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Stem cells in the treatment of diabetes mellitus – focus on mesenchymal stem cells

Günter Päth[1]*, Nikolaos Perakakis[2], Christos S. Mantzoros[2], Jochen Seufert[1]

[1] Division of Endocrinology and Diabetology, Department of Medicine II, Medical Center – University of Freiburg, Faculty of Medicine, University of Freiburg, Germany

[2] Division of Endocrinology, Diabetes and Metabolism, Department of Internal Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

**** Corresponding author***

Günter Päth, PhD

Division of Endocrinology and Diabetology

Department of Medicine II

Medical Center – University of Freiburg

Faculty of Medicine, University of Freiburg

Hugstetter Str. 55

79106 Freiburg

GERMANY

Tel.: +49 761 270 73270

Fax: +49 761 270 33720

E-mail: guenter.paeth@uniklinik-freiburg.de

Keywords

Stem cells, diabetes mellitus, transplantation, embryonic stem cells, induced pluripotent stem cells, mesenchymal stromal cells

Abbreviations

ADA, American Diabetes Association; AE, adverse event; α-SMA, alpha smooth muscle actin; ANXA1, annexin-1; AUC, area under the curve; BAD, Bcl-2 antagonist of cell death; Bcl-2b, B-cell lymphoma 2; bFGF, basic fibroblast growth factor; BM, bone marrow; BMI, body mass index; CAR cell, CXCL12-abundant reticular cell; CCL5/RANTES, CC-chemokine ligand 5; CD, cluster of differentiation; CFU-F, colony forming unit-fibroblast; CXCL12, CXC chemokine ligand 12; DC, dendritic cell; Ebf, early B-cell factor; ECM, extracellular matrix; EMT, epithelial to mesenchymal transition; ERK, extracellular signal-regulated kinases; ESC, embryonic stem cells; FDA, Food and Drug Administration; FGFR1, fibroblast growth factor receptor 1; GAD, glutamic acid decarboxylase; GCV, ganciclovir; GM-CSF, granulocyte-macrophage colony-stimulating factor; GSIS, glucose-stimulated insulin secretion; GVHD, graft-versus-host disease; HbA1c, glycated hemoglobin A1c; HGF, hepatocyte growth factor; HLA, human leukocyte antigen; HNF1A, hepatocyte nuclear factor 1α; HOMA, homeostasis model assessment; HSC, hematopoietic stem cells; HSV-Tk, herpes simplex virus thymidine kinase; ICAM1, intercellular adhesion molecule 1; ICOS, inducible costimulator; IDDM, insulin-dependent diabetes mellitus; IDO, indoleamine 2,3-dioxygnase; IGF, insulin-like growth factor; IL, interleukin; IL-1RA, interleukin-1 receptor antagonist; IPF-1, insulin promoter factor 1 (official name, PDX1); IR, insulin resistance; iPSC, induced pluripotent stem cells; ISC, insulin screting cell; ISL-1, insulin gene enhancer protein 1; IU, international unit; IV, intravenous; MET, mesenchymal to epithelial transition; MHC, major histocompatibility complex; MMP, matrix metalloproteases; MMTT, mixed-meal tolerance test; MNC, mononuclear cells; MODY3, maturity-onset diabetes of the young type 3; MPC, mesenchymal precursor cells; MSC, mesenchymal stem cells; NG2, neural/glial antigen 2; NGF, nerve growth factor; NGN3 neurogenin 3; NKX6.1, NK6 homeobox 1; NO, nitric oxide; NOD, non-obese diabetic; NSG, NOD scid gamma; PAX, paired box protein; PDGF, platelet-derived growth factor; PD-L1, programmed cell death ligand 1; PDX1, pancreatic and duodenal homeobox 1; PEDF, pigment epithelium-derived factor; PGE2, Prostaglandin E2; SC, stem cells; SCF, stem cell factor; SCID, severe combined immunodeficiency; STZ, streptozotocin; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; TGF, transforming growth factor; Th, T helper; TNF, tumour necrosis factor; TRAIL, TNF-related apoptosis inducing ligand; Treg, regulatory T cell; TSG-6, tumour necrosis factor-stimulated gene 6; TUNEL, terminal deoxynucleotidyl transferase mediated dUTP nick end labeling; UC, umbilical cord; VCAM1, vascular cell adhesion molecule 1; VEGF, vascular endothelial growth factor; WHO, world health organisation; WJ, Wharton's Jelly.

Abstract

Diabetes mellitus type 1 and type 2 have become a global epidemic with dramatically increasing incidences. Poorly controlled diabetes is associated with severe life-threatening complications. Beside traditional treatment with insulin and oral anti-diabetic drugs, clinicians try to improve patient's care by cell therapies using embryonic stem cells (ESC), induced pluripotent stem cells (iPSC) and adult mesenchymal stem cells (MSC). ESC display a virtually unlimited plasticity, including the differentiation into insulin producing β-cells, but they raise ethical concerns and bear, like iPSC, the risk of tumours. IPSC may further inherit somatic mutations and remaining somatic transcriptional memory upon incomplete re-programming, but allow the generation of patient/disease-specific cell lines. MSC avoid such issues but have not been successfully differentiated into β-cells. Instead, MSC and their pericyte phenotypes outside the bone marrow have been recognized to secrete numerous immunomodulatory and tissue regenerative factors. On this account, the term 'medicinal signaling cells' has been proposed to define the new conception of a 'drug store' for injured tissues and to stay with the MSC nomenclature. This review presents the biological background and the resulting clinical potential and limitations of ESC, iPSC and MSC, and summarizes the current *status quo* of cell therapeutic concepts and trials.

Introduction

Human organs and tissues possess a limited capacity to completely recover their structure and function in a number of pathologic conditions and degenerative diseases. This fact initiated the multidisciplinary field of regenerative medicine which investigates the potential of stem cells (SC) for tissue repair and restoration of organ function. Based on their nature and origin, SC exhibit features of interest for cell therapies; e.g. targeting functional degeneration and loss of insulin producing pancreatic β-cells in diabetes mellitus (DM). The diverse potential of embryonic SC (ESC), induced pluripotent SC (iPSC) and adult mesenchymal SC (MSC) has been exploited to restore or maintain insulin secretion as well as to investigate patient-specific disease aspects. MSC are currently the most investigated cells in DM-related trials while clinical testing of ESC has just started. This review summarizes the biological aspects and the application strategies for the treatment of DM by stem cell therapy.

β-cell replacement

Patients with autoimmune DM type 1 (T1DM) experience a loss of insulin producing pancreatic β-cells and rely on daily insulin injections. Despite modern insulin therapies, exogenous application of insulin can never be as accurate and dynamic like insulin secretion from endogenous β-cells and therefore can only partially reduce the risk for the development of micro- (i.e. nephropathy, retinopathy) or macrovascular (i.e. coronary artery disease, peripheral artery disease, cerebrovascular disease) complications. Additionally, efforts to develop effective immunosuppressive treatments to prevent β-cell loss before disease onset had limited success so far [1]. Consequently, restoration of endogenous insulin secretion represents an important aim to prevent hyper- and hypoglycemia as well as to reduce or avoid diabetic complications and the patient's requirement for self-management of glycemic control by exogenous insulin administration.

Clinical islet transplantation aims to re-establish endogenous insulin secretion and has been steadily refined since its beginning in the 1980s [2]. An important step was the 'Edmonton Protocol' from 1999 which avoids β-cell toxic glucocorticoids by using sirolimus, tacrolimus and daclizumab for immunosuppression [3]. Ongoing clinical research improved isolation, culture and transplant techniques, and evaluated advanced anti-inflammatory and

immunomodulatory interventions [4-6]. As a result, a multicenter analysis with 18 diabetic patients receiving 34 islet transplantations showed a graft survival (defined by C-peptide concentrations of ≥ 0.3 ng/ml) of about 72.2%, 44.4% and 22.2% after 1, 2 and 5 years, respectively [7]. Subsequently, a multicenter phase 3 trial, which enrolled 48 participants receiving 75 islet transplantations, successfully improved glycemic control to a median glycated hemoglobin A1c (HbA1c) level of 5.6% after 1 and 2 years [8]. Compared to standard insulin therapy, islet transplantation more efficiently improved glycemic control and progression of retinopathy, and resolved hypoglycemia even in patients with only partially remaining graft function [9, 10].

Transplantation of whole pancreases is an established alternative to islets and both procedures display advantages and limitations [11]. The standard procedure of islet infusion into the liver is much safer with less complications than pancreas transplantation which is considered a major surgery with accordingly enhanced risks for the patient. Thus, pancreas transplantation is rarely performed alone and is most commonly combined with a kidney transplantation in patients with T1DM and end-stage renal disease. The major obstacle of the less risky islet transplantation is the limited graft survival. Insulin independence after islet transplantation initially reached barely 10% at 1 year but has been improved by the Edmonton protocol to 10-15% at 5 years [12] and by later developments, such as T cell-depleting agents and blockade of tumour necrosis factor (TNF), to 50% at 5 years [4]. Thereby, at least experienced islet transplantation centers have substantially improved long-term graft function towards the reported 5 year outcomes in pancreas transplantation; 55% for pancreas alone and after kidney, and 72% for pancreas together with kidney [2, 13].

