

## Review Article

# Stem cells for the cell and molecular therapy of type 1 diabetes mellitus (T1D): the gap between dream and reality

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**Abstract:** In spite of intense research, over the past 2-3 decades, targeted to validating methods for the cure of T1D, based on cell substitution therapy in the place of exogenously administered insulin injections, achievement of the final goal continues to remain out of reach. In fact, aside of very limited clinical success of the few clinical trials of pancreatic islet cell transplantation in totally immunosuppressed patients with T1D, the vast majority of these diabetic patients invariably is insulin-dependent. New advances for cell and molecular therapy for T1D, including use of stem cells, are reviewed and discussed in an attempt to clearly establish where we are and where are we may go for the final cure for T1DM.

**Keywords:** Insulin, blood glucose, reversal, complications

### Introduction

Diabetes mellitus, a metabolic disease hall-marked by uncontrolled high blood glucose (BG) levels, may be considered, at this juncture, as a global epidemics with continuous, steep increase in its prevalence and incidence worldwide [1]. Chronic high BG, is responsible, over time, for severe secondary complications. These include, but are not limited to, macro-/microangiopathy, and in particular, retinopathy, leading to premature blindness, chronic renal insufficiency requiring hemodialysis or kidney graft, central and peripheral disabling neuropathy, possibly resulting in limb amputations, and cardiovascular disease. The main forms of this chronic disorder are type 1 (T1D) and type 2 (T2D) diabetes mellitus: the latter is far more prevalent (90-95%) than the former, and it usually, although not necessarily, emerges at mid age, and it derives from a combination of peripheral insulin resistance with progressive pancreatic  $\beta$ -cell failure, often throughout final endogenous insulin secretory failure [2]. Hence T2D, after an initial phase where insulin resistance prevails, might ultimately require exogenous insulin supplementation upon functional

$\beta$ -cells exhaustion. T1D while much less common (5-10%) than T2D, and prevalently affecting the pediatric/adolescent/young age, because of widespread autoimmune destruction of insulin secreting pancreatic  $\beta$ -cells, requires since diagnosis, substitution therapy regimen consisting of daily exogenous insulin multiple injections. Unfortunately, while a life-saving therapy, although a daily highly demanding choir, exogenous insulin may delay but not eliminate the risk for developing the above mentioned, secondary complications of the disease [3]. Hence new approaches, to possibly cure the disease, in both the two main clinical forms, are to be intensively sought.

### Ongoing strategies for $\beta$ -cell substitution: lights and shadows

#### *Whole pancreatic transplant*

The apparent easiest solution to replace the diseased/destroyed islet  $\beta$ -cells, in T1D, with intact and viable tissue, would be to graft a whole pancreatic organ retrieved from cadaveric donors. The organ would be grafted heterotopically under strict recipient's general phar-

macologic immunosuppression therapy regimens to avoid immune rejection. In terms of metabolic control, looking at the data from the International Pancreatic Transplant Registry (IPTR) whole pancreatic grafts usually resulted in rapid control of hyperglycemia in the recipients, with consequential discontinuation of the exogenous insulin supplementation. However, two main drawbacks have shadowed, thus far, the impact of this strategy on the radical treatment of T1D: 1) significant morbidity associated with this major surgery; 2) need for strict and life-long recipient's immunosuppression with all the invariably associated, severe, imminent side effects. These problems have suggested that this therapeutic approach should apply to insulin-dependent diabetic patients who also suffer for end-stage renal disease [4]: this being the case, the patients could undergo either simultaneous kidney + pancreas transplantation or pancreas after kidney graft, which would fully endorse general immunosuppression of the recipients. On the contrary, because of immunosuppression-related risks, pancreatic graft alone is virtually confined to those patients with brittle diabetes and severe deterioration of their quality of life, that would be otherwise untreatable. It is ultimately clear that both procedures (pancreas alone and pancreas + kidney transplantation) remain experimental and are not to be recommended routinely.

### *Isolated pancreatic islet cell transplantation*

At least less traumatizing would appear recourse to isolated islet grafts for cell substitution therapy. In fact, the islets occupy an incomparably smaller volume than whole pancreas, could be lodged by minimally invasive procedures, into an organ (ie, liver) and finally, last but not least, the islet  $\beta$ -cells are the sole tissue that is required for cell replacement in T1D. However, isolated islets, upon retrieval from the whole donor pancreas, get disconnected from their native ECM, and are more difficult to engraft in a different organ, ie, the liver, while they invariably become exposed to not only immune but also not immune, local noxious environmental factors. Moreover, isolated islets like whole pancreases, would elicit immune rejection upon allograft, therefore also requiring strict recipient's general immunosuppression.

Looking at clinical data on islet transplantation (TX), human islet allografts have steadily

improved through the past few decades with special regard to 2007–2010, when, according to the data of the Clinical Islet Transplant (CIT) Consortium, 65% of the treated patients achieved insulin-independency at 1 year of post-TX. Less consistent was the maintenance of insulin independence at 5 years of TX, ranging on as low as 5-6% with few exceptions in few Centers [5].

The actual reasons for such a poor outcome of the islet graft clinical trials likely are multiple, and only partially identified. One reason may relate to progressive decline of the functionally active  $\beta$ -cell mass, by the time of retrieval from donor pancreata, to the grafting procedures, to the immediate post-transplant time period [6].

The islet engraftment process is adversely affected by several immune and not immune specific factors, mainly based on activation of the coagulation system (IBMIR) as well as the hostile liver cell/tissue environment) [7].

