Published in final edited form as:
Expert Opin Biol Ther. 2011 January ; 11(1): 41–53. doi:10.1517/14712598.2011.540235.

Stem cell-based strategies for the treatment of Type 1 diabetes mellitus

Yujie Wen, **Bo Chen**, and **Suzanne T. Ildstad**[¶]
Institute for Cellular Therapeutics, University of Louisville, Louisville, KY, 40202

Abstract

Importance of the field—Beta cell regeneration and beta cell preservation are two promising therapeutic approaches for the management of patients with Type 1 diabetes (T1D). Stem cell-based strategies to address the problems of shortage in beta cells, autoimmune and alloimmune responses have become an area of intense study.

Areas covered in this review—This review focuses on the progress that has been made in obtaining functional, insulin-producing cells from various types of stem/progenitor cells, including the current knowledge on the immunomodulatory roles of hematopoietic stem cell and multipotent stromal cell in the therapies for T1D.

What the reader will gain—A broad overview of recent advancements in this field is provided. The hurdles that remain in the path of using stem cell-based strategies for the treatment of T1D and possible approaches to overcome these challenges are discussed.

Take home message—Stem cell-based strategies hold great promise for the treatment of T1D. In spite of the progress that has been made over the last decade, a number of obstacles and concerns need to be cleared before widespread clinical application is possible. In particular, the mechanism of ESC and iPSC-derived beta cell maturation *in vivo* is poorly understood.

Keywords

Type 1 diabetes; Stem cells therapy; Regenerative medicine; Transplantation

1. Introduction

Type 1 diabetes (T1D), also known as insulin-dependent diabetes, is an autoimmune disease that targets insulin-secreting pancreatic beta cells for destruction. T1D remains a major cause of long-term morbidity and mortality, affecting more than one percent of the population worldwide. All patients with T1D and one-third of the patients with Type 2 diabetes require insulin treatment to prevent death from hyperglycemia resulting from the loss of pancreatic islets. T1D has various complications including renal failure, proliferative retinopathy leading to blindness, peripheral neuropathy, and vascular disease. The discovery of insulin has prevented death from acute diabetes. However, even tight glucose control does not prevent the systemic complications [1]. Transplantation of whole pancreas or purified insulin-producing islets is the preferred approach to achieve glucose homeostasis, especially for a specific population of T1D patients who do not respond to conventional therapy [2].

[¶]Correspondence should be addressed to (STI): Suzanne T. Ildstad, M.D., Director, Institute for Cellular Therapeutics, The Jewish Hospital Distinguished Professor of Transplantation, Distinguished University Scholar, Professor of Surgery, Microbiology and Immunology, Physiology, University of Louisville, 570 South Preston Street, Suite 404, Louisville, Kentucky 40202-1760, USA, Telephone: 502-852-2080, Fax: 502-852-2079, stilds01@gwise.louisville.edu.

Even though promising results, including reduction or freedom from insulin, better glucose stability, and less risk of complications, have been achieved, the major obstacle to widespread use of pancreatic or islet transplantation is the shortage of suitable donor tissues [2,3] and the inferior long-term results, including graft rejection and nephrotoxicity from the immunosuppressive agents [4]. An attractive strategy to address this problem would be to generate islets from other types of cells, such as stem cells. This review focuses on the progress made in obtaining functional, insulin-producing cells from various types of stem/progenitor cells, emphasizes the current knowledge on the immunomodulatory role of hematopoietic stem cells and multipotent stromal cells in the therapies for T1D (Figure 1), and speaks to the continuing obstacles that must be addressed.

2. Pluripotent stem cells: ESC and induced pluripotent stem cells (iPSC)

Embryonic stem cells (ESC) are pluripotent cells derived from the inner cell mass of a blastocyst and have the potential to form derivatives from all three germ cell layers (mesoderm, ectoderm, and endoderm) [5]. Embryology has offered important insights into key developmental pathways regulating ESC differentiation, resulting in advances in modeling gastrulation in culture and in the efficient induction of endoderm, mesoderm, and ectoderm and many of their downstream derivatives [6]. The key signaling pathways regulating differentiation of ESC to pancreatic hormone-expressing endocrine cells include the TGF-beta signaling pathway, the Wnt/beta-catenin signaling pathway, the Hedgehog signaling pathway, Notch signaling pathway, and PI3K signaling pathway [7]. The studies of embryonic pancreas development have been used as the basis for the directed, step-wise differentiation of mouse and human embryonic stem cells into pancreatic endocrine cells [8]. By applying modulators to reproduce the aforementioned developmentally active or inactive signaling pathways, several ESC differentiation protocols recapitulating pancreatic development *in vivo* have been used to guide differentiation of ESC toward definitive endoderm, pancreatic progenitors, followed by endocrine progenitors *in vitro*. Lumelsky et al. first described a five-step protocol for *in vitro* generation of insulin-expressing cells and other pancreatic endocrine hormones from mouse embryonic stem cells (mES) [9]. Although later studies did not confirm insulin production by the differentiated ES cells [10,11], but rather demonstrated uptake of insulin present in the media supplements in which the cells were cultured [12,13], the initial study generated interest in development of future differentiation protocol. D'Amour et al. [14,15] further developed a differentiation process that converts human embryonic stem (hES) cells to endocrine cells capable of synthesizing the pancreatic hormones insulin, glucagon, somatostatin, pancreatic polypeptide and ghrelin. This process mimics *in vivo* pancreatic organogenesis by directing cells through stages resembling definitive endoderm, gut-tube endoderm, pancreatic endoderm and endocrine precursors. Moreover, the hES cell-derived insulin-expressing cells have an insulin content approaching that of adult islets. More recently, Luc Bouwens et al. modified the D'Amour's protocol by shifting developmental pathways from hepatic to pancreatic cell differentiation at the definitive endoderm stage to favor pancreatic over hepatic differentiation, and reported improved and more efficient differentiation of pancreatic progenitors from multiple human embryonic stem cell lines [16,17]. These proof-of-concept studies demonstrate that *in vitro* recapitulating signals controlling the development of the endocrine pancreas *in vivo* offer a promising strategy for beta cell generation [7].

The obvious prerequisite for embryonic stem (ES) cell-based beta cell replacement therapies is that ES cell-derived beta cells must be capable of secreting fully processed insulin in response to glucose in a physiologic manner. However, functional characterization of endocrine cell populations differentiated from hES cells by D'Amour's protocol showed that those cells did not respond to glucose stimulation to secrete insulin *in vitro* [15]. To overcome this limitation, researchers attempted to optimize the protocol and tested the

survival and function of ES cell derived beta-like cells *in vivo*. Shi et al. [18] developed a novel three-stage short-term induction method to induce mES cells into insulin-producing cells, which are able to lower blood glucose in mice rendered diabetic by treatment with the beta cell cytotoxic agent streptozotocin (STZ). By combining activin A and all-trans retinoic acid in a chemically defined medium, and other maturation factors such as basic fibroblast growth factor (bFGF) and nicotinamide in DMEM/F12, this group further demonstrated an optimized approach in serum-free culture medium without a feeder layer of stromal cells to induce hES cells to differentiate into functional insulin-producing cells [19]. The secretion of insulin by these cells was responsive to variations in glucose levels and the percentage of C-peptide positive cells exceeded 15%. However, in *in vivo* functional tests after transplantation of the differentiated cells into the renal capsules of STZ induced diabetic mice, only 30% of animals showed an obvious rescue of their hyperglycemic phenotype. Another group applied a similar serum-free protocol for differentiating hES cells into insulin-producing islet-like clusters (ILC) [20]. The cells that expressed pancreatic endocrine markers within ILC are likely to represent an immature phenotype as they contained significantly less insulin than adult beta cells, however, they responded to high glucose challenge *in vivo* and extended the survival of graft recipients when transplanted them into STZ-induced diabetic mice [21]. Recent research from Baetge and colleagues [22] described pancreatic endoderm derived from hES cells by a modified five-stage protocol efficiently generates glucose-responsive endocrine cells after implantation into mice. The cells are morphologically and functionally similar to normal pancreatic islets. And after a few months of maturation *in vivo*, implantation of hES cell-derived pancreatic endoderm protects against STZ-induced hyperglycemia in the majority (92%) of implanted mice expressing high levels of C-peptide before STZ treatment. Their results provide definitive evidence that hES cell-derived pancreatic endoderm can differentiate further *in vivo* and hES cell are competent to generate glucose-responsive, insulin-secreting cells. Their findings also suggest the need for *in vivo* differentiation to derive functionally mature beta cells from ESC [23]. Two subsequent studies from other groups also support that the presence of *in vivo* factors is important for the final stages of maturation [24,25]. A recent report demonstrates that final maturation to islet-specific cells and a high yield of pancreatic islet cells are achieved by co-culturing the ESC-derived pancreatic endocrine cells with endothelial cells [26], which suggests that the signals received from interactions of pancreatic endocrine cells with endothelial cells and extracellular matrix are necessary for ESC-derived beta cell maturation. Collectively, these *in vivo* studies confirm the potential of using ES cell-derived beta cells for the treatment of diabetes.