As with other organ transplantation, there is a great scarcity of human donor material which evoked intensive research on the generation of insulin producing β-cells from SC. Whole pancreas transplantation strictly depends on high quality organs while generation of insulin producing β-cells from SC has the potential to solve the problem of limited availability of donor material for islet transplantation. However, the need for immunosuppression reserves both pancreas and islet transplantation as a therapeutic choice to a limited patient population such as brittle diabetics with life-threatening hypoglycemic events or subjects who anyway receive immunosuppression; e.g. because of kidney transplantations for renal failure due to diabetic nephropathy.

I. ESC

ESC represent the inner cell mass of the blastocyst and possess a pluripotent differentiation capacity. This makes them capable to form all three germ layers (ectoderm, endoderm and mesoderm) which subsequently give rise to all cell types of the body. On this account, these cells are considered the superior tool for tissue generation but they evoke ethical concerns regarding their origin from human embryos. Moreover, they bear a clinical risk since their pluripotent nature makes undifferentiated ESC capable to form teratomas and malignant teratocarcinomas *in vivo*. The development of clinically safe differentiation protocols and testing routines for tumorigenicity in rodents represents an important challenge in the field [14, 15].

The differentiation of human ESC into functional β-cells is not trivial since transforming processes have to mimic complex embryonal organogenesis *in vitro*. Differentiation protocols therefore established a number of factors and inhibitors that modulate molecular pathways in an exact sequential timing to resemble the natural development of pancreatic β-cells. Generally, human ESC were firstly differentiated into definitive endoderm cells and then sequentially into primitive gut tube and posterior foregut, pancreatic endoderm and finally β-cells using multiple specified media and supplementation in each step [16, 17]. The earlier attempts proved the concept but did not achieve high yields; e.g. a typical early study resulted in an average percentage of insulin+ cells in differentiated human ESC cultures of 7.3% [16]. Meanwhile, the exact media compositions have been further improved and current protocols reached the level of scalable production of β-cell phenotypes with functional insulin secretion [17-20]. However, the complexity of protocols and differences in employed ESC and iPSC lines raise difficulties in reproducibility outside experienced laboratories since stem cell differentiation is despite all progress not a routine procedure.

A major step in differentiation of human ESC towards β-cells is the expression of the transcription factors pancreatic and duodenal homeobox 1 (PDX1) and NK6 homeobox 1 (NKX6.1) which are markers of pancreatic endoderm and endocrine precursor cells. It has been shown that comparable human embryonic pancreas tissue from fetal weeks 6-9, which contained very few β-cells at that stage, is capable to mature into functional β-cells after transplantation into non-obese diabetic mice with severe combined immunodeficiency (NOD/SCID mice) [21]. Based on these findings, *in vivo* maturation of PDX1+/NKX6.1+

progenitors into β-cells has been recognized to be more efficient than mimicking this months-long process *in vitro* [22]. Furthermore, scalable *in vitro* differentiation of ESC into endocrine pancreatic precursor cells is to date more robust and less complicated [23, 24] than generation of fully functional β-cell phenotypes by advanced protocols [17, 19] and therefore the favoured strategy of the company ViaCyte [25].

The resulting improvements in differentiation of ESC towards PDX1+/NKX6.1+ pancreatic progenitors during the last decade have also reduced the risk of tumour formation *in vivo* which occurred in a typically early study of the company NovoCell (name changed to ViaCyte in 2010) with a rate of 7 out of 46 transplanted mice [22]. Since then, ViaCyte has steadily optimized the approach to efficient large-scale production and embedded their pancreatic endoderm and endocrine progenitor cells into a macroencapsulation device to generate immune isolated VC-01 implants [18, 25, 26].

Macroencapsulation improved graft survival and clinical safety. Although ESC-derived progenitors are hypoimmunogenic, transplanted cells are challenged by adaptive immune responses such as local inflammation and rejection. In addition, once maturated into insulin producing β-cells, graft cells will be attacked by persistent autoreactive T cells in patients with T1DM. Several studies have demonstrated that macroencapsulation protects embedded cells by isolation from immune responses and thereby avoids rejection and the need for immunosuppression [27, 28]. Furthermore, macroencapsulation prevents escape of embedded cells into the body. This is an important safety issue since any ESC transplanted in an undifferentiated state bears the potential of malignant transformation. In this view, subcutaneously transplanted devices could be retrieved and removed easily. Using this concept, Viacyte has achieved a milestone by initiating the first-in-man clinical trial to test safety and efficacy of their pancreatic endoderm implant VC-01 in patients with T1DM and hypoglycemic unawareness (ClinicalTrials.gov: NCT02239354, currently enrolling patients, estimated completion in January 2021).

This trial will answer the question whether the established *in vivo* maturation of human ESC-derived PDX1+/NKX6.1+ progenitors in rodents can be comparably recapitulated in human subjects. In addition, two of three further trials initiated by Viacyte aim to test a modified implantation device with reduced immuno isolation that allows vascularisation of the macroencapsulated cells. Provided that outcome of all these trials will prove safety, efficacy

and significant long-term graft function, this approach holds the potential to pave the way towards clinical β-cell replacement without immunosuppression and independent of the limited availability of donor islets.

II. iPSC

The ethical criticism related to the use of human pre-implantation embryos for extraction of ESC inspired researchers to refine the work of John B. Gurdon. His key study demonstrated that enucleated *Xenopus laevis* eggs that were transplanted with nuclei from differentiated intestinal epithelium could, at least in small numbers, develop into living tadpoles [29]. This demonstrated for the first time that somatic cell nuclei have the potential to revert into a pluripotent state. Decades passed by before this finding gained broad acceptance in the scientific community and the progression of this idea culminated in the birth of the first mammal, clone sheep Dolly, on July 5, 1996 [30].

Gurdons basic concept of reprogrammable somatic cells was further developed and one decade after Dolly's birth, Takahashi and Yamanaka induced embryonic-like pluripotency in somatic mouse fibroblasts by viral overexpression of the four transcription factors Sox2, Oct4, Klf4 and c-Myc [31]. For their groundbreaking discoveries Gurdon and Yamanaka were honored by the Noble Prize in 2012 (www.nobelprize.org).

As a major achievement, iPSC overcome the ethic obstacle of using embryos for harvest of ESC. During ongoing research, the original Yamanaka protocol has been diversely modified and viral integration was effectively replaced by treatment with recombinant proteins, small molecules and microRNAs [32, 33]. Following the principle route of pancreatic development as used for ESC, researchers have successfully differentiated iPSC into functional β-cell phenotypes [34, 35] and also established scalable production of both endocrine pancreatic progenitors and β-cells [20, 36].

Nevertheless, further refinement of procedures is still an issue and new tools were created for the identification of compounds and conditions which enhance yield and functionality of generated β-cells. For example, human iPSC expressing the fluorescent reporters Venus and mCherry markers under the control of intrinsic neurogenin 3 and insulin promoters have been

generated for screening of differentiation efficiency [37]. These cells have served to identify an inhibitor of fibroblast growth factor receptor 1 (FGFR1) that, while blocking the early development of pancreatic progenitors, promoted the terminal differentiation of pancreatic endocrine progenitors into endocrine cells including β-cells.

However, due to their origin from adult somatic cells, iPSC can inherit somatic mutations and incomplete reprogramming can maintain somatic transcriptional memory including cancer associated gene activity [38, 39]. These dangers currently do not define them as the first choice for clinical use but, more importantly, iPSC enable the successful generation of patient-specific cell phenotypes that allow to recapitulate disease processes *in vitro* and can serve as platforms for drug development and testing [40-42]. For example, researchers successfully generated an iPSC line from a patient carrying a hepatocyte nuclear factor 1α (HNF1A) mutation resulting in maturity-onset diabetes of the young type 3 (MODY3). In the near future, patient-specific cell lines will help to develop disease-related models that overcome the obstacle of species differences between human subjects and animal models.

III. MSC

For an excellent graphical overview on MSC biology discussed in section III we recommend the poster by Somoza *et al*. [43].

MSC within the bone marrow (BM)

The discovery of MSC has been generally attributed to A. J. Friedenstein who observed that BM explants form plastic adherent fibroblast-like clonogenic cells with a high replicative capacity *in vitro* and named them colony forming unit-fibroblasts (CFU-F) [44]. Friedenstein *et al*. further figured out that culture expanded CFU-F are capable to differentiate into osteoblasts, chondrocytes and adipocytes, and to reconstitute a hematopoietic microenvironment after transplantation in irradiated mice [45, 46]. These findings supported the pioneering study of Tavassoli and Crosby who demonstrated that autologous BM fragments transplanted into extramedullary sites can reconstitute hematopoietic and adventitial structures in rats [47]. The observed process started from a developing network of proliferating reticular cells and was successively followed by the occurrence of osteoblasts,

osteoid tissue, endothelial layers of sinusoidal structure and finally hematopoietic re-population.