Additionally, general immunosuppression results in both local and systemic toxicity that invariably impairs survival and functional competence of the grafted islet cells.

### **Perspectives on B-cell substitution therapy for insulin-dependent diabetes**

In an attempt to circumvent the above mentioned problems two possible alternatives could be envisioned:

-Immunoprotection of islet grafts by microencapsulation

-New strategies for cell and molecular therapy

Microencapsulation consists of entrapping cells/tissue within polymeric and non-cytotoxic membranes that constitute immunoprotective barriers. These would prevent physical, cell-to-cell contact between the host and the transplanted cell/tissue, thereby virtually circumventing the islet graft-directed immune destruction. Based on this concept, a wide spectrum of cells, other than pancreatic islet cells, have been immobilized within the microcapsules in order to broaden applicability of this approach well beyond diabetes [8]. This strategy holds promise to allow for islet grafting, with no recipient's immunosuppression, not only across genetically different individuals from the same species, but eventually, also

across species barriers [9, 10]. Consequently, microcapsules might offer the opportunity to employ non-human tissue as a resource for donor islets, an issue that is gaining growing interest in light of the shortage of cadaveric human donor organs. In summary, should the grafted islets be enveloped within artificial membranes that selectively regulate cross-permeability of noxious soluble factors, while preventing access to immune-reactive cells, the classic immunosuppressive treatment of the recipients could theoretically be obviated. In order to afford clinical feasibility to this technology, the microcapsules, of variable size, should be comprised of highly purified chemical polymers, virtually endotoxin and pyrogen-free, that are applicable to humans following FDA guidelines. Moreover, these capsules should be associated with established physical-chemical membrane's properties so as to comply with the required permeability and porosity selectiveness. The last generation of these alginate acid-based microcapsules has been successfully applied to microencapsulated human islet allograft pilot clinicals, in patients with T1D in our Center [11, 12].

However and unfortunately, microencapsulation would mitigate but not fully overcome the problem of the restricted availability of human donor islets coupled with the finite life-span of the retrieved islet beta-cells. This basic "cell mass shortage" issue, even in the best scenario, would not enable the microencapsulated islet grafts to be an universal treatment for all insulin-requiring diabetic patients.

Prospectively, fully novel attempts to replace the destroyed  $\beta$ -cell mass in T1D could be: expansion of the native pancreatic islet  $\beta$ -cell pool; substitution of  $\beta$ -cells by endogenous or exogenously grafted stem cells that are associated with the potential of self-renewal and multi-lineage cell differentiation, with special regard to insulin producing cells for transplantation purposes.

### *Theoretical bases*

*Molecular regulation of the endocrine pancreas development:* The human pancreas originates from the fusion of two distinct outgrowths of the foregut endoderm, dorsal, and ventral. One of the most important factors in the endocrine pancreas developmental process is PDX-1

gene (pancreatic and duodenal homeobox gene 1) [13]. During development, its expression has been reported in both endocrine and exocrine progenitors, while in the mature organ it is mainly expressed by  $\beta$ -cells [14]. Following the first wave of expression of PDX-1, the cells that will give rise to both acinar and endocrine tissue start co-expressing the bHLH (basic Helix-Loop-Helix) transcription factor Ptf1a (pancreas specific transcription factor 1a) [15, 16]. Absence of Ptf1a usually results in pancreas-deprived animals [17]. The endocrine program is initiated by Ngn-3 (Neurogenin 3), a bHLH transcription factor. Ngn3-null mice do not have islets in their pancreases and die within a short time due to hyperglycaemia [18]. Ngn-3<sup>+</sup> cells are the progenitors of each endocrine cell type in mature islets. Then, specification of single hormone positive cells occurs via other transcription factors, acting either as activators or repressors, e.g. Pax4 (paired box 4), Arx (aristaless related homeobox), Pax6 (paired box 6), and NeuroD1. Among the latter factors, involved in the endocrine cells differentiation, a key regulator of  $\beta$ -cells differentiation is Nkx2.2 (NK2 homeobox 2). Its inactivation gives rise to Islet1<sup>+</sup>/Insulin<sup>-</sup> cells, thus suggesting its role in blocking the definitive differentiation of such endocrine elements [19, 20].

*Sources of stem cells for diabetes:* Various types of stem cells have been isolated, to date, in humans from a variety of tissues including pre-implantation embryos, fetuses, birth-associated tissues and adult organs. Based on biochemical and genomic markers, they can be broadly classified into Embryonic stem cells; (ESC); Mesenchymal stem cells (MSC); Hematopoietic stem cells (HPS); Induced pluripotent stem cells (iPSCs); Adult stem cells derived from adult tissues (ASC).

Embryonic stem cells have the highest differentiation potential into insulin secreting cells. Soria et al. were the first to differentiate mouse ESC into insulin producing cells and to observe that the differentiated cells, upon transplantation in diabetic mice, induced reversal of hyperglycaemia. In fact, insulin secreting cells derived from ESC normalize glycemia in streptozotocin-induced diabetic mice [21]. Lumelsky and co-workers described a protocol to obtain iPSCs from mouse ESC via a nestin expression step [22]. In all experiments, the differentiated ESCs were able to synthesize insulin, by cleav-