To bring ES cell-based beta cell replacement therapies for diabetes closer to reality, one of the hurdles that must be overcome is to generate mature and functional beta cells in sufficient quantity. Current protocols for direct differentiation of ES cells into beta cells employ recombinant protein and growth factors and are not cost-effective to generate therapeutic quality beta cells for clinic transplantation purposes [27]. Therefore, the focus has shifted to the possible use of small molecules as imitators of the cellular signaling events for large-scale, reproducible, directed ES cell differentiation under good manufacturing practice conditions [28]. Implementation of high-throughput and high-content screening assays of chemical libraries to embryonic stem cell research has led to the identification of several small molecules that promote short-term hES maintenance and direct early lineage choice during differentiation [29,30]. Zhu et al. identified a small molecule named stauprimide that increases the efficiency of the directed differentiation of mouse and human ES cells in synergy with defined extracellular signaling cues [31]. Affinity-based methods revealed that stauprimide interacts with NME2 and inhibits its nuclear localization and down-regulates expression of c-Myc. In a screen of 4000 compounds, Melton and colleagues [32] also identified two cell-permeable small molecules that direct differentiation of ESC into the endodermal lineage through induction of Smad2 phosphorylation. These

compounds induce nearly 80% of ESC to form definitive endoderm, a significantly higher efficiency than that achieved by Activin A or Nodal. Moreover, the chemically induced endoderm expresses multiple endodermal markers, can participate in normal development when injected into developing embryos, and can form pancreatic progenitors. For promoting differentiation from definitive endoderm to pancreatic progenitors, the same group identified indolactam V, an agonist of protein kinase C, induces differentiation of a substantial number of Pdx1-expressing cells from human ES cells. In addition, the Pdx1-expressing cells express other pancreatic markers and some of these Pdx1-expressing cells form insulin-expressing cells after transplantation into the kidney capsule of nude mice. Taken together, with the discovery of small molecules that specifically promote endoderm and pancreatic differentiation, the ES cell field is entering a new phase in which the possibility of using stem cells for human therapies edges closer to feasibility [27].

A landmark breakthrough of pluripotent stem cell research was made in 2006 when Takahashi and Yamanaka developed strategies to induce adult somatic cells to become pluripotent stem cells, also known as iPSC, by introducing a few key transcription factors or pluripotency genes including Oct3/4, Sox2, c-Myc, and Klf4 [33]. iPSC derived from mouse or human adult fibroblasts showed similar capacities to ESC in terms of genetic, epigenetic, and developmental criteria. iPSC are attractive cell therapy candidates derived from autologous cells because they avoid the inferior outcomes of beta cell replacement therapy in diabetes for transplanted allogeneic pancreas, isolated islets, and engineered insulin-producing cells that are destroyed by the recipient immune system [34]. Recently, disease-specific iPSC have been generated from a wide variety of adult somatic cell types from donors with various disease conditions, including T1D, to fulfill therapeutic applications [35–42]. Maehr et al. reported that patient-specific iPSC can be generated from patients with T1D by reprogramming their adult fibroblasts with the transcription factors Oct4, Sox2, and Klf4. T1D–specific iPSC derived from patients with T1D, termed DiPS cells, have hallmarks of pluripotency and can be spontaneously differentiated into insulin-producing cells, as well as offering a few significant advantages including containing the genotype responsible for the human disease, providing an immunologically matched autologus cell population and making possible patient-specific disease modeling wherein the initiation and progression of T1D can be studied [37]. Using a serum-free protocol, Tateishi et al. confirmed that human iPSC can be derived from human skin cells by retroviral expression of Oct4, Sox2, c-Myc, and Klf4. Moreover, they successfully generated ILC from the iPSC under feeder-free conditions. ILC not only contain C-peptide-positive and glucagon-positive cells but also release C-peptide upon glucose stimulation [43]. More recently, Alipio et al. demonstrated that iPS derived from skin fibroblast could be differentiated into insulin-secreting beta-like cells, which respond to glucose stimulation under physiological or pathological conditions. These beta-like cells stably engrafted and corrected the hyperglycemia in two mouse models of type 1 and 2 diabetes [44]. These data raise the possibility that patient-specific iPSC could potentially provide a treatment for diabetes in the future.

For improved induction of human iPSC to achieve high reprogramming efficiency, a chemical screening platform was configured to determine the small molecules that have direct impact on the signaling pathways of reprogramming process [45,46]. More recently, Amit et al. developed a suspension culture system for undifferentiated hES cells and iPSC, using medium supplemented with IL6RIL6 chimeras (interleukin-6 receptor fused to interleukin-6), and bFGF. They demonstrate that the IL6RIL6 chimera supports the self-renewal and expansion of undifferentiated ESC and iPSC in suspension, and thus present another efficient system for large-scale propagation of undifferentiated pluripotent cells for clinical and translational applications [47].

The formation of teratomas or other tumors known to arise from ESC and iPSC is a major safety concern [22,48]. Cell-sorting-based approaches may prove suitable for selection of residual undifferentiated ESC and iPSC *ex vivo* prior to transplantation [49], and building reporter constructs engineered to encode a suicide gene expression is a method for tracking and ablating any transformed cells that appear *in vivo* in the patient [50–52]. A recent study identified a new signaling pathway that the undifferentiated cells use for propagation, which depends upon the phosphorylation of the transcription factor Nanog [53]. By inhibiting this pathway with small-molecule compounds, there was a stark reduction in resulting teratomas. Their study suggests that teratomas can be eliminated. The use of iPSC to produce differentiated beta cells induced with retroviral vectors to deliver oncogenes as reprogramming factors may cause intentional mutagenesis and malignant diseases. Zhang et al. [54] developed a novel chemical-defined culture system to induce insulin-producing cells from patient specific human fibroblasts. A panel of human iPSC was generated by transducing the lentivirus containing Oct4, Sox2 and Klf4, cMyc was omitted in order to decrease the risk of tumorigenicity. The generated iPSC expressed pluripotency marker genes, such as Sea4, Nanog, TRA-1–60 and TRA-1–81. These cells were also co-expressed beta cell markers Pdx1, Mafa, Glut2 and insulin. In addition, the possibility that virus-free and transgene-free iPSC might be generated using only valproic acid and recombinant proteins or a non-viral minicircle vector is currently being explored in the murine system [55,56].

3. Organ-specific facultative stem/progenitor cells

Endocrine stem/progenitor cells in the pancreas

The pancreas is comprised of two main compartments: endocrine and exocrine. The exocrine cell compartment consists of acinar and duct cells. As the most abundant cell type in pancreas, the acinar compartment has been regarded as the possible site where endocrine progenitor cells reside in the pancreas. Several studies have suggested that functional insulin producing cells can be generated by *in vitro* transdifferentiation from rat exocrine tissue by adding agonists of the JAK2/STAT3 signaling pathway (epidermal growth factor and leukemia inhibitory factor) to the medium [57]. The acinar cells that undergo exocrine-to-endocrine transdifferentiation first need to re-express a lineage-defining transcription factor Neurogenin 3 (Ngn3) and then need to escape inhibition by Notch signaling. The insulin-expressing cells that are generated with this approach are glucose-regulated and can normalize hyperglycemia after transplantation into immunoincompetent diabetic mice [58]. Analysis by both non-genetic and genetic lineage tracing systems indicated these newly made insulin-producing cells originate from acinar cells [59,60]. Okuno et al. demonstrated that insulin-secreting cells can be generated by transdifferentiation from pancreatic acinar cells of diabetic rodents and further suggests that pancreatic acinar cells represent a potential source of autologous transplantable insulin-secreting cells for treatment of T1D [61].

Substantial evidence has also suggested that the duct compartment contains facultative endocrine stem/progenitor cells that can differentiate into beta cells [62,63]. Yatoh et al. first showed that affinity-purified duct cells from adult human pancreas can differentiate to insulin-producing cells [64]. Hao et al. marked non-endocrine epithelial cells derived from human pancreas and showed that non-endocrine epithelial cells can differentiate into endocrine cells in response to inductive factors present in the fetal pancreas [65]. The potential of duct cells to undergo endocrine differentiation was further confirmed by *in vivo* experiments. Xu et al. found Ngn3-positive endocrine progenitors can be activated in injured adult mouse pancreas following partial duct ligation (PDL) and the Ngn3-positive cells emerge from the pancreatic ducts. Differentiation of the adult progenitors is Ngn3-dependent and gives rise to all islet cell types, including glucose-responsive beta cells that subsequently proliferate, both in situ and when cultured in embryonic pancreas explants

[66]. Recently, Zhou et al. reprogrammed differentiated pancreatic exocrine cells of adult mice into cells that closely resemble beta cells by re-expressing key developmental regulators (a combination of three transcription factors Ngn3, Pdx1 and Mafa) *in vivo* [67]. These *in vivo* experiments showed that mature pancreatic duct cells do contribute to pancreatic regeneration after partial pancreatectomy, and mature ducts can regress and recapitulate the embryonic differentiation program to form all differentiated pancreatic cell types [68].