These findings pointed out that CFU-F include a group of cells with the capacity of multipotent differentiation into mesenchymal lineages. Based on these features, multipotent CFU-F were renamed 'mesenchymal stem cells' by A. I. Caplan in 1991 [48]. Caplan later commented that the term 'stem cell' was provocative at that time but justified by ongoing research displaying that CFU-F could generate bone, cartilage, fat, muscle and other mesodermal phenotypes *in vitro* [49]. Postnatally, the contribution of BM-MSC to bone formation is associated with declining numbers of MSC after birth as indicated by the 10fold drop of CFU-F colony numbers in BM obtained from newborn and skeletally developed teenaged donors and a steady further decline with aging [50].

Thus, bone formation was considered a core function of BM-MSC *in vivo* and their osteogenic potential has been investigated in further detail by using scaffolds. As an example, porous calcium phosphate ceramic cubes of 3 mm in size were loaded with BM-MSC expressing the genetic marker *lacZ* and then subcutaneously transplanted into immunodeficient mice [51]. The MSC monolayers formed osteoblasts, then the scaffold became vascularized by host vessels and mineralized osteocytes developed. Importantly, lacZ+ osteoblasts and osteocytes confirmed that new bone was formed by donor MSC. Testings of this approach in animals and clinical settings showed that transplanted porous scaffolds loaded with BM-MSC significantly contributed to bone repair in rodents and in patients with large bone defects [52, 53].

The cube experiments further demonstrated that lacZ+ cells also occur around blood vessels [51]. In support, cultured CFU-F express the same markers [e.g. cluster of differentiation (CD) 146] as adventitial reticular cells of sinusoids in the intact BM *in vivo* [54]. Consequently, BM-MSC which do not undergo osteogenic differentiation reside at the abluminal surface of endothelial cells. After subcutaneous transplantation, cells sorted for CD146 were capable to organize a hematopoietic microenvironment outside the BM. This confirmed that skeletal progenitors are a functional part of the hematopoietic stem cell (HSC) niche and form a specialized microenvironment as conceptually already conceived by R. Schofield in 1978 [55]. The use of culture expanded BM-MSC to improve outcomes of BM

transplantation in cancer patients after chemo-therapy was tested first-in-man in 1995 and was clinically successful and safe [56].

Lineage tracing, using nestin (*Nes*)- or leptin receptor (*LepR*) promoter driven expression of the fluorescence reporters GFP or tdTomato, was employed to further investigate MSC-related cell fates in the BM [57, 58]. One should keep in mind that characterization of BM lineages is complicated since marker expression like that of *Nes*-GFP is in part variable during cellular development and overlapping between distinct phenotypes. Besides *Nes* and *LepR* gene activation, the *in situ* localization of MSC in the BM has been mainly defined by the differential expression of CD146, CD271, neural/glial antigen 2 (NG2) and alpha smooth muscle actin (α-SMA). Resulting findings indicated the existence of three different MSC populations within the endosteal niche and the perivascular niches at arterioles and sinusoids.

These investigations disclosed that *Nes*+ MSC of the endosteal niche secrete factors or express cell surface molecules that regulate quiescence in nearby HSC [59, 60]. *Nes*+ MSC in the perivascular niches express the key niche factors CXC chemokine ligand 12 (CXCL12), therefore also called CXCL12-abundant reticular (CAR) cells, and stem cell factor (SCF) which both control retention and maintenance of HSC [57]. The perivascular MSC could be divided in rare periarteriolar NG2+ cells with high nestin expression (Nes^{bright}/NG2+) and abundant perisinusoidal *LepR*+ cells with low nestin expression (Nes^{dim}/*Lepr*+). Deletion of *Cxcl12* and *Scf* in *Nes*+ MSC results in the mobilization of HSC to extramedullary organs and a marked reduction of HSCs in the BM [57, 61, 62]. Notably, the secretion of CXCL12 is in part regulated by direct innervation of the sympathetic nervous system and modulated by circadian rhythms [63].

The very low numbers of CFU-F in BM of adult human donors points out that BM-MSC are a minor population [50]. In line with this, it was found that among BM-MSC 'abundant' CAR/*LepR*+ cells account for only 0.3% of mouse BM cells [64]. This small population is the major source of adipocytes and osteoblasts in adult mouse BM but most of these cells remained undifferentiated to maintain the hematopoietic niche. The underlying molecular regulation was unclear until recently Seike *et al.* found that CAR/*LepR*+ cells preferentially express early B-cell factor (Ebf) 3 and analyzed its function [65]. Deletion of *Ebf3* in CAR/*LepR*+ cells severly impaired HSC niche function and BM became osteosclerotic with increased bone in aged mice. Additional deletion of *Ebf1* further increased niche dysfunction

leading to depletion of HSC already in infant marrow. This demonstrated that CAR/*LepR*+ MSC-derived Ebf3 and Ebf1 are required to maintain the HSC niche by inhibition of osteogenesis.

Pericyte-MSC outside the BM

The early view on MSC as BM stroma cells has nowadays completely changed and the occasionally used term 'mesenchymal stromal cell' became misleading. Instead, it became obvious that MSC within the BM are not part of the connective tissue stroma but are forming the endosteal and perivascular niches. Most BM-MSC are of perivascular origin [54]. In a landmark study, Crisan *et al*. clearly documented that MSC phenotypes exist outside the BM in multiple organs as perivascular pericytes expressing typical BM-MSC markers like CD146, NG2 and α-SMA, and being multipotent for osteogenic, chondrogenic, adipogenic and myogenic lineages *in vitro* [66]. Further functional characterization tested whether such pericyte-MSC possess the ability of BM-MSC to restore a hematopoietic niche in irradiated mice [67]. The study revealed that sorted CD146+ perivascular cells, isolated from human adipose tissue, are capable to support the long-term persistence of transplanted human HSC while CD146- perivascular cells did not. This observation clearly demonstrated that BM-MSC and pericytes expressing the same markers are equivalent in function.

Various studies have meanwhile demonstrated that MSC phenotypes could be isolated from virtually all tissues of the body including fat, muscle, cord blood, Wharton's jelly, placenta and others [68]. This initiates the notion that possibly a unique MSC may exists but it became obvious that all these MSC, beside core markers, display differential gene expression profiles in a time and tissue-related manner and thereby affect stemness [69]. For example, muscle pericytes are not spontaneously osteochondrogenic while cord blood-derived MSC phenotypes display the unique capacity to form cartilage spontaneously *in vivo*. Furthermore, there is evidence that also the intrinsic mechanical properties of the extracellular matrix influences cell fate decisions in MSC, as softer matrices that mimic muscle are myogenic while rigid matrices that mimic collagenous bone are osteogenic [70]. Collectively, this raises the notion that mesenchymal stemness of MSC is adapted to or imprinted by tissue microenvironment and that MSC from placenta, Wharton's jelly, umbilical cord blood *etc*. may display the most embryonal-like phenotype [68, 69].

Regarding the notion of developmental and tissue-related differences, Chen *et al*. recently proposed the concept of multiple 'paralogous' stem-cell niches which are progressively and functionally transformed within an individual organism throughout its life span [58]. In their view, delineation of distinct cell phenotypes results from complex multiple interchangeable events of epithelial to mesenchymal transition (EMT) and reverse mesenchymal to epithelial transition (MET). These dynamic processes make it difficult to discern cell identities and to define reliable markers. Therefore, the question whether all pericytes give rise to MSC, or in the alternative view, pericyte-MSC differ from BM-MSC but may derive from a common progenitor, is not finally answered to date [49, 71, 72]. Surely, the answer will be complex and limited by the accuracy and composition of available marker sets; 'true' pericyte-MSC may likely represent a subpopulation among all pericytes.

In vivo function of pericyte-MSC

Pericytes have been discovered in the early 1870s by C.J. Ebert and C.M.B. Rouget [73] and were named by K.W. Zimmermann describing their contractile nature in 1923 [74]. Later characterization specified that pericytes communicate with endothelial cells by both physical contact and secreted factors to regulate growth, stability, architecture and blood flow of microvessels as well as they are important for the integrity of the blood brain barrier and provide clearance and phagocytosis in the brain [75].

Pericytes attach to the epithelium by their tips and their contractile apparatus consisting of microfilaments containing actin, myosin and tropomyosin enables them to regulate the capillary diameter or to move along the microvessels [76]. This indicated that perivascular pericyte-MSC and their BM counterparts are not static but dynamic and their close proximity to the vasculature enables them to readily mobilize and travel in the bloodstream to sites of injury. Consistent with this view, pericytes respond to a series of pro-inflammatory stimuli and are able to sense different types of tissue trauma signals by their expressed functional pattern-recognition receptors and contribute to the onset of innate immune responses by cell-cell contact and paracrine effectors [73]. Similarly, transplanted BM-MSC home to various sites of injury, e.g. stroke [77], pancreatic islet inflammation and diabetic kidney [78, 79] and cancer [80]. Once on site, BM-MSC secrete a variety of immunomodulatory, anti-inflammatory, angiogenic, anti-apoptotic and tissue-regenerative trophic factors [81], and fend off invading microbes by secretion of anti-microbial peptide LL36 that kills bacteria upon contact [82, 83].

