection and cryopreservation of cord blood stem cells or other components of extraembryonic annexes can be useful to cure their own diabetes or that of the unborn child in case of development of the disease in the future. At the state of current knowledge there is no clinical application of cord blood cells for diabetes that justifies its preservation for private use by parents with diabetes. The topic of cord blood, however, deserves a study more in deep in order to better understand its usefulness and limitations.

Why storage of cord blood?

The clinical use of cord blood is related to its content of stem cells, and mainly of hematopoietic stem cells. In fact, the use of hematopoietic stem cells derived from umbilical cord blood is a well-established therapeutic reality for the treatment of patients with various blood disorders, such as leukemia and lymphoma, and non-cancer diseases such as thalassemia, bone marrow aplasia and congenital immune deficiencies in pediatric and adult patients. In Italy the complete list of such diseases is shown in the Annex to the Ministerial Decree of November 18th, 2009 “Disposizioni in materia di conservazione di cellule staminali da sangue del cordone ombelicale per uso autologo-dedicato”, updated in 2014.

The situation in Italy

Is it allowed to store cord blood?

In Italy, the current legislation allows the collection and preservation of umbilical cord blood, and the service is offered by the National Health System (NHS):

- in case of donation for allogeneic use to charitable purposes;
- dedicated to baby with a disease occurring at the time of the birth or highlighted in the prenatal period, or for use in dedicated consanguineous with a disease present at the time of collection, in case this disease is treatable with hematopoietic stem cell transplantation;
- dedicated to families at risk of having children with defined genetic diseases for which there is proven evidence of use of stem cells derived from umbilical cord blood;
- dedicated to autologous-use in clinical trials, approved under current regulations, aimed at gathering evidence of a possible use of cord blood in case of particular diseases;

while the legislation prohibits:

- conservation for exclusive autologous use, in the absence of the above conditions;
- the establishment of private banks in the national territory;
- all forms of advertising related to private banks.

It is however allowed the collection of umbilical cord blood for personal use and its export in private facilities outside the Italian territory according to the rules defined by a specific legislative act. For more information, consult the document “Normativa in tema di conservazione e donazione del sangue cordonale” which gives full information about cord blood donation and storage in Italy.

Where can I donate cord blood?

On national territory, umbilical cord blood is stored in public facilities (Umbilical Cord Blood Banks) and it remains available to transplant centers in case of need. The list of Umbilical Cord Blood Banks is public and available at the following link: http://www.centronazionalesangue.it/pagine/rete-banche-sangue-cordonale. The Italian National Blood Centre with the National Transplant Center work to ensure safety and reliability of the units preserved to protect the health of the giver and of the receiver

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(http://www.centronazionalesangue.it/pagine/sangue-cordonale#sthash .lkRERMt6.dpuf).

Why the preservation of cord blood for autologous use only is not allowed?

Cord blood storage for autologous use is not permitted in Italy, because currently there is no scientific evidence regarding its use for personal purposes outside the cases indicated by current regulations. For more information, see the Position Paper "Appropriate use of stem cells" and the Position Statement "Collection and storage of cord blood in Italy" from the Ministry of Health. Even ADISCO (Italian Umbilical Cord Blood Donors Association) has produced a Position Statement about the collection and storage of cord blood in Italy.

Are there private cell banks for the conservation of umbilical cord blood?

In Italy the establishment of private banks for the storage of umbilical cord blood is not allowed, but there is a network of "brokers" who organize the pickup, transport and delivery service of the cord blood from Italy to a bank abroad.

Are there private banks for the conservation of umbilical cord blood for the treatment of diabetes?

Browsing the web we identified 32 private bank websites that promote the preservation of umbilical cord blood in Italy for "personal" use. These companies have their registered offices in the United States, in San Marino, in the UK, Slovakia, Belgium, Switzerland, Poland, Germany and Greece, while the physical locations where stem cells are preserved are scattered around the world. The average cost for the collection and cryopreservation of cord blood cells for about 20 years is of 2370 € (with a range between 1570 and 3100 Euros). A review of the information provided on the websites on the benefits of cryopreservation of cord blood cells reveals a confusing and potentially misleading information model. All private banks publish a list of diseases that "could be treated" with the umbilical cord blood transplantation, including tumors, bone marrow deficiencies and genetic disorders. Most of these diseases are treatable only with an allogeneic umbilical cord cell transplant, but many commercial banks do not explain the difference between autologous and allogeneic transplant with sufficient clarity, implying that the indications for allogeneic also apply to autologous transplants. Most commercial banks also list several conditions that could be treated in the future with cellular therapies which are currently at an early stage of research. Twenty-eight of the 32 banks considered report on their websites the usefulness of cryopreservation of cord blood stem cells for the cure of diabetes. In most cases, diabetes appears as one of the diseases that could be cured in the future and for which there are ongoing clinical trials to determine its effectiveness. In some cases, the indication of a potential use of the stored stem cells in the field of diabetes is linked to the description of clinical trials (often with reference to the NIH website clinicaltrials.gov), or to the publication of a list of scientific articles in which the potential of hematopoietic stem cells in diabetes has been tested or, finally, to expert testimonials. Some banks report direct experience of transplantation of stem cells cryopreserved by individuals with type 1 or type 2 diabetes, without specific references to registered clinical trials or scientific publications.

Conclusion

The evolution of regenerative medicine and the study of stem cell biology is opening innovative scenarios even in the therapeutic field. Despite this, the treatments covered in this document cannot be considered clinical standards and therefore should only be carried out within clinical studies approved by ethics committees and by competent regulatory authorities. In order to better inform patients, the International Society for Stem Cell Research has compiled the online guide for patients about participation to clinical trials with cell therapy, translated into many languages, that can be found at this address: http://www.closerlookatstemcells.org/patient-resources.

Appendix 1. Scientific societies providing information about bone marrow and cord blood cell donation.

International references

- European Group for Blood and Marrow Transplantation (EBMT; https://www.ebmt.org/Contents/Pages/Default.aspx)
- International Society for Cell Therapy (ISCT; http://www.celltherapysociety.org/)
- International Bone Marrow Transplant Registry (IBMTR; http://www.cibmtr.org/pages/index.aspx)
- Joint Accreditation Committee of ISHAGE and EBMT (JACIE; http://www.jacie.org/)
- Bone Marrow Donor Worldwide (BMDW; http://www.bmdw.org/)
- World Marrow Donor Association (WMDA; https://www.wmda.info/)
- International Society of Blood Transfusion (ISBT; http://www.isbtweb.org/)
- International NetCord Foundation (NETCORD; http://www.netcord.org/)

Italian references:

- Gruppo Italiano Trapianto Midollo Osseo (GITMO; http://www.gitmo.it/),
- Italian Bone Marrow Donor Registry (IBMDR; http://ibmdr.galliera.it/presentazione)
- Associazione Donatori Midollo Osseo (ADMO; http://www.admo.it/)
- Società Italiana di Ematologia (SIE; http://www.siematologia.it/)

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- Associazione Italiana diOncoematologia Pediatrica (AIEOP; http://www.aieop.org/web/index.php)
- Società Italiana di Medicina Trasfusionale e di Immunoematologia (SIMTI; http://www.simti.it/)
- Società Italiana di Emaferesi (SIDE; http://www.emaferesi.it/)

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