However, the ductal origin of endocrine cell formation in adult injured pancreas remains controversial. By genetically marking ductal cells using carbonic anhydrase II (CAII) as a duct-cell specific promoter to drive Cre recombinase in the Cre/loxP-based direct cell lineage tracing system, the Bonner-Weir group showed that CAII-positive pancreatic cells are progenitors for both endocrine and exocrine pancreas after birth or PDL in adult mice [69]. The Ferrer group used a similar system with the Hnf1-beta promoter to label ductal epithelium and found pancreatic exocrine duct cells give rise to insulin-producing beta cells during embryogenesis but not after birth. By genetically marking duct and acinar cells according to their expressing the mucin gene Muc1, another recent study also came to the same conclusion [70]. The contradictory results may be explained by the hypothesis that there is functional heterogeneity of the morphologically defined ductal cells and duct cells are heterogeneous in their potential to act as pancreatic progenitors [71]. A recent report in the American Diabetes Association's 70th Scientific Sessions by the Bonner-Weir group supported this hypothesis [72]. They found heterogeneity of protein expression for Sox9 and Hnf1-beta in duct cells within the ductal tree of adult mice and humans and provided evidence for ductal subpopulations serving as postnatal pancreatic progenitors.

Beta cell regeneration might be achieved from other endocrine cells. Collombat et al. demonstrated that ectopic and conditional expression of the transcription factor Pax4 in mouse pancreas using different cell-specific promoters converts progenitor cells into alpha and subsequently beta cells. Notably, ectopic expression of Pax4 in alpha cells promotes the reconstitution of the insulin-expressing cell mass and cures diabetes in animals that have been chemically depleted of beta cells [73]. Chung et al. recently developed a new mouse model of beta cell regeneration by combining PDL with elimination of preexisting beta cells with alloxan. In this model, they found that virtually all beta cells observed are neogenic and large numbers of beta cells were generated within two weeks. Most importantly, the neogenic beta cells arose primarily from alpha cells [74]. In a transgenic mouse model of diphtheria-toxin-induced acute selective near-total beta cell ablation, under the condition of insulin treatment and in the absence of autoimmunity, mature glucagon-producing alpha cells are identified as the origin of beta cells regeneration by genetic lineage tracing [75]. Whether the observed beta cell regeneration through spontaneous conversion of alpha cells also occurs in human pancreas of T1D patients with pre-existing autoimmunity to beta cells is not known, but the approaches to modulate immune system of T1D patients might enhance beta cell preservation by promoting spontaneous conversion of alpha cells to beta cells. In summary, the results of these studies confirms the potential plasticity in adult pancreatic cells reported previously and reveals the unrecognized function of alpha cells as beta cell progenitors.

Liver cells

Liver and pancreas arise from the same region of ventral foregut endoderm in response to the same signals during development. They share some specific differentiation pathways and expression of many specialized genes such as transcription factors, glucose transporter type 2 and glucokinase [76–78]. It is hypothesized that because liver cells share many transcription factors with beta cells, they may retain the potential to produce endocrine pancreatic hormones. These characteristics make liver cells an attractive source for

transdifferentiation into beta-like cells. A variety of liver cells including hepatocytes, intra-/extrahepatic biliary epithelial cells, and gall-bladder epithelium have been demonstrated to show the phenotype of pancreatic lineages after viral introduction of different pancreatic transcription factors such as Pdx1 [79–81]. Recently, Yechoor et al. reported transfer of a single lineage-defining transcription factor Ngn3 is sufficient to induce a transdetermination from a hepatic progenitor cells to an islet lineage, and resulted in a rapid reversal of hyperglycemia as well as the significantly reduced blood glucose [82].

Spleen cells

Whether spleen-derived stem cells can be used to generate beta cells to treat T1D is still controversial. In 2003, Kodama et al. reported the existence of stem cells in the spleen of mice that could increase the speed of pancreas regeneration if the underlying autoimmune disease was controlled by administration of complete Freund's adjuvant, donor spleen cells, and temporary islet transplantation. More than 90% of non-obese diabetic (NOD) mice maintained euglycemia after the transplanted islets had been removed [83]. These results were so attractive since the spleen is not as essential to humans compared to islets, and it would dramatically increase the donor supply. In addition, spleen cells also share a very close developmental relationship with the pancreas, as the splenic mesenchyme budding off from the pancreatic mesenchyme early in development [84]. Some research teams also identified that splenic stem cells contribute to the regeneration of many tissues including bone, heart, and salivary glands in animals[85,86]. Faustman et al. recently reported that the spleen of a variety of adult species (including humans) possesses a reservoir of multilineage adult CD45 negative stem cells that express the developmental transcription factor Hox11/Tlx1, which is essential for development of the spleen, pancreas, and portions of the nervous system [87]. However, other groups failed to reproduce their findings using a similar treatment protocol. The results achieved by three independent efforts were remarkably consistent to Kodama's conclusion: no splenocyte contribution to the islets was observed, even though diabetes could be reversed in this mouse model. Therefore, the recovered host beta cells, rather than spleen cells, were believed be responsible for this reversal [88–90]. Additional studies are underway to confirm and enhance the role of splenocytes in islet neogenesis.

Very small embryonic-like (VSEL) cells

The infusion of bone marrow cells has been reported to improve damaged pancreas function in STZ-treated mice, and restored blood glucose and serum insulin levels to almost normal within a relatively short period [91,92]. Recent studies suggest that hematopoietic stem cells (HSC) may not be the main candidate for beta cell differentiation [93,94] but that another type of stem cell, $CD45^{-}/Sca\text{-}1^{+}/Lin^{-}$ VSEL stem cells, may contribute to tissue repair and regeneration [95]. Using a low-dose STZ induced moderate pancreatic damage and hyperglycemia model, we recently reported that VSEL stem cells are mobilized in response to injured pancreatic tissue and contribute to beta cell regeneration, which were significantly enriched for developmental pancreatic transcription factors including Pdx1, Nkx6.1, and Ptf1. However, normoglycemia was not sustained long term, suggesting that additional stimuli are required for a more durable and robust effect [96].

4. Hematopoietic stem cells

The best-known and best-studied example of stem cells is hematopoietic stem cells (HSC). HSC are located in the bone marrow niche and are characterized by the expression of cell-surface markers, which allows for isolation by cell-sorting-based approaches[97]. HSC can be readily harvested from bone marrow and umbilical cord blood. It also can be collected

from peripheral blood after mobilization from the marrow with growth factor mobilization agents [98].

Hematopoietic stem cell transplantation (HSCT) has been used for the treatment of autoimmune disease [99]. The transient lymphoablation by conditioning regimens before autologous HSCT allows immune regeneration and a resetting of immune self-tolerance from the multipotent HSC [100]. The Diabetes Control and Complications Trial demonstrated a reverse relationship between residual C-peptide production and development of chronic complications [101]. It suggests preservation of endogenous beta cell function is a potential target in the management of patients with T1D. The first autologous HSCT study in T1D patients was performed at a center in Brazil. After a mean follow-up of 29.8 months post autologous HSCT, C-peptide levels increased significantly and the majority of patients (20 of 23 newly diagnosed T1D patients) achieved insulin independence with good glycemic control, in which twelve of them stayed free of insulin for an average of 31 months and eight patients had periods ranging from 6 to 47 months in which they were free from insulin [102,103]. More recently, a group in Poland reported the results of 8 patients receiving autologous HSCT for treatment of T1D [104]. Patients became independent of exogenous insulin after transplantation. One patient resumed low-dose insulin 7 months after transplantation. Six out of eight patients were given acarbose for better glycemic control after transplantation. All patients exhibited good glycemic control: the average HbA1c concentrations were 12.3% at diagnosis, and 5.6 and 6.2% at 3 and 6 months after transplantation, respectively. Both studies suggest that independence from exogenous insulin can be achieved in most new-onset T1D patients following immunoablation and reconstitution of the immune system with autologous HSCT.

The clinical experience of allogeneic HSCT in T1D patients is limited but shows promise. When patients with insulin-dependent T1D received bone marrow transplants due to leukemia or other blood-borne diseases, there was no improvement of diabetes after the transplant[105]. In a T1D mouse model, allogeneic bone marrow transplant reversed autoimmune process and prevented development of T1D if allogeneic HSCT was performed in pre-diabetic animals [106–108]. In overtly diabetic animals, allogeneic HSCT induced hematopoietic chimerism without causing graft-versus-host disease and reversed hyperglycemia in new-onset diabetic animals [109,110]. Notably, recovery of endogenous beta cell function occurs in both pre-diabetic and new-onset diabetic animals rendered chimeric [110,111], which may suggest that residual beta cells play a significant role in determining the outcome of allogeneic HSCT. On the other hand, islet transplantation is currently limited by the toxicity of immunosuppressive agents and failure to control the autoimmune response. Induction of tolerance to alloreactive and auto-reactive response by allogeneic HSCT has been achieved in T1D animal models [112–115]. However, combined islet and HSC allotransplantation under an ‘Edmonton-like’ immunosuppression, without ablative conditioning, did not lead to stable chimerism and graft tolerance in a clinical pilot trial [116]. Taken together, these results suggest that durable mixed chimerism may prove to be an important approach to halt the autoimmune process and induce tolerance in T1D. As nonmyeloablative conditioning approaches become refined and less toxic, the establishment of mixed chimerism may not only induce tolerance to islet allografts, but also halt the progression of the autoimmune process.