Altogether the ability of migration and humoral tissue restoration is a common feature of MSC independent of their BM or pericyte origin. A.I. Caplan, who once coined the term MSC, has meanwhile suggested to rename these cells 'medicinal signalling cells' to more accurately reflect the new conceptional view on MSC as a 'drug store' for injured tissues *in vivo* and to preserve the MSC nomenclature [84].

MSC in cancer

Besides MSC and HSC, the perivascular niche also accommodates tumour SC and its microenvironment has been shown to regulate tumour dormancy and growth [85-87]. Tumours recruit pericytes by e.g. platelet-derived growth factor (PDGF) to maintain their tumour vessels [88] and consequently, inhibition of PDGF receptor signalling causes pericyte detachment and vessel regression, and diminishes tumour growth in several cancer models [89-91]. Tumour cells further interact with the surrounding stroma leading to a chronically increased release of inflammatory cytokines and growth factors [92] that has been described as a 'wound that never heals' [93]. The chronic inflammatory state drives the recruitment of responsive cell types including MSC [94, 95] which account for 0.01–1.1% of total cells in prostatectomies from human prostate tumours [96].

It is now understood that MSC interact with tumour cells at various stages of progression but it is not finally clear whether their role is tumour promoting or suppressive. Several cancer models implicated that MSC promote tumour progression and invasiveness as well as having a role in the creation of a metastatic niche at the secondary site [97-100]. In contrast, MSC suppressed tumour growth in several cancer models including breast cancer, Kaposi's sarcoma, hepatoma and melanoma [101-104]. Reasons for conflicting findings may result from the heterogeneity of tested MSC populations, differences in experimental design and varying responses dependent on the stimuli [105].

However, it seems unlikely that transplanted MSC have a significant role in inducing or promoting tumours in human subjects, as their clinical use has been considered safe since 1995 [56] and clinicians did not notice a tumour risk. In support of this notion, a meta-analysis has studied 1012 participants who received MSC for treatment of ischemic stroke,

Crohn's disease, cardiomyopathy, myocardial infarction, graft versus host disease or served as healthy volunteers but did not find any indication of malignancy [106].

Meanwhile, research has employed the recruitment of MSC to tumours in order to target malignant diseases with genetically modified MSC that, for example, overexpress pigment epithelium-derived factor (PEDF) to reduce angiogenesis or overexpress TNF-related apoptosis inducing ligand (TRAIL) to induce apoptosis [107]. Despite using MSC from different sources, different transfection methods and a wide array of expressed proteins, the data consistently showed a reduction in tumor growth and prolonged survival in rodents. These promising pre-clinical outcomes initiated the first-in-man trial TREAT-ME-1 which aimed to target advanced gastrointestinal cancer (ClinicalTrials.gov: NCT02008539).

The trial used ganciclovir (GCV) in combination with autologous BM-MSC overexpressing herpes simplex virus thymidine kinase (HSV-Tk) under the control of the CC-chemokine ligand 5 (CCL5/RANTES) promoter. Mechanistically, engineered MSC migrate to tumours where they become activated to express CCL5 [98]. Subsequently induced HSV-Tk phosphorylates GCV which then inhibits DNA polymerases and thereby induces apoptosis in transfected cells and, due to a bystander effect, also in nearby tumour and stromal cells [108]. The primary study aim was to evaluate safety and tolerability, and both features were found generally favorable with stable disease in four patients, and progressive disease in 2 patients after one year follow-up [109]. Slowed tumour progression and enhanced survival are of great importance in the field and engineered MSC may contribute in the future to prolong the life of cancer patients. Side note: the HSV-Tk/GCV suicide gene technique has also been tested as an 'emergency switch' that would allow to eliminate transplanted iPSC in case of malfunction [110].

Transdifferentiation of MSC into insulin producing pancreatic β-cells

There was initially great optimism that MSC could be easily transdifferentiated across the germ layer border into insulin producing pancreatic β-cells and thereby avoid the ethical and tumorigenic obstacles of ESC and iPSC. Generation of insulin producing cells from MSC employed genetic engineering including overexpression of PDX1, neurogenin 3 (NGN3) and paired box 4 (PAX4) [111-113] and/or complex *in vitro* protocols using various conditions and factors to resemble pancreatic development [114-118]. Depletion of β-cells in rodents by a high dosage of the β-cell toxic compound streptozotocin (STZ) has frequently been used to

test the functional capacity of transplanted insulin producing cells. As a variant related to aspects of T2DM [119], multiple low-dose STZ-treatment causes islet inflammation for testing of MSC-mediated recovery of β-cell dysfunction and partial loss. Aspects related to the immunology of T1DM have been investigated in female NOD mice with spontaneously occurring autoimmune insulitis [120].

Several studies reported that transplanted MSC-derived insulin producing cells can improve glycemia in STZ-diabetic rodents [111, 114, 118, 121]. Nevertheless, stem cell specialists remained sceptic concerning *in vitro* transdifferentiation of MSC beyond mesodermal lineages. In this regard, the efficiency of MSC transdifferentiation was generally very low and resulting insulin producing phenotypes frequently possessed an acurate secretory capacity but were not further expandable or *vice versa*. To date, transdifferentiation of MSC has not reached clinically significant large scale production of pancreatic progenitors or β-cells as it has been established for ESC and iPSC.

The study of Ianus *et al*. initiated the notion that injected BM cells contain a subpopulation of cells that engraft into islets and are capable to transdifferentiate into insulin producing phenotypes *in vivo* [122]. It was observed that injection of BM cells with insulin gene 2 promoter driven GFP expression into sublethally irradiated mice gave rise to a small proportion of 1.7-3% glucose-responsive GFP+/insulin+ cells within islets which, after isolation and sorting, show a functional insulin secretion comparable to control β-cells. Insulin+ phenotypes could be reproduced in a subsequent study. Hess *et al*. transplanted BM cells from GFP mice in NOD/SCID mice with multiple low-dose STZ-induced islet inflammation and noted partial recovery of diabetic blood glucose levels [78]. GFP-BM-cells significantly migrate to the inflamed endocrine pancreas and their occurrence within islets was associated with enhanced local proliferation and 2.5% GFP+/insulin+ cells. Since the insulin+/GFP+ cells did not express PDX1, a major marker of a mature and functional β-cell, the authors concluded that amelioration of hyperglycemia was not caused by incompletely differentiated GFP+/insulin+ cells but by the proliferative increase in β-cell mass.

In further testing, using injection of GFP-BM cells into single-dose STZ-treated mice, only 2 GFP+/insulin+ cells out of more than 100,000 screened β-cells could be retrieved [123]. These very rare events were considered to rather result from cell fusion than transdifferentiation [124, 125]. Importantly, an elaborated lineage tracing study from Douglas

Melton's group strongly suggested that new β-cells and islets only derive from pre-existing β-cells and not from adult pancreatic stem cells or progenitors [126]. In this regard, a later study reported that up to 3% of injected human BM-MSC engrafted into inflamed pancreatic islets of multiple low-dose STZ-diabetic NOD/SCID and improved hyperglycemia by reduction of β-cell loss and partly maintained mouse insulin blood levels in the absence of detectable human insulin [79]. In addition, up to 11% of injected human BM-MSC engrafted into the STZ-injured kidneys and improved glomerular morphology as well as decreased mesangial thickening and macrophage infiltration.

Collectively, these studies exclude significant transdifferentiation of MSC into insulin producing cells *in vivo* and pave the way for the new understanding that MSC migrate to and engraft at site of injury to support tissue repair by secretion of numerous tissue regenerative factors [81].

Humoral potential of MSC

After isolation, pancreatic islets suffer from hypoxic culture stress due to loss of blood supply and consequently impeded transport of oxygen to the inner cell layers of the three-dimensional islet structure [127, 128]. After transplantation, islets were further challenged by local inflammation and rejection processes [129]. Fast dynamics of revascularisation and downregulation of immune responses have been considered important for long-term graft function and generated interest on the angiogenic and immunomodulatory potential of MSC in the context of islet transplantation.

Kinnaird *et al*. displayed that human MSC express a wide array of arteriogenic cytokine genes and that MSC conditioned media promoted smooth muscle cell proliferation and migration in a dose-dependent manner *in vitro* [130]. *In vivo*, using a murine hindlimb ischemia model, murine MSC conditioned media enhanced collateral flow recovery and remodeling, improved limb function, reduced the incidence of autoamputation, and attenuated muscle atrophy compared with control media. In this regard, Figliuzzi *et al*. tested the angiogenic effects of BM-MSC on co-transplanted islets in STZ-diabetic rats and noted that improved graft survival and function in association with increased numbers of new capillaries and expression of vascular endothelial growth factor (VEGF) [131]. Upcoming studies confirmed the angiogenic capacity of MSC and its association with VEGF [132-135]. In this regard, it was shown *in vitro* that VEGF inhibition partially blocked the enhanced formation of

anastomosing tubule networks by co-cultured endothelial cells [134]. Therefore, VEGF appears to be an important player which is supported by other MSC-derived factors such as nerve growth factor (NGF) [136] and factors inducing angiopoietin receptor Tie-2 expression in islets [135]. In sum, these studies established that MSC-mediated revascularisation contributes to islet graft survival by shortening the post-transplantation ischemia period.