age of pro-insulin into C-peptide and insulin, and expressed voltage-activated calcium channels. Despite the fact that these ESC-derived cells showed features resembling  $\beta$ -cells, they were unable to secrete insulin exclusively in response to high glucose levels, and did not show the presence of insulin-containing secretory granules, with these results having been confirmed also by others [23]. Hence the inability to secrete insulin in response to glucose stimulation, which is the key function of  $\beta$ -cells, remains an issue to be tackled by future research [24]. As far as pro's and con's are concerned, a first hurdle to use of ESCs would be the potential tumorigenesis of such cells. In fact, ESCs can give rise to teratomas and teratocarcinomas in humans [25]. The substantial number of rounds of replication which these cells undergo before transplantation may lead to the accumulation of potentially oncogenic chromosomal abnormalities [26]. Moreover, self-renewal, rapid proliferation, lack of contact inhibition and telomerase activity are some of the shared characteristics between ESCs and cancer cells [27]. An efficient way to reduce the risk of tumor/teratoma development could be increasing differentiation status and commitment to the cell type of interest before transplantation into the patient. Even cell sorting procedures, using surface antigen markers for undifferentiated (negative selection) or committed (positive selection) cells can partially contribute some oncological risk. However, these approaches are unlikely to be viable for the production of large numbers of cells needed for clinical use. A "kill-gene" strategy (such as thymidine kinase) or the packaging in micro/macrocapsules are other strategies under evaluation.

iPSCs (induced pluripotent stem cells) *Pros*-Human iPSCs are generated by transfection of somatic cells like fibroblasts [28], stomach cells and hepatocytes [29] human keratinocytes and blood cells [30] with a group of key transcription factors, including OCT4, SOX2, cMYC, KLF4, SOX2, LIN28, and NANOG [5]. Like hESCs, hiPSCs can be differentiated into insulin-secreting cells upon step-wise differentiation protocols into SOX17-positive cells, PDX1-positive cells (pancreatic progenitors), and NGN3-positive cells (endocrine progenitors) [32-36], using the same protocols applied to hESCs [32-34]. The first report showing dif-

ferentiation of hiPSCs into pancreatic  $\beta$  cells was communicated in 2008 [37]. Using a four-stage differentiation procedure, skin fibroblast-derived hiPSCs can differentiate into insulin secreting cells, which are responsive to glucose stimulation. Although hiPSC clones generated from T1D patients have similar capacities to differentiate into DE cells, they showed intra-assay variability in their differentiation potential into pancreatic  $\beta$  cells, which was more evident in the final stage of differentiation [36]. Furthermore, the variations in pancreatic differentiation abilities have been observed among hiPSC clones too [38-40]. The ability of iPSCs to differentiate into fully functional pancreatic  $\beta$  cells, remains controversial [41], with the most important feature being consistent generation of pancreatic  $\beta$  cell-like that retain insulin secretory competence upon glucose stimulation. Also, as a sign of incomplete maturation, the hiPSC-derived  $\beta$  cells may co-express multi-hormones, such as INS, GCG (glucagon) and C-peptide, but they lack the expression of specific mature pancreatic  $\beta$  cell markers such as NKX6-1 and MAFA [37]. Although several studies successfully generated insulin-secreting cells in vitro from hESCs using step-wise differentiation protocols [33, 39, 41, 43-47], the functionality of the produced  $\beta$  cells is very low, since they showed blunted glucose responsiveness. An important role has been ascribed to the in vivo microenvironment, for maturation of pancreatic  $\beta$ -cells intended for transplantation. In fact, transplantation of still immature pancreatic  $\beta$  cells or pancreatic progenitors cells into experimental animals, may lead to pancreatic  $\beta$  cells maturation, a result otherwise not achievable. The transplanted iPSC-derived  $\beta$  cells into two mouse models of T1D and T2D, were able to proficiently secrete insulin in response to glucose and improved the hyperglycemic phenotype [48]. Furthermore, transplantation of monkey iPSC-derived  $\beta$  cells could rescue hyperglycemia in diabetic mouse models [49]. Finally, the iPSC-derived insulin-secreting cells obtained from pancreatic epithelial cells in non-obese diabetic (NOD) mice, a model of human T1D, produced insulin in response to glucose stimulation, and their transplantation into a kidney of non-obese diabetic mice led to functional response to glucose stimulation [50]. All together, these findings suggest that in vivo maturation is essential for the functionality of iPSC-derived pancreatic  $\beta$  cells. Possibly,

there are specific signals at the transplantation sites that promote  $\beta$  cell differentiation and maturation.

Cons- The reprogramming of somatic cells into iPSCs is usually obtained by using viral transfection of transcription factors. The major limitation of this technology is the use of harmful genome integrating viruses, where the vector backbone and transgenes are permanently incorporated into the genome. This incorporation can cause mutations, which may hinder the normal function of iPSCs, their differentiation ability, or cause tumorigenesis [51]. To overcome this problem, some studies have generated iPSCs using an adenoviral reprogramming method, where non-integrating adenoviruses transiently expressing Oct4, Sox2, Klf4 and c-Myc are used. This procedure requires longer exposure to reprogramming factors [52, 53] and establishment of disease models. The relationship between iPSC pluripotency and tumorigenicity lies on the fact that some genes used to generate iPSCs are oncogenes such as MYC [54, 55]. Recently, additional techniques have been developed to exclude the tumorigenic gene MYC so as to avoid genetic modifications, by use of microribonucleic acids (mir-) [56, 57]. Furthermore, iPSCs have been generated from patients with T1D by using three factors (OCT4, SOX2, and KLF4), and excluding the tumorigenic gene C-MYC [32]. Obviously, further studies are required to confirm the efficiency of methods to generate iPSCs from different human somatic cells.