5. Multipotent mesenchymal stromal/stem cells

Multipotent Mesenchymal stromal/stem cells (MSCs) are a heterogeneous population of adult stem cells originally isolated from bone marrow (BM). They have also been identified in cord blood, the perivascular niche, and adipose tissue [117–121]. MSC have been recognized as a promising source for regenerative medicine due to their ability to undergo

multilineage differentiation to various cell types of mesenchymal origin, and their ability to stimulate proliferation and differentiation of resident progenitor cells, as well as secrete multiple cytokines and chemokines [122]. MSC can be isolated from the bone marrow and be easily separated from the HSC by their adherence to plastic [123]. However, there are no uniform phenotypic markers to characterize MSC, and investigators are using diverse methods to isolate and expand. The International Society for Cellular Therapy recommended that their name be changed to “multipotent mesenchymal stromal cells” because the majority of MSC lack complete “stemness” property. They also proposed minimal criteria for standardization of preparations[124], in which MSC must express CD105, CD73 and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79-alpha or CD19 and histocompatibility complex antigen (HLA)-DR surface molecules. Recently, evidence suggests that MSC also play an important role in immunomodulation mainly through the direct inhibition of cell differentiation or proliferation, the alteration of cytokine secretion profiles of T cells, B cells, dendritic cells (DC), and natural killer cells, and the induction of T regulatory cells (T_{reg}) [125–129]. These immunomodulatory properties make MSC a promising source for therapeutic applications to treat numerous autoimmune diseases and induce allograft transplant tolerance [128,130]. Madec et al. recently reported that MSC could prevent autoimmune beta cell destruction and subsequent diabetes by inducing IL-10-secreting $CD4^+$ $FoxP3^+$ T_{reg} [131]. Using a monkey model of islet/bone marrow transplantation, Berman et al. demonstrated that MSC may provide an important approach for enhancement of islet engraftment and function, and the mechanism was associated with increased T_{reg} numbers in peripheral blood [132].

MSC offer an attractive source of stem cells for generation of surrogate beta cells[121,133]. Expression of Pdx1 gene has been found in bone marrow derived MSC from human donors, followed by the activation of expression of all four islet hormones *in vitro*. Transplantation of these cells into streptozotocin-diabetic immunodeficient mice resulted in further differentiation to insulin producing cells, including induction of Neurogenic differentiation 1, and reduction of hyperglycemia [133]. Neshati et al. also demonstrated that bone marrow derived MSC expressed characteristics of insulin-producing cells including spherical, grape-like morphology, secretion of insulin, and being positive for dithizone in an *in vitro* environment with high glucose concentration, nicotinamide and beta-mercaptoethanol [134]. MSC treatment of experimental T1D resulted in long-term reversal of hyperglycemia, and therapy was shown to alter diabetogenic cytokine profile, to diminish T cell effector frequency in the pancreatic lymph nodes, to alter antigen-presenting cell frequencies, and to augment the frequency of the plasmacytoid subset of DC [134,135]. MSC from human umbilical cord blood and murine adipose tissue were also studied and showed the capacity to differentiate into the pancreatic lineage with insulin secretion functions [136,137]. However, this cell lineage transition has not been confirmed. Lee et al. found that the infusion of human bone marrow derived MSC to diabetic NOD/SCID mice resulted in significantly increased mouse insulin levels accompanied by improved pancreatic islets, even though rare human cells were found within the islets. Therefore, they proposed the possibility that MSC may be useful in enhancing insulin secretion instead of differentiation to insulin producing cells [138,139]. Sordi et al. indicate that MSC express negligible levels of islet-specific genes both in basal and stimulated conditions and might not optimal candidates for generation of physiologically competent beta cells [140]. MSC could exert an indirect role of “helper” cells and provide trophic support to the injured tissues in tissue repair process. Park et al. reported that co-culture of islets and MSC improved the ATP / ADP ratio and insulin secretory function *in vitro* [141]. Ding et al. demonstrated that MSC could prevent islet allograft rejection leading to stable and long-term normoglycemia. These functions mainly through the matrix metalloproteinases (MMP) secreted by MSC, in particular MMP-2 and MMP-9, to reduce surface expression of CD25 on responding T cells [142]. Other mechanisms underlying MSC’s protective role in experimental autoimmune

T1D include the T cell cytokine secretion pattern shift toward IL-10/IL-13 production and the induction of T_{reg} [143], as well as the promotion of pancreatic islet graft vascularization [144,145].

6. Expert Opinion

Generation of insulin-secreting cells from ESC and iPSC holds great promise for the treatment of T1D. Despite recent progress in research on beta cell regeneration from ESC and iPSC, a number of scientific issues and medical limitations need to be addressed and be overcome before ESC or iPSC-derived beta cells can be considered safe for clinical applications. The first hurdle is the tumorigenic potential of ESC and iPSC. One strategy for dealing with this problem is to select and develop pure populations of more committed cells for transplantation. Therefore, future development of an ESC and iPSC cell-based therapy will require the purification of properly specified endodermal populations and the elimination of inappropriate cell types. Further research to study mechanisms of beta cell maturation *in vivo* and signals that drive differentiation to endocrine progenitors and/or mature beta cells is required to realize the full potential of this therapy. The second hurdle is the fact that both generation of iPSC and differentiation of ESC or iPSC to mature beta cells still suffer from low efficiency and high cost. Small-molecule library screening identifies simple imitators of the cellular signaling events that normally guide formation of the pancreas and its insulin-secreting beta cells, enabling cost-effective differentiation methods for large-scale generation of transplantation quality beta cell from ESC or iPSC [146]. Marked differences in differentiation propensity among hES cell lines and iPSC generated from various tissues have been reported[147,148]. Therefore, screening and deriving lines is important to achieve efficient lineage-specific differentiation. The third hurdle is prevention of alloimmune rejection and recurrent autoimmune destruction. Transplantation of hES cell-derived beta cells into patients is limited by potential HLA incompatibility. Strategies to reduce immune rejection include: 1) establishing a large ‘bank’ of ESC for the purpose of matching its HLA phenotype to recipient transplant patients[149]; 2) microencapsulation techniques to protect grafted tissues from the immune system [28]; and 3) using ESC generated from parthenogenetic embryos as a source of histocompatible cells and tissues for cell therapy [150]. Although generating autologous patient-specific beta cells from iPSC solves the allorecognition issue, and bypasses some of the ethnical problems of ESC, both iPSC and ESC-derived beta cells will likely be the targets of persistent autoimmune response in T1D patients. In this regard possible approaches to suppress pre-existing autoimmunity will be developed and integrated for induction of long-term tolerance to autoantigens. Recent studies suggest that hES cells derived $CD34^+$ cells not only have a potential for long-term *in vivo* endocrine cellular activity without teratomas [151], but also prevent diabetes in a T1D mouse model by induction of hematopoietic mixed chimerism [152]. Thus, combined ESC-derived beta cells islet and ESC-derived HSC transplantation may become an approach to suppress pre-existing autoimmunity. Most recently, MSCs have been derived from hESC through several different methodologies[153]. Human MSC derived from hESC have been shown to possess characteristics very similar to BM-derived MSC. Thus, immunomodulatory properties of hESC-derived MSC are likely to prove valuable for inducing immune tolerance toward other cells or tissues derived from the same hESC lines[118]. The fourth hurdle is ethical and policy issue rose by hES cells research. Although in 2009 the Obama administration overturned the restriction access and use of hES cells by revoking statements and orders made during the former President Bush's administration, a federal judge recently issued an injunction barring the Obama's stem cell policy from funding research involving hES cells [154,155], which leads to the limitation of grants by NIH for the stem cell research. At the same time, the guidelines are recommended by the major stem cell stem cell organizations to enforce the regulation of stem cell therapies[156,157].

Experimental data suggest that the adult human pancreas contains facultative endocrine stem/progenitor cells that can differentiate to beta cells under specific circumstances [59,68,75]. Induction of facultative endocrine stem/progenitor cells in pancreas and other organs to differentiation into beta cells could become a viable therapeutic approach. However, any effort to replace beta cells in diabetes, in particular by endogenous progenitors, will require an approach to dealing with recurrent autoimmunity in patients with T1D [62]. Thus, the amelioration of autoimmune stress together with stimulus for regeneration of endogenous beta cells could be a feasible approach to improve endogenous insulin production in a substantial number of patients with T1D.

A recent clinical study suggests residual functional beta cells remain after as long as 50 years in the majority of patients with T1D [158]. In T1D patients, autologous HSCT may allow the residual beta cells to recover and may induce facultative endocrine stem/progenitor cells in pancreas to transdifferentiate into beta cells [58,66,75]. Induction of tolerance to alloreactive and autoreactive response by allogeneic HSCT has been achieved in experimental models of T1D. Future studies need to address the possible immunomodulatory effects of donor HSC and to optimize reduced-intensity nonmyeloablative conditioning protocols.

MSC have the capacity to differentiate into various cell types of mesenchymal origin, and also are well recognized for their immunomodulatory roles through direct inhibiting cell differentiation or proliferation and the altering of cytokine secretion profiles of many immune cell types. These unique characteristics make them an attractive source for cellular therapy of T1D based on the combined benefits not only from insulin-producing cells regeneration but also the negative regulation of autoimmunity. Although an emerging body of evidence shows the infusion of MSC resulted in increased insulin levels accompanied by improved pancreatic islets, some unaddressed questions may limit their clinical application. A standard protocol must be established to produce MSC and the optimal passage and doses of cells should be defined since these may have significant influence on MSC's immunomodulatory properties. Clinically applicable assays should also be considered to monitor the genetic and phenotypic stability of transplanted cells and the safety of recipients.