Improved revascularization and functional outcome of co-transplanted islet grafts have been further associated with reduced numbers of terminal deoxynucleotidyl transferase mediated dUTP nick end labeling (TUNEL)+ and caspase-3+ apoptotic cells [137, 138]. Angiogenic VEGF and several other MSC-released trophic factors including hepatocyte growth factor (HGF), insulin-like growth factor (IGF)-1, transforming growth factor (TGF)-β, basic fibroblast growth factor (bFGF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) display anti-apoptotic properties [81, 139]. The potential of MSC to mediate survival was tested by direct interactions with β-cells *in vitro* in the absence of third party cells from surrounding tissues.

In line with the variety of released growth factors, we and others displayed that MSC-conditioned medium or co-cultured MSC preserve Akt signaling in cultured islets undergoing hypoxic culture stress and additional treatment with alloxan and STZ [135, 140]. Akt signaling promotes survival and reduces intrinsic apoptosis by its influence on B-cell lymphoma 2 (Bcl-2) family proteins such as phosphorylation of Bcl-2 antagonist of cell death (BAD) and caspase-9 [141]. MSC-released factors also activate mitogenic extracellular signal–regulated kinases (ERK)1/2 signaling which, similar to Akt, promotes survival by inhibition of intrinsic apoptosis [142, 143]. Interestingly, MSC induced ERK1/2 signaling only in highly proliferative endothelial cells and INS-1E insulinoma cells but not in primary mouse islets with a low proliferation rate [135, 140, 144]. These observations indicate an important role for the Akt pathway in MSC-mediated survival of pancreatic islets.

MSC-released factors have been further reported to improve insulin secretion and glucose-response (see Table 1 in [145]). Experiments may indicate a beneficial effect of cell-cell contacts since humoral improvement of glucose-stimulated insulin secretion (GSIS) in indirect co-cultures with cells separated by membranes [135] was not well reproducible by other studies unless cells were cultured in direct contact [146, 147]. In this respect, MSC enhance GSIS *in vitro* by release of annexin-1 (ANXA1) while MSC from *Anxa1-/-* mice had

no functional capacity [148]. Hence, heterogeneous effects of MSC on GSIS may partly result from different expression levels of ANXA1. Likely, the very close proximity of MSC and islets in direct co-cultures enhanced local effector levels and involves the extracellular matrix (ECM) since MSC co-cultured with HSC maintain the vascular niche by upregulated expression of intercellular adhesion molecule 1 (ICAM1) and vascular cell adhesion molecule 1 (VCAM1) [149]. Such supportive processes are an important topic in tissue engineering and it has been recognized that islets, which lost ECM during enzymatic isolation, show improved survival and function after treatment with ECM molecules [150].

Moreover, numerous MSC-released factors exhibit potent immunomodulatory characteristics; e.g. transforming growth factor-β1 (TGF-β1), indoleamine 2,3-dioxygnase (IDO), nitric oxide (NO), human leukocyte antigen-G (HLA-G), Prostaglandin E2 (PGE2), interleukin-1 receptor antagonist (IL-1RA) and tumour necrosis factor-stimulated gene 6 (TSG-6) [81, 139, 151]. As a result, MSC have been described to induce regulatory T cells and anti-inflammatory M2 macrophages, and to inhibit T cells, natural killer cells and T helper (Th)17 cell differentiation as well as maturation of dendritic cells (DC).

Consequently, MSC substantially reduced co-transplanted islet graft inflammation and rejection in BALB/c mice [138, 152], humanized NOD scid gamma (NSG) mice [153] and a cynomolgus monkey model [154]. All these studies showed MSC-improved engraftment in association with reduced infiltration of T cells and neutrophils, and increased numbers of circulating regulatory T cells. Inhibitors established that MSC-mediated prevention of T cell proliferation and islet graft rejection was not related to IDO and heme oxignase-1, partially related to NO and profoundly mediated by matrix metalloproteases (MMP)-2 and MMP-9 via reduction of IL-2 receptors on T cells [152]. MSC further suppress the proliferation and activation of T cells by interaction with IL-10-producing CD14+ monocytes [153]. Remarkably, systemically injected MSC in female NOD mice reduced the incidence of spontaneous T1DM [155] or reversed recent-onset hyperglycemia via release of programmed cell death ligand 1 (PD-L1) and inhibition of myeloid/inflammatory DC through an IL-6-dependent mechanism [156]. Moreover, treatment of NOD mice with CD4+CD62L+ regulatory T cells (Treg), which have been cocultured with cord blood-derived MSC before, resulted in a marked reduction of spontaneous autoimmune insulitis, restored Th1/Th2 cytokine balance in blood and induced apoptosis of infiltrated leukocytes in pancreatic islets

[157]. This concept has been translated into clinic as 'Stem Cell Educator' therapy (see below) [158, 159].

In addition, MSC have been further tested for their ability to ameliorate wound healing which is a frequent diabetic complication. Endogenous MSC, present in the skin as dermal sheath cells surrounding hair follicle units [160] and as perivascular pericytes [161]. Skin injury induces MSC to recruit and activate epithelial cells, fibroblasts and keratinocytes to revascularize and re-populate the wounded area during the proliferative healing phase [162]. Wounds treated with MSC show acceleration of angiogenesis and re-epithelialisation [163] which, according to the notion of paracrine factors, could also be achieved by treatment with MSC-conditioned medium [164-166]. Wound healing is impaired in DM patients which show degraded micro- and macrovessels in association with early occurring detachment and loss of vascular pericytes at capillaries [167, 168]. Importantly, MSC-treatment successfully improved wound healing under diabetic conditions such as in diabetic db/db mice with mutated leptin receptor [169] and a rat model of diabetic foot ulceration [170].

MSC in clinical trials

The complex and wide-ranged humoral potential of MSC attracted much attention among researchers and clinicians. MSC can be isolated from various tissues, frequently from BM and adipose tissue, by minimal invasive puncture and they also allow noninvasive retrieval from often discarded 'medical waste' such as placenta, cord blood and umbilical cord [68, 171]. It is further known from early CFU-F studies and numerous studies since then that MSC could be easily expanded *in vitro* without significant loss of their mesenchymal differentiation capacity or their humoral secretion. Moreover, MSC are immune-privileged because they express very low levels of major histocompatibility complex (MHC) class I and no MHC class II which normally prevents or strongly reduces immune responses [172, 173]. In clinical use since 1995 [56], MSC are considered clinically safe [106] and both administration of autologous and also allogenic MHC-mismatched MSC is generally well tolerated and clinically effective [174-176].

To date, CinicalTrials.gov listed over 850 therapeutic approaches using MSC to target a broad array of diseases including hematological disease, graft-versus-host disease (GVHD), organ transplantation, cardiovascular and neurological diseases, bone and cartilage repair as well as inflammatory and autoimmune diseases [177]. Among these, more than 60 trials address

T1DM and T2DM, and from these we have summarized all trials with reported outcome in Table 1. In these trials, the various humoral features of MSC address different disease aspects (Fig. 1). In many patients with T1DM a minor portion of insulin producing β-cells survive but can not recover unless thereby induced autoimmune responses are blocked [178]. MSC mediate immune tolerance that aims to enable partial recovery of remaining β-cell mass [158, 159] or to reduce and delay the β-cell destruction during new-onset of T1DM [179, 180]. In T2DM, the anti-inflammatory features of MSC were used to ameliorate chronic low-grade inflammation which has been recognized as an important cause of insulin resistance and β-cell dysfunction [119]. These features in combination with secretion of pro-angiogenic factors should improve engraftment and survival of transplanted islets [181].

Interestingly, there is only one completed trial investigating the effect of MSC co-transplantation on islet graft survival and function. Potentially, there are concerns on the additional expense needed for generation, testing and application of clinical-grade MSC since established immunosuppression regimes should prevent graft rejection. In this regard, Wang *et al*. tested combined autotransplantation of BM-MSC and islets in chronic pancreatitis patients undergoing pancreatectomy without immunosuppression [181]. Patients showed reduced insulin requirement in the peritransplantation period, reduced decline of C-peptide levels after 6 month and lowered fasting blood glucose levels after 12 month. This suggests that co-transplanted MSC reduced loss of islet graft function. Additional studies and long-term observations are needed to verify these very limited results from 3 patients.