Mesenchymal Stem Cells (MSCs)- MSCs are multipotent cells localized in several tissues including cord blood, bone marrow, and adipose tissue [58]; however, we will primarily refer to the cells derived from the most widely used source: the bone marrow-derived MSCs. BM-MSC are multipotent progenitor cells, capable of self-renewal and “vocational” differentiation into adipogenic, chondrogenic, and osteogenic cell lineages [59]. The cells can be isolated from bone marrow in a low-density cellular culture by removal of non-adherent cells [60]. BM-MSC express a typical set of cell markers including CD29, CD44, CD73, CD90, CD105, CD166 CD49e, CD51, CD54, CD59, CD71 [61]. Recently Delorme and colleagues also suggested the expression of CD200 [62]. Unlike endothelial and hematopoietic cells,

BM-MSC do not express CD14, CD31, CD34, CD45, CD79, CD86, CD117 and glycophorin A [63, 64]. In addition, these cells express molecules of class I major histocompatibility complex (MHC), but not class II (such as HLA-DR) such as human leukocyte antigen-DR) which may be very advantageous, graft-wise [65]. Although BM-MSCs are ones of the most studied and well-documented adult stem cells, they have limitations in terms of procured cell mass, thereby possibly requiring in vitro expansion. Unfortunately, the latter could expose the cells to the risk of either losing stemness properties, or inducing artifactual chromosomal changes, or finally, microbial contamination [66].

### *Towards generation of a $\beta$ cell-like cell phenotype from BM-MSC*

Experiments aimed at inducing BM-MSC to differentiate into insulin producing cells (IPC) were conceived to correctly reprogram these cells by activating “ad hoc” differentiation pathways. Oh and co-workers suggested that rat BM-derived cells, cultured in a medium supplemented with DMSO (dimethylsulfoxide) and high glucose concentration, could trans-differentiate into IPC. In particular, this group highlighted the formation of cell aggregates by differentiated cells, which, upon transplantation into hyperglycemic mice, would acquire a three-dimensional architecture mimicking the islets of Langerhans. These cells expressed typical pancreatic endocrine genes as insulin, glucagon and somatostatin, therefore giving rise to a mixed population of islet-like cells. These cells possessed granules with relatively low insulin content and, when transplanted into diabetic mice, favored normalization of blood glucose levels for over almost 3 months [67]. More recently, Xie and co-workers demonstrated that hBM-MSC were able to give rise to IPC by a three-step differentiation protocol, with the final addition of Activin A as a key differentiating agent. The acquisition of a  $\beta$  cell-like phenotype by these cells was attested by morphological analyses and the expression of typical pancreatic genes such as Nkx6.1, Isl-1, Beta2/NeuroD, Glut2, Pax6, nestin, Pdx-1, ngn3, insulin, glucagon and C-peptide. More importantly, differentiated cells released insulin in a glucose-dependent manner and improved hyperglycaemia for over 1 month in streptozotocin-induced diabetic rats [68].

Adipose tissue (AT)- AT is gaining progressive attention as a primary source of MSCs for cell therapy purposes, by virtue of its ready availability, excellent expandability, and ease of isolation with minimal patient discomfort [69, 70]. AT is a remarkable organ that regulates the fat mass and nutrient homeostasis in the body. It releases a large number of bioactive mediators (adipokines) modulating fuel homeostasis, blood pressure, and lipid and glucose metabolism. Adipokines like leptin, adiponectin, and andvisfatin are well known insulin sensitizers and play a major role in glucose pathophysiology [71]. Adipocytes from the carp have been also reported to express insulin, which is termed carp adipocyte insulin. Studies have also shown that the transcription factor ISL-1 and PAX-6, which play important roles in pancreatic endocrine development, are also expressed in the proliferative population of ASCs [72, 73]. All these features make ASCs a prominent cell candidate for cell differentiation into pancreatic endocrine tissue for use in cell-based therapies for diabetes. ASCs can easily be obtained from the patient's own tissue, isolated *ex vivo*, expanded, differentiated into insulin-producing cells, and transplanted back into the patient as an autologous transplant.

Miscellaneous sources- Recent data suggest that in particular the post-partum umbilical cord-extracted Wharton Jelly (WJ) contains adult mesenchymal stem cells that can differentiate into ectodermal, mesodermal and endodermal cellular lineages and successfully be expanded *ex vivo* and cryopreserved [74]. WJ-derived MSC (WJ-MSC) have a gene expression profile similar to BM-MSC, although they also express additional markers (e.g. for CD117) [75, 76]. A differentiation study attempted conversion of adult stem cells from umbilical cord blood-derived mesenchymal cells (UCB-MSC), WJ-MSC and amniotic epithelial stem cells (AE-SC) into insulin-producing cells. WJ-MSC immune features resemble those of BM-MSC, since they do not express type II MHC (HLA-DR). Moreover, as we and others observed, these cells are further characterized by the expression of key molecules that are associated with immunomodulatory properties [77, 78]. These clearly enhance the MSCs potential, in terms of either cells that can transdifferentiate into IPCs or ancillary cells that may help islet cells or IPC of different origin to engraft and be functionally active in a diabetic

host [79]. WJ-MSCs could be very useful in allogeneic cell transplantation settings. In fact, an important requisite for allogeneic transplantation is low immunogenicity. A recent study assessed the long-term effects of the implantation of WJ-MSC in newly-onset T1D, with initial interesting results [80]. Ongoing studies where WJ-MSCs are combined with normal islet  $\beta$  cells, within a newly generated syncytium-like complex, possibly enveloped by alginate-based microcapsules, could not only enhance  $\beta$ -cell survival and function, but also potentiate cell graft acceptance by the host. All this could translate in efficient and performing cell grafts for T1D.