In conclusion, stem cell-based therapies continue to show great promise for treatment of T1D, and significant progress has brought these therapies closer to a clinical reality through research efforts.

Article highlights

- Several ESC differentiation protocols recapitulating pancreatic development *in vivo* have been used to guide differentiation of ESC toward definitive endoderm, pancreatic progenitors, followed by endocrine progenitors *in vitro*. The *in vivo* studies confirm the potential of using ES cell-derived beta cells for the treatment of diabetes.
- T1D-specific iPSC could potentially provide a treatment for diabetes in the future.
- Potential plasticity in adult pancreatic cells was reported previously and recent studies reveal the previously unrecognized function of alpha cells as beta cell progenitors.
- Independence from exogenous insulin can be achieved in most new-onset T1D patients following immunoablation and reconstitution of the immune system with autologous HSCT.

- As nonmyeloablative conditioning approaches become refined and less toxic, the establishment of mixed chimerism may not only induce tolerance to islet allografts, but also halt the progression of the autoimmune process.
- MSC offer an attractive source of stem cells for generation of surrogate beta cells. MSC also play an important role in immunomodulation.

Abbreviations

T1D	Type 1 diabetes
ESC	embryonic stem cells
iPSC	induced pluripotent stem cells
STZ	streptozotocin
bFGF	basic fibroblast growth factor
ILC	insulin-producing islet-like clusters
Ngn3	Neurogenin 3
PDL	partial duct ligation
VSEL	Very small embryonic-like cells
HSC	hematopoietic stem cells
MSC	multipotent mesenchymal stromal/stem cells
DC	dendritic cells
T_{reg}	T regulatory cells
MMP	matrix metalloproteinases
HLA	histocompatibility complex antigen

Acknowledgments

The authors thank Yiming Huang for critically reading the manuscript and Carolyn DeLautre for manuscript preparation.

We apologize to the colleagues whose work we could not cite here because of space limits.

Declaration of interest

This work was supported in part by the following: The American Diabetes Association-Takeda Pharmaceuticals Beta Cell postdoctoral fellowship (07–09-BETA-03), NIH R01 DK069766, and the W. M. Keck Foundation. ST Ildstad and B Chen are sponsored by the Department of Defense, Office of Army Research, National Institute of Health. ST Ildstad is CEO of Regenerex LLC.

Bibliography

1. Nathan DM. Finding new treatments for diabetes--how many, how fast… how good? N Engl J Med 2007;356(5):437–440. [PubMed: 17267901]
2. Vardanyan M, Parkin E, Gruessner C, Rodriguez Rilo HL. Pancreas vs. islet transplantation: a call on the future. Curr Opin Organ Transplant 2010;15(1):124–130. [PubMed: 20009930]
3. Shapiro AM, Ricordi C, Hering BJ, et al. International trial of the Edmonton protocol for islet transplantation. N Engl J Med 2006;355(13):1318–1330. [PubMed: 17005949]

4. Bromberg JS, Kaplan B, Halloran PF, Robertson RP. The islet transplant experiment: time for a reassessment. Am J Transplant 2007;7(10):2217–2218. [PubMed: 17845557]
5. McCall MD, Toso C, Baetge EE, Shapiro AMJ. Are stem cells a cure for diabetes? Clin Sci 2010;118(2):87–97. [PubMed: 19807695]
6. Murry CE, Keller G. Differentiation of embryonic stem cells to clinically relevant populations: lessons from embryonic development. Cell 2008;132(4):661–680. [PubMed: 18295582]
7. Champeris Tsaniras S, Jones PM. Generating pancreatic beta-cells from embryonic stem cells by manipulating signaling pathways. The Journal of endocrinology 2010;206(1):13–26. [PubMed: 20385725]
8. Spence JR, Wells JM. Translational embryology: using embryonic principles to generate pancreatic endocrine cells from embryonic stem cells. Developmental dynamics 2007;236(12):3218–3227. [PubMed: 17973329]
9. Lumelsky N, Blondel O, Laeng P, Velasco I, Ravin R, McKay R. Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. Science 2001;292(5520):1389–1394. [PubMed: 11326082]
10. Sipione S, Eshpeter A, Lyon JG, Korbutt GS, Bleackley RC. Insulin expressing cells from differentiated embryonic stem cells are not beta cells. Diabetologia 2004;47(3):499–508. [PubMed: 14968299]
11. Courtney ML, Jones PM, Burns CJ. Importance of quantitative analysis in the generation of insulin-expressing cells from human embryonic stem cells. Pancreas 2010;39(1):105–107. [PubMed: 20019566]
12. Rajagopal J, Anderson WJ, Kume S, Martinez OI, Melton DA. Insulin staining of ES cell progeny from insulin uptake. Science 2003;299(5605):363. [PubMed: 12532008]
13. Hansson M, Tonning A, Frandsen U, et al. Artifactual insulin release from differentiated embryonic stem cells. Diabetes 2004;53(10):2603–2609. [PubMed: 15448090]
14. D'Amour KA, Agulnick AD, Eliazer S, Kelly OG, Kroon E, Baetge EE. Efficient differentiation of human embryonic stem cells to definitive endoderm. Nature biotechnology 2005;23(12):1534–1541.
15. D'Amour KA, Bang AG, Eliazer S, et al. Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. Nature biotechnology 2006;24(11):1392–1401.
16. Mfopou JK, Chen B, Mateizel I, Sermon K, Bouwens L. Noggin, retinoids, and fibroblast growth factor regulate hepatic or pancreatic fate of human embryonic stem cells. Gastroenterology 2010;138(7) 2233-14.
17. Kaestner KH. From embryonic stem cells to beta-cells: new hope for diabetics? Gastroenterology 2010;138(7):2211–2212. [PubMed: 20435010]
18. Shi Y, Hou L, Tang F, et al. Inducing embryonic stem cells to differentiate into pancreatic beta cells by a novel three-step approach with activin A and all-trans retinoic acid. Stem cells 2005;23(5):656–662. [PubMed: 15849173]
19. Jiang W, Shi Y, Zhao D, et al. In vitro derivation of functional insulin-producing cells from human embryonic stem cells. Cell research 2007;17(4):333–344. [PubMed: 17426693]
20. Jiang J, Au M, Lu K, et al. Generation of insulin-producing islet-like clusters from human embryonic stem cells. Stem cells 2007;25(8):1940–1953. [PubMed: 17510217]
21. Eshpeter A, Jiang J, Au M, et al. In vivo characterization of transplanted human embryonic stem cell-derived pancreatic endocrine islet cells. Cell Proliferation 2008;41(6):843–858. [PubMed: 19040565]
22. Kroon E, Martinson LA, Kadoya K, et al. Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. Nature biotechnology 2008;26(4):443–452.
23. Aguayo-Mazzucato C, Bonner-Weir S. Stem cell therapy for type 1 diabetes mellitus. Nature Reviews Endocrinology 2010;6(3):139–148.
24. Shiraki N, Yoshida T, Araki K, et al. Guided differentiation of embryonic stem cells into Pdx1-expressing regional-specific definitive endoderm. Stem cells 2008;26(4):874–885. [PubMed: 18238854]

25. Naujok O, Francini F, Picton S, Bailey C, Lenzen SAJ. Changes in gene expression and morphology of mouse embryonic stem cells on differentiation into insulin-producing cells in vitro and in vivo. Diabetes/Metabolism Research and Reviews 2009;25(5):464–476. [PubMed: 19425055]
26. Banerjee I, Sharma N, Yarmush M. Impact of co-culture on pancreatic differentiation of embryonic stem cells. J Tissue Eng Regen Med. 2010
27. Zaret KS. Using small molecules to great effect in stem cell differentiation. Cell stem cell 2009;4(5):373–374. [PubMed: 19427285]
28. Mayhew CN, Wells JM. Converting human pluripotent stem cells into beta-cells: recent advances and future challenges. Current opinion in organ transplantation 2010;15(1):54–60. [PubMed: 19855279]
29. Schugar RC, Robbins PD, Deasy BM. Small molecules in stem cell self-renewal and differentiation. Gene therapy 2008;15(2):126–135. [PubMed: 17989703]
30. Desbordes SC, Placantonakis DG, Ciro A, et al. High-throughput screening assay for the identification of compounds regulating self-renewal and differentiation in human embryonic stem cells. Cell stem cell 2008;2(6):602–612. [PubMed: 18522853]
31. Zhu S, Wurdak H, Wang J, et al. A small molecule primes embryonic stem cells for differentiation. Cell stem cell 2009;4(5):416–426. [PubMed: 19427291]
32. Borowiak M, Maehr R, Chen S, et al. Small molecules efficiently direct endodermal differentiation of mouse and human embryonic stem cells. Cell stem cell 2009;4(4):348–358. [PubMed: 19341624]
33. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006;126(4):663–676. [PubMed: 16904174]
34. Todd JA. Stem cells and a cure for type 1 diabetes? Proc Natl Acad Sci U S A 2009;106(37): 15523–15524. [PubMed: 19805208]
35. Dimos JT, Rodolfa KT, Niakan KK, et al. Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. Science 2008;321(5893):1218–1221. [PubMed: 18669821]
36. Ebert AD, Yu J, Rose FF Jr, et al. Induced pluripotent stem cells from a spinal muscular atrophy patient. Nature 2009;457(7227):277–280. [PubMed: 19098894]
37. Maehr R, Chen S, Snitow M, et al. Generation of pluripotent stem cells from patients with type 1 diabetes. Proc Natl Acad Sci U S A 2009;106(37):15768–15773. [PubMed: 19720998]
38. Soldner F, Hockemeyer D, Beard C, et al. Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors. Cell 2009;136(5):964–977. [PubMed: 19269371]
39. Raya A, Rodríguez-Pizà I, Guenechea G, et al. Disease-corrected haematopoietic progenitors from Fanconi anaemia induced pluripotent stem cells. Nature 2009;460(7251):53–59. [PubMed: 19483674]
40. Hanna J, Wernig M, Markoulaki S, et al. Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin. Science 2007;318(5858):1920–1923. [PubMed: 18063756]
41. Carvajal-Vergara X, Sevilla A, D'Souza SL, et al. Patient-specific induced pluripotent stem-cell-derived models of LEOPARD syndrome. Nature 2010;465(7299):808–812. [PubMed: 20535210]
42. Moretti A, Bellin M, Welling A, et al. Patient-Specific Induced Pluripotent Stem-Cell Models for Long-QT Syndrome. N Engl J Med 2010;363(15):1397–1409. [PubMed: 20660394]
43. Tateishi K, He J, Taranova O, Liang G, D'Alessio AC, Zhang Y. Generation of insulin-secreting islet-like clusters from human skin fibroblasts. J Biol Chem 2008;283(46):31601–31607. [PubMed: 18782754]
44. Alipio Z, Liao W, Roemer EJ, et al. 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
45. Li W, Ding S. Generation of novel rat and human pluripotent stem cells by reprogramming and chemical approaches. Methods Mol Biol 2010;636:293–300. [PubMed: 20336530]
46. Lin T, Ambasudhan R, Yuan X, et al. A chemical platform for improved induction of human iPSCs. Nat Meth 2009;6(11):805–808.