The other studies addressed T1DM (7 trials) as well as severe T2DM (8 trials) in patients who required insulin and/or oral anti-diabetic drugs to control glycemia. Currently the total number of investigated patients is relatively small. In total, 276 patients were investigated in small groups of 6-22 subjects and 3 studies [159, 174, 182] analyzed groups of 31-45 subjects. The enrolled patients show diversity regarding age, BMI and other aspects as well as duration and severeness of the disease. In this view, also applied MSC came from different sources including BM, adipose tissue and umbilical cord, have been differentially processed and applied in different dosages. Though MSC did not cure the disease and despite much heterogeneity regarding applied MSC, it is quite astonishing that studies reported varying positive aspects of partially improved glycemia. Only two T2DM trials reported on diabetic complications. Jiang *et al*. noted without further details that renal and cardiac functions showed varying degrees of improvement [183] and Hu *et al*. found no rise of diabetic

complications in MSC-treated patients while placebo-treated patients displayed higher incidences of diabetic retinopathy, neuropathy and nephropathy during 36 months follow-up [182]. In general, MSC were well tolerated and it could be noted as the quintessence of outcome that all trials except one [179] reported reduced requirement for exogenous insulin and/or anti-diabetic drugs.

Two studies addressed the question whether MSC treatment could delay development of newly-onset T1DM. Hu *et al*. reported that both the HbA1c and C-peptide were improved compared to the pre-therapy values and to control patients during 2 years follow-up [180]. Consequently, MSC-patients required smaller insulin dosages. This outcome indicated a reduced loss of insulin producing β-cells but was not fully reproduced by Carlsson *et al*. who observed improved C-peptide levels in response to a mixed-meal tolerance test but no changes in HbA1c, fasting C-peptide and daily insulin dosages after 1 year [179]. More trials with prolonged observation periods are needed to clarify the potential of MSC to delay the development of T1DM.

An interesting aspect is the so-called 'Stem Cell Educator' [158, 159]. While studies normally apply MSC by intravenous injection, the 'Stem Cell Educator' approach routed the patient's blood through a closed-loop system that separates lymphocytes from the whole blood and briefly co-cultures them with adherent cord blood-derived MSC before returning them into the patient’s circulation. Though MSC are not delivered into the body, their temporary contact to patient's lymphocytes was sufficient to induce immune tolerance which ameliorates the disturbed Th1/Th2/Th3 cytokine balance with increased Treg numbers in type 1 diabetic patients and decreased CD86+/CD14+ monocytes and reduced markers of inflammation in type 2 diabetic patients. As a result, all patients displayed a generally reduced requirement for insulin and metformin and improved HbA1c values after 10-12 months follow-up. In addition, homeostasis model assessment of insulin resistance (HOMA-IR) demonstrated that insulin sensitivity was improved post-treatment in type 2 diabetics. The potential of the 'Stem Cell Educator' is currently further investigated by 2 further trials (ClinicalTrials.com: NCT02624804 and NCT03390231).

Collectively, the trials show that even heterogeneous MSC populations could be clinically effective. The question, however, remains whether clinical outcome could be improved by optimized MSC batches since actually no standardized method exists for isolation,

characterization, expansion, potency testing or pathogen screening [184-186]. A basic issue is the donor heterogeneity which has the potential to dramatically influence therapeutical properties of MSC. This includes the question whether autologous MSC are a good choice for cell therapy of DM since, for eample, hyperglycemia induces pericyte dysfunction via activation of p75 neurotrophin receptor/NF-kB-mediated release of microparticles carrying miR-503 from neighbouring endothelial cells [187]. Also subclinical inflammation present in subjects with metabolic syndrome and T2DM [119] impairs the vascular stem cell niche and leads to MSC dysfunction [188]. MSC batches could be further influenced by isolation and expansion since several studies suggested that outgrowth from donor tissue generates less heterogeneous cell populations with increased proliferation rates and cell viability than isolation by enzymatic tissue digestion [189-191]. Moreover, MSC may lose their functions due to increased cellular senescence during longer expansion and passaging since a direct positive link between early passages of MSC and clinical outcomes in GVHD has been demonstrated [192]. One should finally keep in mind that only a small proportion of systemically injected MSC engraft at site of injury while most rapidly embolise in the lungs and disappear with a half-life of about 24 h to an unclear fate [193, 194].

The International Society for Cell Therapy (ISCT) defined clinically useful MSC by mesenchymal differentiation (into bone, cartilage and fat), plastic-adherent growth *in vitro* and expression of CD73, CD90 and CD105 in the absence of hematopoietic surface markers [195]. Notably, expansion of plastic-adherent BM cells favours the expansion of nonclonal stromal cell-enriched populations, often misinterpreted as pure SC fractions, which contain varying percentages of true MSC, e.g. depending on donor age [50], and thereby plausibly exhibit different clinical effectiveness [196, 197]. This demands a reliable assay, such as the CFU-F assay, and careful evaluation of each MSC batch may allow the identification of the percentage of stem cells and their multilineage potential in each batch of nonclonal MSC. Potentially, efficacy of MSC populations could be further enhanced by selection via additional markers such as stromal (STRO)-1, CD146, alkaline phosphatase, CD49a, CD271 and VCAM1 [197]. Against this background, the US Food and Drug Administration (FDA) demands registering of tissue processing facilities which should report on (i) prevention of transmitting communicable disease via contaminated tissue, (ii) proper handling and processing of tissue and (iii) demonstration of clinical safety and effectiveness of cells, especially after extensive manipulation.

Conclusion on the current state of stem cell therapy of diabets mellitus

Stem cell therapy has to deal with a wide array of limitations which are still the subject of current research. See Table 2 for summary and comparison of SC in cell therapy of DM.

Differentiation of ESC and iPSC has meanwhile reached clinical large-scale production and current developments of macroencapsulation may provide clinical safe usage of these cells that demonstrate otherwise potentials for tumor development. Macroencapsulation prevents the escape of embedded cells into the body and subcutaneously transplanted devices could be retrieved and removed easily. The outcome of Viacyte's first-in-man trial of their pancreatic endoderm implant VC-01 will clarify whether the use of ESC and iPSC is an option in DM therapy in the near future.

MSC are clinically safe and several trials exist though they are limited in number and investigated patients. Currently, MSC-based therapy is no cure but shows a potential to ameliorate DM since most studies report decreased requirement of exogenous insulin and/or anti-diabetic drugs. In this regard, MSC may be best used with diabetic patients that have severe problems in controlling glycemia by conventional therapies; e.g. patients with brittle DM. We see potential in optimizing their therapeutical performance by standardizing isolation, characterization, selection, expansion, potency testing and pathogen screening. However, increased efforts will increase costs and MSC have to prove their value.

Declarations of interest

None.

Author contributions

All authors have participated in manuscript preparation and have approved the final article.

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Table 1. Clinical trials with reported outcome using MSC to treat DM.

Section A. T1DM

Reference, Trial number, Country, Patient number, Duration	Disease Objective	Patient characteristics	Treatment	Main outcomes
Wang *et al*. 2018 NCT02384018 United States n = 3 12 months	Testing safety and effects of BM-MSC and islet autotransplantation in patients with chronic pancreatitis undergoing total pancreatectomy	Aged 26, 29 and 42 years with chronic pancreatitis	Infusion of 20 ± 2.6 x 10^6 BM-MSC together with 5,107 ± 1,920 islet equivalents/kg via the portal vein 101 patients with pancreatectomy due to chronic pancreatitis who received islets alone served as historical controls	No AE directly related to MSC Insulin requirement lowered in the peri-transplantation period Fasting blood glucose lowered Fasting C-peptide with smaller declines during months 1-6 (mean C-peptide levels comparable to control values at 6 months) HbA1c not different Improved life quality
Cai *et al*. 2016 NCT01374854 China n = 21 12 months	Testing safety and effects of combined UC-MSC plus autologous BM-MNC without immunotherapy on C-peptide	Aged 18-40 years with established T1DM for 2-16 years HbA1c 7.5-10.5% Fasting serum C-peptide < 0.1 pmol/ml Daily insulin requirement < 100 IU	Infusion of UC-MSC (1.1 x 10^6/kg) and BM-MNC (106.8 x 10^6/kg) through supraselective pancreatic artery cannulation Controls received standard care (n = 21)	MSC-treatment was well tolerated 1 patient with puncture site bleeding 1 patient with abdominal pain Serum C-Peptide AUC 105.7% increase, controls 7.7% decrease Serum insulin AUC 49.3% increase, controls 5.7% decrease HbA1c decreased 12.6%, controls increased by 1.2% Reduced insulin requirement (-29.2%) with no change in controls
Carlsson *et al*. 2015	Testing safety and effects of	Aged 18-40 years	IV infusion of 2.1–3.6 x	No side effects of MSC treatment