### Conclusion

T1D and T2D lead, by different mechanisms, involving autoimmune destruction pathways in the former and progressive toxicity (by lipid infiltration, and activation of cell apoptosis) in the latter, to destruction of the original pancreatic endocrine  $\beta$ -cell pool. The field of  $\beta$ -cell regeneration to substitute the diseased/destroyed cells, has fueled increasing hopes and enthusiasm as an alternative to either conventional insulin replacement therapy regimens or the more recent attempt to graft whole pancreatic or isolated islets from cadaveric donors. Either endogenous endocrine pancreatic regeneration by possibly stimulating organ-specific progenitor cells or use of cells derived from organs that share with the pancreas similar developmental pathways are very appealing, but still require seminal work to be performed. "Exogenous" stem cells such as MSCs derived from bone marrow or other sources, like the umbilical cord Wharton Jelly are extremely competitive due to their trans-differentiation capability coupled with potent immunomodulatory properties. Once safety issues were surmounted, use of recipient specific iPSC in conjunction with MSCs isolated from the same patient could be auspicious. Still under investigation is the possibility that by eliminating the  $\beta$ -cell directed autoimmune destruction vicious cycle, with the addition of proper stimuli, the original  $\beta$ -cell reservoir of the pancreas could be regenerated in T1D. Endogenous regeneration of the  $\beta$  cells would be the most physiological way to reconstitute a sufficient mass of IPC, with less invasive methods. This would also represent the ultimate frontier to cure T1D and T2D, the latter, in particular, when advanced  $\beta$ -cell dys-

fuction would make exogenous insulin treatment an ineludible choice.

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### References

- [1] Bluestone JA, Herold K and Eisenbarth G. Genetics, pathogenesis and clinical interventions in type 1 diabetes. *Nature* 2010; 464: 1293-1300.
- [2] Ashcroft FM and Rorsman P. Diabetes mellitus and the  $\beta$  cell: the last ten years. *Cell* 2012; 148: 1160-1171.
- [3] Jensen RA, Agardh E, Lernmark A, Gudbjörnsdottir S, Smith NL, Siscovick DS, Törn C and DISS Group. HLA genes, islet auto antibodies and residual C-peptide at the clinical onset of type 1 diabetes mellitus and the risk of retinopathy 15 years later. *PLoS One* 2011; 6: e17569.
- [4] Cefalu WT. American diabetes association-European association for the study of diabetes position statement: due diligence was conducted. *Diabetes Care* 2012; 35: 1201-1203.
- [5] CITR Research Group. 2007 Update on allogeneic islet transplantation from the Collaborative Islet Transplant Registry (CITR). *Cell Transplant* 2009; 18: 753-767.
- [6] Balamurugan AN, Naziruddin B, Lockridge A, Tiwari M, Loganathan G, Takita M, Matsumoto S, Papas K, Trieger M, Rainis H, Kin T, Kay TW, Wease S, Messinger S, Ricordi C, Alejandro R, Markmann J, Kerr-Conti J, Rickels MR, Liu C, Zhang X, Witkowski P, Posselt A, Maffi P, Secchi A, Berney T, O'Connell PJ, Hering BJ and Barton FB. Islet product characteristics and factors related to successful human islet transplantation from the Collaborative Islet Transplant Registry (CITR) 1999-2010. *Am J Transplant* 2014; 14: 2595-2606.
- [7] Nilsson B, Ekdahl KN and Korsgren O. Control of instant blood-mediated inflammatory reaction to improve islets of Langerhans engraftment. *Curr Opin Organ Transplant* 2011; 16: 620-626.
- [8] Murua A, Portero A, Orive G, Hernández RM, de Castro M and Pedraz JL. Cell microencapsulation technology: towards clinical application. *J Control Release* 2008; 132: 76-83.
- [9] Elliott RB, Escobar L, Tan PL, Muzina M, Zvain S and Buchanan C. Live encapsulated porcine islets from a type 1 diabetic patient 9.5 yr after xenotransplantation. *Xenotransplantation* 2007; 14: 157-161.
- [10] Dufrane D and Gianello P. Pig islet xenotransplantation into non-human primate model. *Transplantation* 2008; 86: 753-760.
- [11] Calafiore R, Basta G, Luca G, Lemmi A, Montanucci MP, Calabrese G, Racanicchi L, Mancuso F and Brunetti P. Microencapsulated pancreatic islet allografts into nonimmunosuppressed patients with type 1 diabetes: first two cases. *Diabetes Care* 2006; 29: 137-138.
- [12] Basta G, Montanucci P, Luca G, Boselli C, Noya G, Barbaro B, Qi M, Kinzer KP, Oberholzer J and Calafiore R. Long-term metabolic and immunological follow-up of nonimmunosuppressed patients with type 1 diabetes treated with microencapsulated islet allografts: four cases. *Diabetes Care* 2011; 34: 2406-2409.
- [13] Puri S and Hebrok M. Cellular plasticity within the pancreas—lessons learned from development. *Dev Cell* 2010; 18: 342-356.
- [14] Shao S, Fang Z, Yu X and Zhang M. Transcription factors involved in glucose-stimulated insulin secretion of pancreatic beta cells. *Biochem Biophys Res Commun* 2009; 384: 401-404.
- [15] Burlison JS, Long Q, Fujitani Y, Wright CV and Magnuson MA. Pdx-1 and Ptf1a concurrently determine the fate specification of pancreatic multipotent progenitor cells. *Dev Biol* 2008; 316: 74-86.
- [16] Kawaguchi Y, Cooper B, Gannon M, Ray M, MacDonald RJ and Wright CV. The role of the transcriptional regulator Ptf1a in converting intestinal to pancreatic progenitors. *Nat Genet* 2002; 32: 128-134.
- [17] Sellick GS, Barker KT, Stolte-Dijkstra I, Fleischmann C, Coleman RJ, Garrett C, Gloyn AL, Edghill EL, Hattersley AT, Wellauer PK, Goodwin G and Houlston RS. Mutations in PTF1A cause pancreatic and cerebellar agenesis. *Nat Genet* 2004; 36: 1301-1305.
- [18] Gradwohl G, Dierich A, LeMeur M and Guillemot F. Neurogenin3 is required for the development of the four endocrine cell lineages of the pancreas. *Proc Natl Acad Sci U S A* 2000; 97: 1607-1611.
- [19] Sussel L, Kalamaras J, Hartigan-O'Connor DJ, Meneses JJ, Pedersen RA, Rubenstein JL and German MS. Mice lacking the homeodomain transcription factor Nkx2.2 have diabetes due to arrested differentiation of pancreatic beta cells. *Development* 1998; 125: 2213-2221.
- [20] Servitja JM and Ferrer J. Transcriptional networks controlling pancreatic development and beta cell function. *Diabetologia* 2004; 47: 597-613.
- [21] Soria B, Roche E, Berná G, León-Quinto T, Reig JA and Martín F. Insulin secreting cells derived from embryonic stem cells normalize glycemia in streptozotocin-induced diabetic mice. *Diabetes* 2000; 49: 157-162.