47. Amit M, Chebath J, Margulets V, et al. Suspension Culture of Undifferentiated Human Embryonic and Induced Pluripotent Stem Cells. Stem Cell Reviews and Reports 2010;6(2):248–259. [PubMed: 20431964]
48. Belmonte JCI, Ellis J, Hochedlinger K, Yamanaka S. Induced pluripotent stem cells and reprogramming: seeing the science through the hype. Nature Reviews Genetics 2009;10(12):878–883.
49. King FW, Liszewski W, Ritner C, Bernstein HS. High throughput tracking of pluripotent human embryonic stem sells with dual FRET molecular beacons. Stem Cells and Development. 2010
50. Ellis J, Bruneau BG, Keller G, et al. Alternative induced pluripotent stem cell characterization criteria for in vitro applications. Cell Stem Cell 2009;4(3):198–199. [PubMed: 19265656]
51. Sallam K, Wu JC. Embryonic stem cell biology: insights from molecular imaging. Methods Mol Biol 2010;660:185–199. [PubMed: 20680820]
52. Schuldiner M, Itskovitz-Eldor J, Benvenisty N. Selective ablation of human embryonic stem cells expressing a "suicide" gene. Stem Cells 2003;21(3):257–265. [PubMed: 12743320]
53. Moretto-Zita M, Jin H, Shen Z, Zhao T, Briggs SP, Xu Y. Phosphorylation stabilizes Nanog by promoting its interaction with Pin1. Proc Natl Acad Sci USA 2010;107(30):13312–13317. [PubMed: 20622153]
54. Zhang D, Jiang W, Liu M, et al. Highly efficient differentiation of human ES cells and iPS cells into mature pancreatic insulin-producing cells. Cell Res 2009;19(4):429–438. [PubMed: 19255591]
55. Zhou H, Wu S, Joo JY, et al. Generation of induced pluripotent stem cells using recombinant proteins. Cell Stem Cell 2009;4(5):381–384. [PubMed: 19398399]
56. Jia F, Wilson KD, Sun N, et al. A nonviral minicircle vector for deriving human iPS cells. Nat Methods 2010;7(3):197–199. [PubMed: 20139967]
57. Baeyens L, De Breuck S, Lardon J, Mfopou JK, Rooman I, Bouwens L. In vitro generation of insulin-producing beta cells from adult exocrine pancreatic cells. Diabetologia 2005;48(1):49–57. [PubMed: 15616797]
58. Baeyens L, Bouwens L. Can beta-cells be derived from exocrine pancreas? Diabetes Obes Metab 2008;10 Suppl 4:170–178. [PubMed: 18834444]
59. Baeyens L, Bonné S, Bos T, et al. Notch signaling as gatekeeper of rat acinar-to-beta-cell conversion in vitro. Gastroenterology 2009;136(5):1750–1760. e13. [PubMed: 19208356]
60. Minami K, Okuno M, Miyawaki K, et al. Lineage tracing and characterization of insulin-secreting cells generated from adult pancreatic acinar cells. Proc Natl Acad Sci USA 2005;102(42):15116–15121. [PubMed: 16210247]
61. Okuno M, Minami K, Okumachi A, et al. Generation of insulin-secreting cells from pancreatic acinar cells of animal models of type 1 diabetes. Am J Physiol Endocrinol Metab 2007;292(1):E158–E165. [PubMed: 16926384]
62. Demeterco C, Hao E, Lee S-H, Itkin-Ansari P, Levine F. Adult human beta-cell neogenesis? Diabetes Obes Metab 2009;11 Suppl 4:46–53. [PubMed: 19817788]
63. Dor Y, Melton DA. Facultative endocrine progenitor cells in the adult pancreas. Cell 2008;132(2): 183–184. [PubMed: 18243094]
64. Yatoh S, Dodge R, Akashi T, et al. Differentiation of affinity-purified human pancreatic duct cells to beta-cells. Diabetes 2007;56(7):1802–1809. [PubMed: 17473224]
65. Hao E, Tyrberg B, Itkin-Ansari P, et al. Beta-cell differentiation from nonendocrine epithelial cells of the adult human pancreas. Nat Med 2006;12(3):310–316. [PubMed: 16491084]
66. Xu X, D'Hoker J, Stangé G, et al. Beta cells can be generated from endogenous progenitors in injured adult mouse pancreas. Cell 2008;132(2):197–207. [PubMed: 18243096]
67. Zhou Q, Brown J, Kanarek A, Rajagopal J, Melton DA. In vivo reprogramming of adult pancreatic exocrine cells to beta-cells. Nature 2008;455(7213):627–632. [PubMed: 18754011]
68. Li W-C, Rukstalis JM, Nishimura W, et al. Activation of pancreatic-duct-derived progenitor cells during pancreas regeneration in adult rats. Journal of Cell Science 2010;123(Pt 16):2792–2802. [PubMed: 20663919]

69. Inada A, Nienaber C, Katsuta H, et al. Carbonic anhydrase II-positive pancreatic cells are progenitors for both endocrine and exocrine pancreas after birth. Proc Natl Acad Sci U S A 2008;105(50):19915–19919. [PubMed: 19052237]
70. Kopinke D, Murtaugh LC. Exocrine-to-endocrine differentiation is detectable only prior to birth in the uninjured mouse pancreas. BMC Dev Biol 2010;10:38. [PubMed: 20377894]
71. Kushner JA, Weir GC, Bonner-Weir S. Ductal origin hypothesis of pancreatic regeneration under attack. Cell Metabolism 2010;11(1):2–3. [PubMed: 20085728]
72. Ouziel-Yahalom L, Li WC, Straussman S, et al. Ductal heterogeneity suggests a subpopulation serves as postnatal pancreatic progenitors. Diabetes 2010;59 Suppl 1:A25.
73. Collombat P, Xu X, Ravassard P, et al. The ectopic expression of Pax4 in the mouse pancreas converts progenitor cells into alpha and subsequently beta cells. Cell 2009;138(3):449–462. [PubMed: 19665969]
74. Chung C-H, Hao E, Piran R, Keinan E, Levine F. Pancreatic beta-Cell Neogenesis by Direct Conversion from Mature alpha-Cells. Stem cells. 2010
75. Thorel F, Népote V, Avril I, et al. Conversion of adult pancreatic alpha-cells to beta-cells after extreme beta-cell loss. Nature 2010;464(7292):1149–1154. [PubMed: 20364121]
76. Zaret KS, Grompe M. Generation and regeneration of cells of the liver and pancreas. Science 2008;322(5907):1490–1494. [PubMed: 19056973]
77. Wells JM, Melton DA. Early mouse endoderm is patterned by soluble factors from adjacent germ layers. Development 2000;127(8):1563–1572. [PubMed: 10725233]
78. Shen CN, Slack JM, Tosh D. Molecular basis of transdifferentiation of pancreas to liver. Nat Cell Biol 2000;2(12):879–887. [PubMed: 11146651]
79. Zalzman M, Anker-Kitai L, Efrat S. Differentiation of human liver-derived, insulin-producing cells toward the beta-cell phenotype. Diabetes 2005;54(9):2568–2575. [PubMed: 16123344]
80. Aviv V, Meivar-Levy I, Rachmut IH, Rubinek T, Mor E, Ferber S. Exendin-4 promotes liver cell proliferation and enhances the PDX-1-induced liver to pancreas transdifferentiation process. J Biol Chem 2009;284(48):33509–33520. [PubMed: 19755420]
81. Nagaya M, Katsuta H, Kaneto H, Bonner-Weir S, Weir GC. Adult mouse intrahepatic biliary epithelial cells induced in vitro to become insulin-producing cells. J Endocrinol 2009;201(1):37–47. [PubMed: 19168505]
82. Yechoor V, Liu V, Espiritu C, et al. Neurogenin3 is sufficient for transdetermination of hepatic progenitor cells into neo-islets in vivo but not transdifferentiation of hepatocytes. Dev Cell 2009;16(3):358–373. [PubMed: 19289082]
83. Kodama S, Kuhtreiber W, Fujimura S, Dale EA, Faustman DL. Islet regeneration during the reversal of autoimmune diabetes in NOD mice. Science 2003;302(5648):1223–1227. [PubMed: 14615542]
84. Asayesh A, Sharpe J, Watson RP, et al. Spleen versus pancreas: strict control of organ interrelationship revealed by analyses of Bapx1−/− mice. Genes Dev 2006;20(16):2208–2213. [PubMed: 16912273]
85. Macias MP, Fitzpatrick LA, Brenneise I, McGarry MP, Lee JJ, Lee NA. Expression of IL-5 alters bone metabolism and induces ossification of the spleen in transgenic mice. J Clin Invest 2001;107(8):949–959. [PubMed: 11306598]
86. Swirski FK, Nahrendorf M, Etzrodt M, et al. Identification of splenic reservoir monocytes and their deployment to inflammatory sites. Science 2009;325(5940):612–616. [PubMed: 19644120]
87. Lonyai A, Kodama S, Burger D, Davis M, Faustman DL. The promise of Hox11+ stem cells of the spleen for treating autoimmune diseases. Horm Metab Res 2008;40(2):137–146. [PubMed: 18283632]
88. Chong AS, Shen J, Tao J, et al. Reversal of diabetes in non-obese diabetic mice without spleen cell-derived beta cell regeneration. Science 2006;311(5768):1774–1775. [PubMed: 16556844]
89. Nishio J, Gaglia JL, Turvey SE, Campbell C, Benoist C, Mathis D. Islet recovery and reversal of murine type 1 diabetes in the absence of any infused spleen cell contribution. Science 2006;311(5768):1775–1778. [PubMed: 16556845]