NCT01068951 Sweden n = 9 12 months	autologous BM-MSC in treatment of patients with recently diagnosed T1DM	T1DM newly diagnosed < 3 weeks before enrollment MMTT-stimulated serum C-peptide > 0.1 nmol/l	10^6 autologous BM-MSC/kg Sham controls (n = 9)	HbA1c, fasting C-peptide and insulin requirements not different compared to controls MMTT-induced C-peptide AUC and peak values were preserved/increased by MSC
Dave *et al*. 2015 India n = 10 36 months	Testing co-infusion of ISC (*in vitro* differentiated from adipose tissue-derived MSC) together with BM-HSC	Aged 8-45 years IDDM for at least 6 months C-peptide levels < 0.5 ng/ml	Infusion into portal circulation, thymus and subcutaneous tissue ISC: mean of 3.34 ml cell inoculums with 5.25 x 10^4 cells/µl; ISC expressed ISL1, PAX6 and IPF1 with mean C-peptide and insulin-secretion of 1.03 ng/ml and 17.48 l IU/l after 2 h in glucose medium HSC: mean of 103.5 mL with 2.66 x 10^6/µL	No untoward effect of stem cell infusion Improved serum C-peptide, Hb1Ac, blood sugar levels Reduced exogenous insulin requirement Patients returned to normal lifestyle and unrestricted diet
Thakkar *et al*. 2015 India n = 20 24 months	Testing co-infusion of ISC (*in vitro* differentiated from adipose tissue-derived MSC) together with BM HSC Comparison of autologous vs. allogenic stem cells	Aged 8-45 years Group 1: mean age 20.2 years, mean disease duration 8.1 years Group 2: mean age 19.7 years, mean disease duration 7.9 years	Infusion into portal circulation, thymus and subcutaneous tissue Group 1 received 2.65 ± 0.8 x 10^4 autologous ISC/kg (n = 10); ISC expressed ISL1, PAX6 and IPF1 Group 2 received 2.07 ± 0.67 x 10^4 allogenic ISC/kg (n = 10) HSC not reported	No untoward effect, morbidity (pulmonary embolism, sepsis) or mortality caused by therapy Reduced insulin requirement Sustained improvement in serum C-peptide and HbA1c
Hu *et al*. 2013 China	Testing the long-term effects of WJ-MSC for newly-onset T1DM	Aged 17.6 ± 8.7 years T1DM according to ADA criteria for less than 6	2 IV infusions with a mean of 1.5-3.2 × 10^7 WJ-MSC with an interval of	No acute or chronic side effects compared to control group Fasting plasma glucose levels not different

n = 15 24 months		months Fasting C-peptide ≥ 0.3 ng/ml	1 month Control group received saline (n = 14)	from controls Improved HbA1c, fasting C-peptide and postprandial blood glucose levels Reduced insulin requirement
Zhao *et al*. 2012 NCT01350219 China, Spain n = 12 10 months	Testing safety and efficacy of Stem Cell Educator therapy	Aged 15 to 41 years with a diabetic history of 1 to 21 years Group A: with some residual pancreatic β-cell function (n = 6) Group B: without (n = 6)	Stem Cell Educator therapy: patient's blood circulated through a closed-loop system that separates lymphocytes from the whole blood and briefly co-cultures them with adherent CB-MSC before returning them to the patient's circulation	No AE, minimal pain from two venipunctures Reduced insulin requirement (24 weeks) Improved fasting C-peptide levels and reduced HbA1C (12 weeks) in groups A and B Increased in basal and glucose-stimulated C-peptide levels (40 weeks, group B) Increased expression of co-stimulating molecules CD28 and ICOS, increased numbers of Tregs and restored Th1/Th2/Th3 cytokine balance (4 weeks, groups A and B) Sham controls (n = 3) without significant changes
Vanikar *et al*. 2010 India n = 11 12 months	Testing co-infusion of allogenic ISC (*in vitro* differentiated from adipose tissue-derived MSC) together with BM HSC	Aged 5-45 years IDDM for 1-24-years Low serum C-peptide levels < 0.5 ng/ml	Intraportal infusion ISC: mean of 1.5 ml with 2.1 x 10^3/μL; ISC expressed ISL1, PAX6 and IPF1 HSC: mean of 96.3 ml with 28.1 × 10^3/μL	No adverse side effect related to stem cell infusion or therapy Reduced insulin requirement HbA1c decreased Serum C-peptide levels increased Patients became free of diabetic ketoacidosis events

Section B. T2DM

Reference, Trial number, Country, Patient number, Duration	**Disease Objective**	**Patient characteristics**	**Treatment**	**Main outcomes**
Bhansali *et al*. 2017	Comparison of safety and	Aged 30-60 years with	Infusion of 10^6 BM-MSC	1 patient with local extravasation of blood

NCT01759823 India n = 10 12 months	effects of autologous BM-MSC and BM-MNC in reducing on insulin requirement	T2DM ≥ 5 years and failure to achieve HbA1c ≤ 7.5% while receiving triple oral anti-diabetic drugs in optimal doses along with insulin for the last 6 months	(group I, n = 10) or 10^9 BM-MNC (group II, n = 10) via transfemoral route into the celiac trunk Sham controls were infused into the femoral artery (group III, n = 10)	following infusion Reduced insulin requirement in BM-MSC and BM-MNC groups (6 of 10 patients in both BM-MSC and BM-MNC groups, but none in the control group achieved the primary end point of ≥ 50% reduced insulin requirement) Increased 2nd-phase C-peptide response in BM-MNC group Improvement in insulin sensitivity index with increased insulin receptor substrate-1 gene expression in ABM-MSC group
Hu *et al*. 2016 China n = 31 36 months	Testing safety and long-term effects of WJ-MSC on T2DM	Aged 18-60 years with T2DM according to ADA criteria	2 infusions of 10^6 WJ-MS with an interval of 1 month (group I) through veins in the back of the hand Controls (group II) received normal saline (n = 30)	No serious AE No chronic side effects or lingering effects Fasting plasma glucose almost unchanged Reduced insulin requirement Fasting C-peptide improved HbA1c and postprandial plasma glucose improved by trend after 36 months HOMA of β-cell secretory function improved HOMA-IR unchanged Incidence of diabetic retinopathy, neuropathy and nephropathy only increased in controls
Skyler *et al*. 2015 NCT01576328 United States n = 45 3 months	Testing safety, tolerability, and feasibility of allogeneic BM-derived STRO-3-selected subset of BM-derived MPC (Rexlemestrocel-L, Mesoblast Inc.; product expressed MSC markers STRO-1 and CD146) in T2DM inadequately controlled with metformin or one additional oral anti-	Aged < 80 years with T2DM for 10.1 ± 6 years HbA1c 7.0-10.5% Metformin either alone or in combination with one other oral antidiabetic medication (except a thiazolidinedione) for at least 3 months	IV infusion of 0.3, 1 or 2 x 10^6 BM-derived MPC (n = 15 each) Sham controls (n = 15)	No serious acute AE due to infusion No serious hypoglycemia events or discontinuations due to AE, comparable AE in MSC and placebo groups, 1 subject with severe abdominal pain in MSC group No immunologic responses to MSC HbA1c reduced by trend, more pronounced in patients with baseline HbA1c ≥ 8% Insulin requirement reduced by trend

	diabetic drug			
Guan *et al*. 2015 China n = 6 24-43 months	Testing safety and effects of allogenic UC-MSC	Aged 27-51 years Time from hyperglycemia to first infusion was 4-157 weeks Patients treated with insulin and poorly controlled blood glucose levels and HbA1c	2 IV infusions of 10^6 UC-MSC/kg through the cubital vein with an interval of 14-17 days	No safety issues during infusion and the long-term monitoring period Reduced insulin requirement (significant during months 1-6), 3 patients became insulin-free for 25 to 43 months Insulin-free patients displayed reduced HbA1c and increased fasting C-peptide during months 1-24 Relative stable fasting plasma glucose and 2 h postprandial blood glucose levels
Liu *et al*. 2014 Chinese Clinical Trial Register ChiCTR-ONC-10000985 China n = 22 12 months	Testing safety and effects of treatment with allogenic WJ-MSC	Aged 18–70 years T2DM according to ADA criteria Poor glycemic control with recent antidiabetic therapies, including drugs and/or insulin injection for at least 3 months GAD antibody negative Fasting blood glucose level ≥ 7.0 mmol/L HbA1c ≥ 7%	2 infusions of 10^6/kg WJ-MSC 1st infusion via peripheral vein on day 5 2nd infusion directly delivered to the pancreas via the splenic artery using endovascular catheters on day 10	3 patients with fever after operative day 1 patient with subcutaneous hematoma 1 patient with nausea, vomiting and headache Improved HbA1c, fasting C-peptide levels, HOMA of β-cell secretory function, postprandial blood glucose levels Reduced insulin requirement and oral hypoglycemic drugs Reduced serum levels of IL-1β and IL-6, and reduced numbers of CD3+ and CD4+ T lymphocyte numbers at 6 months
Kong *et al*. 2014 NCT01413035 China n = 18 6 months	Testing safety and effects of allogenic UC-MSC	Aged 23-65 years T2DM according to WHO criteria Patients received insulin and oral anti-diabetic drugs to control	3 IV infusions of 1-3 x 10^5 UC-MSC/kg with an interval of 1 week	4 patients with slight transient fever 8 patients respond to treatment (efficacy group); these show: reduced fasting and postprandial blood glucose levels, and by trend increased plasma C-peptide levels and Treg numbers All patients had a feeling of well-being and are more active
Zhao *et al*. 2013 NCT01415726 United States	Testing safety and efficacy of Stem Cell Educator therapy	Aged 29-68 years with long-standing T2DM Group A: oral medications	Stem Cell Educator therapy: patient's blood circulated through a	No AE, mild discomfort during venipunctures Improved metabolic control and reduced inflammation

n = 36 12 months		(n = 18) Group B: oral medications + insulin injections (n = 11) Group C: impaired β-cell function with oral medications + insulin injections (n = 7)	closed-loop system that separates lymphocytes from the whole blood and briefly co-cultures them with adherent CB-MSC before returning them to the patient's circulation	Reduced HbA1C in groups A and B Improved insulin sensitivity (HOMA-IR, 4 weeks) Recovery of fasting C-peptide levels in Group C (56 weeks) and HOMA of β-cell secretory function/C-peptide (12 weeks) Improved serum TGF-β, reduced CD86+CD14+ monocytes, no effect on Treg numbers and restored Th1/Th2/Th3 cytokine balance (4 weeks)
Jiang *et al*. 2011 China n = 10 6 months	Testing safety and effects of placenta-derived MSC in patients with longer duration of disease	Aged 30-85 years Duration of DM ≥ 3 years Insulin requirement for optimal glycemic control of ≥ 0.7 IU/kg/day at least for 1 year	3 IV infusions of 1.22-1.51 x 10^6/kg placenta-derived MSC with an interval of 1 month	No fever, chills, liver damage and other side effects Insulin and C-peptide levels increased HbA1c decreased Insulin requirement decreased, 4 of 10 patients achieved reduction of > 50% Improved renal and cardiac function (no details)