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- [22] Lumelsky N, Blondel O, Laeng P, Velasco I, Ravin R and McKay R. Differentiation of embryonic stem cells to insulin secreting structures similar to pancreatic islets. *Science* 2001; 292: 1389-1394.
- [23] Sipione S, Eshpeter A, Lyon JG, Korbitt GS and Blackely RC. Insulin expressing cells from differentiated embryonic stem cells are not beta cells. *Diabetologia* 2004; 47: 499-508.
- [24] Rolletschek A, Kania G and Wobus AM. Generation of pancreatic insulin-producing cells from embryonic stem cells-‘proof of principle’, but questions still unanswered. *Diabetologia* 2006; 49: 2541-2545.
- [25] Blum B and Benvenisty N. The tumorigenicity of diploid and aneuploid human pluripotent stem cells. *Cell Cycle* 2009; 8: 3822-3830.
- [26] Knoepfler PS. Deconstructing stem cell tumorigenicity: a roadmap to safe regenerative medicine. *Stem Cells* 2009; 27: 1050-1056.
- [27] Kooreman NG and Wu JC. Tumorigenicity of pluripotent stem cells: biological insights from molecular imaging. *J R Soc Interface* 2010; 7 Suppl 6: S753-S763.
- [28] Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K and Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007; 131: 861-872.
- [29] Aoi T, Yae K, Nakagawa M, Ichisaka T, Okita K, Takahashi K, Chiba T and Yamanaka S. Generation of pluripotent stem cells from adult mouse liver and stomach cells. *Science* 2008; 321: 699-702.
- [30] Hanna J, Markoulaki S, Schorderet P, Carey BW, Beard C, Wernig M, Creighton MP, Steine EJ, Cassady JP, Foreman R, Lengner CJ, Dausman JA and Jaenisch R. Direct reprogramming of terminally differentiated mature B lymphocytes to pluripotency. *Cell* 2008; 133: 250-264.
- [31] Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, Slukvin II and Thomson JA. Induced pluripotent stem cell lines derived from human somatic cells. *Science* 2007; 318: 1917-1920.
- [32] Maehr R, Chen S, Snitow M, Ludwig T, Yagasaki L, Goland R, Leibel RL and Melton DA. Generation of pluripotent stem cells from patients with type 1 diabetes. *Proc Natl Acad Sci U S A* 2009; 106: 15768-15773.
- [33] Zhang D, Jiang W, Liu M, Sui X, Yin X, Chen S, Shi Y and Deng H. Highly efficient differentiation of human ES cells and iPSC cells into mature pancreatic insulin-producing cells. *Cell Res* 2009; 19: 429-438.
- [34] D’Amour KA, Bang AG, Eliazar S, Kelly OG, Agulnick AD, Smart NG, Moorman MA, Kroon E, Carpenter MK and Baetge EE. Production of pancreatic hormone expressing endocrine cells from human embryonic stem cells. *Nat Biotechnol* 2006; 24: 1392-1401.
- [35] Hua H, Shang L, Martinez H, Freeby M, Gallagher MP, Ludwig T, Deng L, Greenberg E, Leduc C, Chung WK, Goland R, Leibel RL and Egli D. iPSC-derived  $\beta$  cells model diabetes due to glucokinase deficiency. *J Clin Invest* 2013; 123: 3146-3153.
- [36] Thatava T, Kudva YC, Edukulla R, Squillace K, De Lamo JG, Khan YK, Sakuma T, Ohmine S, Terzic A and Ikeda Y. Intrapatient variations in type 1 diabetes specific iPS cell differentiation into insulin-producing cells. *Mol Ther* 2013; 21: 228-239.
- [37] Tateishi K, He J, Taranova O, Liang G, D’Alessio AC and Zhang Y. Generation of insulin-secreting islet-like clusters from human skin fibroblasts. *J Biol Chem* 2008; 283: 31601-31607.
- [38] Thatava T, Nelson TJ, Edukulla R, Sakuma T, Ohmine S, Tonne JM, Yamada S, Kudva Y, Terzic A and Ikeda Y. Indolactam V/GLP-1-mediated differentiation of human iPS cells into glucose-responsive insulin-secreting progeny. *Gene Ther* 2011; 18: 283-293.
- [39] Nostro MC, Sarangi F, Ogawa S, Holtzinger A, Corneo B, Li X, Micallef SJ, Park IH, Basford C, Wheeler MB, Daley GQ, Elefanty AG, Stanley EG and Keller G. Stage-specific signaling through TGF $\beta$  family members and WNT regulates patterning and pancreatic specification of human pluripotent stem cells. *Development* 2011; 138: 861-871.
- [40] Liew CG, Shah NN, Briston SJ, Shepherd RM, Khoo CP, Dunne MJ, Moore HD, Cosgrove KE, Andrews PW. PAX4 enhances beta-cell differentiation of human embryonic stem cells. *PLoS One* 2008; 3: e1783.
- [41] Teo AK, Wagers AJ and Kulkarni RN. New opportunities: harnessing induced pluripotency for discovery in diabetes and metabolism. *Cell Metab* 2013; 18: 775-791.
- [42] Jiang J, Au M, Lu K, Eshpeter A, Korbitt G, Fisk G and Majumdar AS. Generation of insulin-producing islet-like clusters from human embryonic stem cells. *Stem Cells* 2007; 25: 1940-1953.
- [43] Kroon E, Martinson LA, Kadoya K, Bang AG, Kelly OG, Eliazar S, Young H, Richardson M, Smart NG, Cunningham J, Agulnick AD, D’Amour KA, Carpenter MK and Baetge EE. Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin secreting cells in vivo. *Nature Biotechnol* 2008; 26: 443-452.
- [44] Shim JH, Kim SE, Woo DH, Kim SK, Oh CH, McKay R and Kim JH. Directed differentiation of human embryonic stem cells towards a pan-