90. Suri A, Calderon B, Esparza TJ, Frederick K, Bittner P, Unanue ER. Immunological reversal of autoimmune diabetes without hematopoietic replacement of beta cells. Science 2006;311(5768): 1778–1780. [PubMed: 16556846]

91. Hess D, Li L, Martin M, et al. Bone marrow-derived stem cells initiate pancreatic regeneration. Nat Biotechnol 2003;21(7):763–770. [PubMed: 12819790]

92. Ianus A, Holz GG, Theise ND, Hussain MA. In vivo derivation of glucose-competent pancreatic endocrine cells from bone marrow without evidence of cell fusion. J Clin Invest 2003;111(6):843–850. [PubMed: 12639990]

93. Wagers AJ, Sherwood RI, Christensen JL, Weissman IL. Little evidence for developmental plasticity of adult hematopoietic stem cells. Science 2002;297(5590):2256–2259. [PubMed: 12215650]

94. Taneera J, Rosengren A, Renstrom E, et al. Failure of transplanted bone marrow cells to adopt a pancreatic beta-cell fate. Diabetes 2006;55(2):290–296. [PubMed: 16443759]

95. Kucia M, Reca R, Campbell FR, et al. A population of very small embryonic-like (VSEL) CXCR4(+)SSEA-1(+)Oct-4+ stem cells identified in adult bone marrow. Leukemia 2006;20(5): 857–869. [PubMed: 16498386]

96. Huang Y, Kucia M, Hussain LR, et al. Bone marrow transplantation temporarily improves pancreatic function in streptozotocin-induced diabetes: potential involvement of very small embryonic-like cells. Transplantation 2010;89(6):677–685. [PubMed: 20110858]

97. Brignier AC, Gewirtz AM. Embryonic and adult stem cell therapy. J Allergy Clin Immunol 2010;125(2 Suppl 2):S336–S344. [PubMed: 20061008]

98. Cottler-Fox MH, Lapidot T, Petit I, et al. Stem cell mobilization. Hematology Am Soc Hematol Educ Program 2003:419–437. [PubMed: 14633793]

99. Couzin-Frankel J. Immunology. Replacing an immune system gone haywire. Science 2010;327(5967):772–774. [PubMed: 20150460]

100. Milanetti F, Abinun M, Voltarelli JC, Burt RK. Autologous hematopoietic stem cell transplantation for childhood autoimmune disease. Pediatric Clinics of North America 2010;57(1):239–271. [PubMed: 20307720]

101. The Diabetes Control and Complications Trial Research Group. Effect of intensive therapy on residual beta-cell function in patients with type 1 diabetes in the diabetes control and complications trial. A randomized, controlled trial. The Diabetes Control and Complications Trial Research Group. Ann Intern Med 1998;128(7):517–523. [PubMed: 9518395]

102. Voltarelli JC, Couri CEB, Stracieri ABPL, et al. Autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus. JAMA 2007;297(14): 1568–1576. [PubMed: 17426276]

103. Couri CEB, Oliveira MCB, Stracieri ABPL, et al. C-peptide levels and insulin independence following autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus. JAMA 2009;301(15):1573–1579. [PubMed: 19366777]

104. Snarski E, Milczarczyk A, Torosian T, et al. Independence of exogenous insulin following immunoablation and stem cell reconstitution in newly diagnosed diabetes type I. Bone Marrow Transplant. 2010

105. Voltarelli JC, Couri CEB. Stem cell transplantation for type 1 diabetes mellitus. Diabetol Metab Syndr 2009;1(1):4. [PubMed: 19825196]

106. Yasumizu R, Sugiura K, Iwai H, et al. Treatment of type 1 diabetes mellitus in non-obese diabetic mice by transplantation of allogeneic bone marrow and pancreatic tissue. Proceedings of the National Academy of Sciences of the United States of America 1987;84(18):6555–6557. [PubMed: 3114751]

107. Li H, Kaufman CL, Boggs SS, Johnson PC, Patrene KD, Ildstad ST. Mixed allogeneic chimerism induced by a sublethal approach prevents autoimmune diabetes and reverses insulitis in nonobese diabetic (NOD) mice. J Immunol 1996;156(1):380–388. [PubMed: 8598488]

108. Ildstad ST, Chilton PM, Xu H, Domenick MA, Ray MB. Preconditioning of NOD mice with anti-CD8 mAb and costimulatory blockade enhances chimerism and tolerance and prevents diabetes, while depletion of alpha beta-TCR+ and CD4+ cells negates the effect. Blood 2005;105(6):2577–2584. [PubMed: 15498851]

109. Nikolic B, Takeuchi Y, Leykin I, Fudaba Y, Smith RN, Sykes M. Mixed hematopoietic chimerism allows cure of autoimmune diabetes through allogeneic tolerance and reversal of autoimmunity. Diabetes 2004;53(2):376–383. [PubMed: 14747288]
110. Zhang C, Todorov I, Lin C-L, et al. Elimination of insulitis and augmentation of islet beta cell regeneration via induction of chimerism in overtly diabetic NOD mice. Proc Natl Acad Sci USA 2007;104(7):2337–2342. [PubMed: 17267595]
111. Zorina TD, Subbotin VM, Bertera S, et al. Recovery of the endogenous beta cell function in the NOD model of autoimmune diabetes. Stem Cells 2003;21(4):377–388. [PubMed: 12832692]
112. Zhang C, Wang M, Racine JJ, et al. Induction of chimerism permits low-dose islet grafts in the liver or pancreas to reverse refractory autoimmune diabetes. Diabetes 2010;59(9):2228–2236. [PubMed: 20530743]
113. Seung E, Iwakoshi N, Woda BA, et al. Allogeneic hematopoietic chimerism in mice treated with sublethal myeloablation and anti-CD154 antibody: absence of graft-versus-host disease, induction of skin allograft tolerance, and prevention of recurrent autoimmunity in islet-allografted NOD/Lt mice. Blood 2000;95(6):2175–2182. [PubMed: 10706892]
114. Wu T, Levay-Young B, Heuss N, et al. Inducing tolerance to MHC-matched allogeneic islet grafts in diabetic NOD mice by simultaneous islet and bone marrow transplantation under nonirradiative and nonmyeloablative conditioning therapy. Transplantation 2002;74(1):22–27. [PubMed: 12134094]
115. Li H, Inverardi L, Molano RD, Pileggi A, Ricordi C. Nonlethal conditioning for the induction of allogeneic chimerism and tolerance to islet allografts. Transplantation 2003;75(7):966–970. [PubMed: 12698081]
116. Mineo D, Ricordi C, Xu X, et al. Combined islet and hematopoietic stem cell allotransplantation: a clinical pilot trial to induce chimerism and graft tolerance. Am J Transplant 2008;8(6):1262–1274. [PubMed: 18444924]
117. Motaln H, Schichor C, Lah TT. Human mesenchymal stem cells and their use in cell-based therapies. Cancer 2010;116(11):2519–2530. [PubMed: 20301117]
118. Hematti P. Human embryonic stem cell-derived mesenchymal progenitors: an overview. Methods Mol Biol 2011;690:163–174. [PubMed: 21042992]
119. Corselli M, Chen CW, Crisan M, Lazzari L, Peault B. Perivascular ancestors of adult multipotent stem cells. Arterioscler Thromb Vasc Biol 2010;30(6):1104–1109. [PubMed: 20453168]
120. Francese R, Fiorina P. Immunological and regenerative properties of cord blood stem cells. Clin Immunol 2010;136(3):309–322. [PubMed: 20447870]
121. Anzalone R, Lo Iacono M, Loria T, et al. Wharton's Jelly Mesenchymal Stem Cells as Candidates for Beta Cells Regeneration: Extending the Differentiative and Immunomodulatory Benefits of Adult Mesenchymal Stem Cells for the Treatment of Type 1 Diabetes. Stem Cell Rev. 2010
122. Battiwalla M, Hematti P. Mesenchymal stem cells in hematopoietic stem cell transplantation. Cytotherapy 2009;11(5):503–515. [PubMed: 19728189]
123. Zhang L, Chan C. Isolation and enrichment of rat mesenchymal stem cells (MSCs) and separation of single-colony derived MSCs. J Vis Exp 2010;22(37):1852. [PubMed: 20308982]
124. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006;8(4):315–317. [PubMed: 16923606]
125. Ghannam S, Pene J, Torcy-Moquet G, Jorgensen C, Yssel H. Mesenchymal stem cells inhibit human Th17 cell differentiation and function and induce a T regulatory cell phenotype. J Immunol 2010;185(1):302–312. [PubMed: 20511548]
126. Sato K, Ozaki K, Oh I, et al. Nitric oxide plays a critical role in suppression of T-cell proliferation by mesenchymal stem cells. Blood 2007;109(1):228–234. [PubMed: 16985180]
127. Corcione A, Benvenuto F, Ferretti E, et al. Human mesenchymal stem cells modulate B-cell functions. Blood 2006;107(1):367–372. [PubMed: 16141348]
128. Bartholomew A, Polchert D, Szilagyi E, Douglas GW, Kenyon N. Mesenchymal stem cells in the induction of transplantation tolerance. Transplantation 2009;87(9 Suppl):S55–S57. [PubMed: 19424008]