Abbreviations. ADA, American Diabetes Association; AE, adverse event; AUC, area under the curve; BM, bone marrow; BMI, body mass index; CD, cluster of differentiation; DM, diabetes mellitus; GAD, glutamic acid decarboxylase; HbA1c, glycated hemoglobin A1c; HOMA, homeostasis model assessment; HSC, hematopoietic stem cells; IDDM, insulin-dependent diabetes mellitus; IL, interleukin; ICOS, inducible costimulator; ISL-1, insulin gene enhancer protein 1; IPF1, insulin promoter factor 1 (official name, PDX1, pancreatic and duodenal homeobox 1); IR, insulin resistance; ISC, insulin secreting cell; IU, international unit; IV, intravenous; MNC, mononuclear cells; MMTT, mixed-meal tolerance test; MPC, mesenchymal precursor cells; MSC, mesenchymal stem cells; PAX6, paired box protein 6; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; TGF, transforming growth factor; Th, T helper cell; Treg, regulatory T cell; UC, umbilical cord; WHO, world health organisation; WJ, Wharton's Jelly.

Table 2. Summary and comparison of SC in cell therapy of DM.

	ESC	iPSC	MSC
Cell type and origin	Embryonic SC Inner cell mass of the blastocyst	Adult somatic cells Reprogramming *in vitro*	Adult SC Endosteal (BM) and perivascular niches (all tissues)
Characteristics	Pluripotent Generates all germ layers: ectoderm, endoderm and mesoderm	Pluripotent Generates all germ layers: ectoderm, endoderm and mesoderm	Multipotent Generates mesenchymal lineages: bone, cartilage, fat and muscle Maintain HSC niche and hematopoiesis
Ethical concerns	Use of embryos	No	No
Differentiation into pancreatic β cells	Yes	Yes	Insulin+ cells with limited secretory or proliferative capacity (experimental)
Cell therapeutic options	β-cell replacement	β-cell replacement Patient-specific cell lines	Secreted factors with immunomodulatory, angiogenic and tissue regenerative properties
Advantages	Large-scale production of pancreatic endoderm, endocrine progenitors and fully functional β-cells	Large-scale production of pancreatic endoderm, endocrine progenitors and fully functional β-cells	Easy isolation and *in vitro* expansion Low immunogenicity allows allogenic transplantation without immunosuppression Minimal-invasive application Clinical safe and well tolerated
Limitations	Tumorigenic if incompletely differentiated	Tumorigenic if incompletely differentiated Somatic mutations Incomplete reprogramming maintains somatic transcriptional memory	ISCT minimal criteria for clinical MSC favor expansion of nonclonal stromal cell-enriched populations with varying proportions of true SC Current clinical protocols are not standardized and exhibit potential for improvements Only a small proportion of systemically injected cells engrafts in injured target tissues
Status	First-in-man trial currently investigates clinical safety and efficacy of macroencapsulated ESC-derived pancreatic endoderm Macroencapsulation avoids	Currently not safe enough for clinical usage Patient-specific cell lines allow investigation of disease processes *in vitro* and represent a platform for drug testing	Completed clinical trials collectively report on reduced requirement for exogenous insulin Greatest benefit for patients with problems in controlling glycemia by conventional therapy More clinical trials in progress

	tumorigenicity by preventing the escape of embedded cells into the body and allows easy graft removal if necessary

Abbreviations. BM, bone marrow; DM, diabetes mellitus; HSC, hematopoietic stem cell; ISCT, International Society for Cell Therapy; SC, stem cells.

Highlights

Large-scale production of pancreatic endoderm and β-cells from embryonic stem cells

First-in-man trial investigates macroencapsulated embryonic stem cell-derived β-cells

Induced pluripotent stem cells allow patient/disease-specific cell lines

Mesenchymal stem cells secrete immunomodulatory and tissue regenerative factors

Transplanted mesenchymal stem cells ameliorate human type 1 and type 2 diabetes

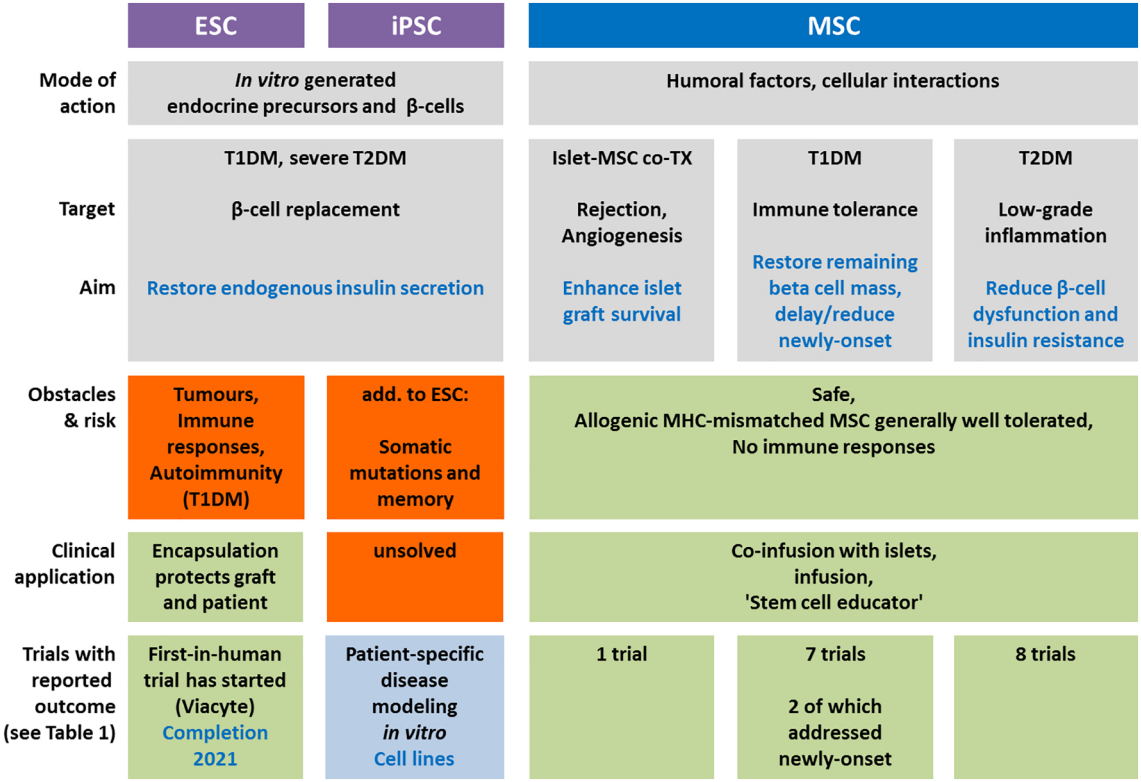
ESC
iPSC
MSC
Mode of action
In vitro generated endocrine precursors and β-cells
Humoral factors, cellular interactions
Target
T1DM, severe T2DM
β-cell replacement
Islet-MSC co-TX
Rejection, Angiogenesis
T1DM
Immune tolerance
T2DM
Low-grade inflammation
Aim
Restore endogenous insulin secretion
Enhance islet graft survival
Restore remaining beta cell mass, delay/reduce newly-onset
Reduce β-cell dysfunction and insulin resistance
Obstacles & risk
Tumours, Immune responses, Autoimmunity (T1DM)
add. to ESC: Somatic mutations and memory
Safe, Allogenic MHC-mismatched MSC generally well tolerated, No immune responses
Clinical application
Encapsulation protects graft and patient
unsolved
Co-infusion with islets, infusion, 'Stem cell educator'
Trials with reported outcome (see Table 1)
First-in-human trial has started (Viacyte) Completion 2021
Patient-specific disease modeling in vitro Cell lines
1 trial
7 trials
2 of which addressed newly-onset
8 trials

Figure 1