## Stem cells for the cell and molecular therapy of T1DM

- creatic cell fate. *Diabetologia* 2007; 50: 1228-1238.
- [45] Mfopou JK, Chen B, Mateizel I, Sermon K and Bouwens L. Noggin, retinoids, and fibroblast growth factor regulate hepatic or pancreatic fate of human embryonic stem cells. *Gastroenterology* 2010; 138: 2233-2245, e1-14.
- [46] Kelly OG, Chan MY, Martinson LA, Kadoya K, Ostertag TM, Ross KG, Richardson M, Carpenter MK, D'Amour KA, Kroon E, Moorman M, Baetge EE and Bang AG. Cell-surface markers for the isolation of pancreatic cell types derived from human embryonic stem cells. *Nat Biotechnol* 2011; 29: 750-756.
- [47] Xu X, Browning VL and Odorico JS. Activin, BMP and FGF pathways cooperate to promote endoderm and pancreatic lineage cell differentiation from human embryonic stem cells. *Mech Dev* 2011; 128: 412-427.
- [48] Alipio Z, Liao W, Roemer EJ, Waner M, Fink LM, Ward DC and Ma Y. Reversal of hyperglycemia in diabetic mouse models using induced-pluripotent stem (iPS)-derived pancreatic beta-like cells. *Proc Natl Acad Sci U S A* 2010; 107: 13426-13431.
- [49] Zhu FF, Zhang PB, Zhang DH, Sui X, Yin M, Xiang TT, Shi Y, Ding MX and Deng H. Generation of pancreatic insulin-producing cells from rhesus monkey induced pluripotent stem cells. *Diabetologia* 2011; 54: 2325-2336.
- [50] Jeon K, Lim H, Kim JH, Thuan NV, Park SH, Lim YM, Choi HY, Lee ER, Kim JH, Lee MS and Cho SG. Differentiation and transplantation of functional pancreatic beta cells generated from induced pluripotent stem cells derived from a type 1 diabetes mouse model. *Stem Cells Dev* 2012; 21: 2642-2655.
- [51] Okita K, Ichisaka T and Yamanaka S. Generation of germline-competent induced pluripotent stem cells. *Nature* 2007; 448: 313-317.
- [52] Stadtfeld M, Nagaya M, Utikal J, Weir G and Hochedlinger K. Induced pluripotent stem cells generated without viral integration. *Science* 2008; 322: 945-949.
- [53] Okita K, Nakagawa M, Hyenjong H, Ichisaka T and Yamanaka S. Generation of mouse induced pluripotent stem cells without viral vectors. *Science* 2008; 322: 949-953.
- [54] Yamanaka S. Strategies and new developments in the generation of patient-specific pluripotent stem cells. *Cell Stem Cell* 2007; 1: 39-49.
- [55] Knoepfler PS. Why myc? An unexpected ingredient in the stem cell cocktail. *Cell Stem Cell* 2008; 2: 18-21.
- [56] Nakagawa M, Koyanagi M, Tanabe K, Takahashi K, Ichisaka T, Aoi T, Okita K, Mochiduki Y, Takizawa N and Yamanaka S. Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. *Nat Biotechnol* 2008; 26: 101-106.
- [57] Wernig M, Meissner A, Cassady JP and Jaenisch R. c-Myc is dispensable for direct reprogramming of mouse fibroblasts. *Cell Stem Cell* 2008; 2: 10-12.
- [58] Kern S, Eichler H, Stoeve J, Klüter H and Bieback K. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. *Stem Cells* 2006; 24: 1294-1301.
- [59] Oswald J, Boxberger S, Jørgensen B, Feldmann S, Ehninger G, Bornhäuser M and Werner C. *Stem Cells* 2004; 22: 377-384.
- [60] Pittenger MF and Martin BJ. Mesenchymal stem cells and their potential as cardiac therapeutics. *Circ Res* 2004; 95: 9-20.
- [61] Hung SC, Chen NJ, Hsieh SL, Ma HL and Lo WH. Isolation and characterization of size-sieved stem cells from human bone marrow. *Stem Cells* 2002; 20: 249-258.
- [62] Delorme B, Ringe J, Gallay N, Le Vern Y, Kerboeuf D, Jørgensen C, Rosset P, Sensebé L, Layrolle P, Häupl T and Charbord P. Specific plasma membrane protein phenotype of culture amplified and native human bone marrow mesenchymal stem cells. *Blood* 2008; 111: 2631-2635.
- [63] Reger RL, Tucker AH and Wolfe MR. Differentiation and characterization of human MSCs. *Methods Mol Biol* 2008; 449: 93-107.
- [64] Turnovcova K, Ruzickova K, Vanecek V, Sykova E and Jendelova P. Properties and growth of human bone marrow mesenchymal stromal cells cultivated in different media. *Cytherapy* 2009; 11: 874-885.
- [65] Weiss ML, Medicetty S, Bledsoe AR, Rachakattala RS, Choi M, Merchav S, Luo Y, Rao MS, Velagaleti G and Troyer D. Human umbilical cord matrix stem cells: preliminary characterization and effect of transplantation in a rodent model of Parkinson's disease. *Stem Cells* 2006; 24: 781-792.
- [66] Baksh D, Yao R and Tuan RS. Comparison of proliferative and multilineage differentiation potential of human mesenchymal stem cells derived from umbilical cord and bone marrow. *Stem Cells* 2007; 25: 1384-1392.
- [67] Oh SH, Muzzonigro TM, Bae SH, LaPlante JM, Hatch HM and Petersen BE. Adult bone marrow-derived cells trans-differentiating into insulin-producing cells for treatment of type I diabetes. *Lab Invest* 2004; 84: 607-617.
- [68] Xie QP, Huang H, Xu B, Dong X, Gao SL, Zhang B and Wu YL. Human bone marrow mesenchymal stem cells differentiate into insulin-producing cells upon microenvironmental manipulation in vitro. *Differentiation* 2009; 77: 483-491.