129. Ozawa K, Sato K, Oh I, et al. Cell and gene therapy using mesenchymal stem cells (MSCs). J Autoimmun 2008;30(3):121–127. [PubMed: 18249090]
130. Tyndall A, Uccelli A. Multipotent mesenchymal stromal cells for autoimmune diseases: teaching new dogs old tricks. Bone Marrow Transplant 2009;43(11):821–828. [PubMed: 19308035]
131. Madec AM, Mallone R, Afonso G, et al. Mesenchymal stem cells protect NOD mice from diabetes by inducing regulatory T cells. Diabetologia 2009;52(7):1391–1399. [PubMed: 19421731]
132. Berman DM, Willman MA, Han D, et al. Mesenchymal Stem Cells Enhance Allogeneic Islet Engraftment in Nonhuman Primates. Diabetes 2010;59(10):2558–2568. [PubMed: 20622174]
133. Karnieli O, Izhar-Prato Y, Bulvik S, Efrat S. Generation of insulin-producing cells from human bone marrow mesenchymal stem cells by genetic manipulation. Stem Cells 2007;25(11):2837–2844. [PubMed: 17615265]
134. Neshati Z, Matin MM, Bahrami AR, Moghimi A. Differentiation of mesenchymal stem cells to insulin-producing cells and their impact on type 1 diabetic rats. J Physiol Biochem 2010;66(2):181–187. [PubMed: 20422338]
135. Jurewicz M, Yang S, Augello A, et al. Congenic mesenchymal stem cell therapy reverses hyperglycemia in experimental type 1 diabetes. Diabetes. 2010
136. Chandra VGS, Phadnis S, Nair PD, Bhonde RR. Generation of pancreatic hormone-expressing islet-like cell aggregates from murine adipose tissue-derived stem cells. Stem Cells 2009;27(8):1941–1953. [PubMed: 19544426]
137. Kang HM, Kim J, Park S, et al. Insulin-secreting cells from human eyelid-derived stem cells alleviate type I diabetes in immunocompetent mice. Stem Cells 2009;27(8):1999–2008. [PubMed: 19544420]
138. Lee RH, Seo MJ, Reger RL, et al. Multipotent stromal cells from human marrow home to and promote repair of pancreatic islets and renal glomeruli in diabetic NOD/scid mice. Proc Natl Acad Sci U S A 2006;103(46):17438–17443. [PubMed: 17088535]
139. Fiorina P, Jurewicz M, Augello A, et al. Immunomodulatory function of bone marrow-derived mesenchymal stem cells in experimental autoimmune type 1 diabetes. J Immunol 2009;183(2):993–1004. [PubMed: 19561093]
140. Sordi V, Melzi R, Mercalli A, et al. Mesenchymal cells appearing in pancreatic tissue culture are bone marrow-derived stem cells with the capacity to improve transplanted islet function. Stem Cells 2010;28(1):140–151. [PubMed: 19924826]
141. Park KS, Kim YS, Kim JH, et al. Influence of human allogenic bone marrow and cord blood-derived mesenchymal stem cell secreting trophic factors on ATP (adenosine-5'-triphosphate)/ADP (adenosine-5'-diphosphate) ratio and insulin secretory function of isolated human islets from cadaveric donor. Transplant Proc 2009;41(9):3813–3818. [PubMed: 19917393]
142. Ding Y, Xu D, Feng G, Bushell A, Muschel RJ, Wood KJ. Mesenchymal stem cells prevent the rejection of fully allogenic islet grafts by the immunosuppressive activity of matrix metalloproteinase-2 and -9. Diabetes 2009;58(8):1797–1806. [PubMed: 19509016]
143. Boumaza I, Srinivasan S, Witt WT, et al. Autologous bone marrow-derived rat mesenchymal stem cells promote PDX-1 and insulin expression in the islets, alter T cell cytokine pattern and preserve regulatory T cells in the periphery and induce sustained normoglycemia. J Autoimmun 2009;32(1):33–42. [PubMed: 19062254]
144. Figliuzzi M, Cornolti R, Perico N, et al. Bone marrow-derived mesenchymal stem cells improve islet graft function in diabetic rats. Transplant Proc 2009;41(5):1797–1800. [PubMed: 19545731]
145. Ito T, Itakura S, Todorov I, et al. Mesenchymal stem cell and islet co-transplantation promotes graft revascularization and function. Transplantation 2010;89(12):1438–1445. [PubMed: 20568673]
146. Yang YP, Wright C. Chemicals turn human embryonic stem cells towards beta cells. Nature chemical biology 2009;5(4):195–196.
147. Sun N, Longaker MT, Wu JC. Human iPS cell-based therapy: considerations before clinical applications. Cell Cycle 2010;9(5):880–885. [PubMed: 20160515]
148. Osafune K, Caron L, Borowiak M, et al. Marked differences in differentiation propensity among human embryonic stem cell lines. Nature biotechnology 2008;26(3):313–315.

149. Thomson JA, Odorico JS. Human embryonic stem cell and embryonic germ cell lines. Trends Biotechnol 2000;18(2):53–57. [PubMed: 10652509]

150. Hao J, Zhu W, Sheng C, Yu Y, Zhou Q. Human parthenogenetic embryonic stem cells: one potential resource for cell therapy. Sci China, C, Life Sci 2009;52(7):599–602. [PubMed: 19641863]

151. Goodrich AD, Ersek A, Varain NM, et al. In vivo generation of beta-cell-like cells from CD34(+) cells differentiated from human embryonic stem cells. Exp Hematol 2010;38(6):516–525. e4. [PubMed: 20227460]

152. Verda L, Kim DA, Ikehara S, et al. Hematopoietic mixed chimerism derived from allogeneic embryonic stem cells prevents autoimmune diabetes mellitus in NOD mice. Stem Cells 2008;26(2):381–386. [PubMed: 17975228]

153. Karlsson C, Emanuelsson K, Wessberg F, et al. Human embryonic stem cell-derived mesenchymal progenitors-Potential in regenerative medicine. Stem Cell Res. 2009

154. Cash G. Embryonic stem cells: court decision a threat to science itself. Nature 2010;467(7313): 271. [PubMed: 20844515]

155. Wadman M. Stem-cell work thrown into limbo. Nature 2010;467(7311):12–13. [PubMed: 20811425]

156. Hyun I, Lindvall O, Ahrlund-Richter L, et al. New ISSCR guidelines underscore major principles for responsible translational stem cell research. Cell Stem Cell 2008;3(6):607–609. [PubMed: 19041777]

157. Dolgin E. Survey details stem cell clinics ahead of regulatory approval. Nat Med 2010;16(5):495. [PubMed: 20448552]

158. Keenan HA, Sun JK, Levine J, et al. Residual insulin production and pancreatic beta cell turnover after 50 years of diabetes: Joslin Medalist Study. Diabetes. 2010

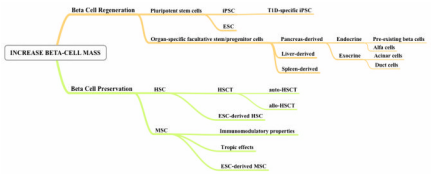

Figure 1.