## Stem cells for the cell and molecular therapy of T1DM

- [69] Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, Alfonso ZC, Fraser JK, Benhaim P and Hedrick MH. Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell* 2002; 13: 4279-4295.
- [70] Schäffler A and Büchler C. Concise review: Adipose tissue-derived stromal cells-basic and clinical implications for novel cell-based therapies. *Stem Cells* 2007; 25: 818-827.
- [71] Kojima H, Fujimiya M, Matsumura K, Nakahara T, Hara M and Chan L. Extrapancreatic insulin-producing cells in multiple organs in diabetes. *Proc Natl Acad Sci U S A* 2004; 101: 2458-2563.
- [72] Timper K, Seboek D, Eberhardt M, Linscheid P, Christ-Crain M, Keller U, Müller B and Zulewski H. Human adipose tissue derived mesenchymal stem cells differentiate into insulin, somatostatin, and glucagon expressing cells. *Biochem Biophys Res Commun* 2006; 341: 1135-1140.
- [73] Chandra V, GS, Phadnis S, Nair PD and Bhonde RR. Generation of pancreatic hormone-expressing islet-like cell aggregates from murine adipose tissue-derived stem cells. *Stem Cells* 2009; 27: 1941-1953.
- [74] Romanov YA, Svintsitskaya VA and Smirnov VN. Searching for alternative sources of postnatal human mesenchymal stem cells: candidate MSC-like from umbilical cord. *Stem Cells* 2003; 21: 105-110.
- [75] La Rocca G, Anzalone R, Corrao S, Magno F, Loria T, Lo Iacono M, Di Stefano A, Giannuzzi P, Marasà L, Cappello F, Zummo G and Farina F. Isolation and characterization of Oct-4+/HLA-G+ mesenchymal stem cells from human umbilical cord matrix: differentiation potential and detection of new markers. *Histochem Cell Biol* 2009; 131: 267-282.
- [76] Montanucci P, Basta G, Pescara T, Pennoni I, Di Giovanni F and Calafiore R. New simple and rapid method for purification of mesenchymal stem cells from the human umbilical cord Wharton jelly. *Tissue Eng Part A* 2011; 17: 2651-2661.
- [77] Weiss ML, Anderson C, Medicetty S, Sesharedy KB, Weiss RJ, VanderWerff I, Troyer D and McIntosh KR. Immune properties of human umbilical cord Wharton's jelly-derived cells. *Stem Cells* 2008; 26: 2865-2874.
- [78] Deuse T, Stubbendorff M, Tang-Quan K, Phillips N, Kay MA, Eiermann T, Phan TT, Volk HD, Reichenspurner H, Robbins RC and Schrepfer S. Immunogenicity and immunomodulatory properties of umbilical cord lining mesenchymal stem cells. *Cell Transplant* 2011; 20: 655-667.
- [79] Ricordi C, Inverardi L and Domínguez-Bendala J. From cellular therapies to tissue reprogramming and regenerative strategies in the treatment of diabetes. *Regen Med* 2012; 7: 41-48.
- [80] Hu J, Yu X, Wang Z, Wang F, Wang L, Gao H, Chen Y, Zhao W, Jia Z, Yan S and Wang Y. Long term effects of the implantation of Wharton's jelly-derived mesenchymal stem cells from the umbilical cord for newly-onset type 1 diabetes mellitus. *Endocr J* 2013; 60: 347-357.