Concise Review: Pancreas Regeneration: Recent Advances and Perspectives

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Key Words. Diabetes • Pancreas • Progenitor cells • Transplantation • Cellular therapy

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ABSTRACT

The replacement of functional pancreatic β-cells is seen as an attractive potential therapy for diabetes, because diabetes results from an inadequate β-cell mass. Inducing replication of the remaining β-cells and new islet formation from progenitors within the pancreas (neogenesis) are the most direct ways to increase the β-cell mass. Stimulation of both replication and neogenesis have been reported in rodents, but their clinical significance must still be shown. Because human islet transplantation is limited by the scarcity of donors and graft failure within a few years, efforts have recently concentrated on the use of stem cells to replace the deficient β-cells. Currently, embryonic stem cells and induced pluripotent stem cells achieve high levels of β-cell differentiation, but their clinical use is still hampered by ethical issues and/or the risk of developing tumors after transplantation. Pancreatic epithelial cells (duct, acinar, or α-cells) represent an appealing alternative to stem cells because they demonstrate β-cell differentiation capacities. Yet translation of such capacity to human cells after significant in vitro expansion has yet to be achieved. Besides providing new β-cells, cell therapy also has to address the question on how to protect the transplanted cells from destruction by the immune system via either allo- or autoimmunity. Encouraging developments have been made in encapsulation and immunomodulation techniques, but many challenges still remain. Herein, we discuss recent advances in the search for β-cell replacement therapies, current strategies for circumventing the immune system, and mandatory steps for new techniques to be translated from bench to clinics. STEM CELLS TRANSLATIONAL MEDICINE *2012;1:150–159*

INTRODUCTION

Diabetes results from an inadequate mass of functional β-cells. In type 1 diabetes (T1D), the immune system attacks and destroys the β-cells by mechanisms still incompletely understood [1]. Type 2 diabetes (T2D) accounts for >95% of diabetes cases worldwide and is associated with obesity leading to insulin resistance and β-cell dysfunction. Exogenous insulin or oral agents can provide tight control of the disease, but only the replacement of β-cells allows physiological control of glycemia. Human islet transplantation is successful in restoring normal glycemia but is limited by the need for toxic immunosuppressive drugs, the scarcity of donors, and graft failure usually within a few years [2]. Alternative ways for replacing β-cells are needed and may theoretically be obtained by (a) replication of remaining β-cells, (b) neogenesis, or the differentiation of new islet cells from pancreatic progenitors or stem cells, and (c) transplantation of β-cells derived from stem or somatic precursor cells. These areas of research, still being mostly experimental, will be addressed in this review.

Evidence of In Vivo Regeneration Capacity of β-Cells

Much enthusiasm about regenerating pancreatic β-cells in situ has been driven by evidence of the impressive proliferation capacity of postnatal rodent β-cells in situations of increased metabolic demand [3]. For example, in the mouse, pre-existing β-cells were shown to be responsible for the pancreas regeneration that occurs after 70% pancreatectomy, in a seminal work by Melton and coworkers [4]. In the human, normal expansion of the β-cell mass occurs during the neonatal period but fades early in childhood [5]. β-Cell regeneration has been suggested by the observation of residual β-cells in T1D patients after onset [6] or even many years after diagnosis [7–9]. Whether this represents a replication capacity, neogenesis, or resistance to apoptosis is unknown. Adult β-cells have some capacity to expand with obesity [10, 11] whereas β-cell replication is usually negligible when analyzed at autopsy [10, 12, 13]. However, an interesting equivalent of the "honeymoon period" has been described in T1D patients during pregnancy with measurable C-peptide levels and transient reduction of the insulin requirements [14]. Replication was initially suggested as the mechanism of this phenomenon [15], but a recent autopsy study on pancreases during or after pregnancy suggested neogenesis by showing increased relative volume of β-cells, increased proportion of

small islets, and increased number of insulin$^+$ cells in the ducts but no change in β-cell replication, cell size, or apoptosis frequency [16]. Thus, the question remains open as to whether β-cell regeneration could be exploited for therapy.

Neogenesis has recently been a source of intense debate, with many lineage tracing studies in mice showing contradictory results [17]. Controversy reached its climax when two recent reports obtained distinctly different results using similar tracing methods for Sox9-expressing populations [18, 19]. Kopp et al. described derivation of non-β-endocrine cells from the ducts in early postnatal life but no endocrine or acinar cell neogenesis in adult mice either physiologically or after pancreatic duct ligation (PDL) [18]. However, Furuyama et al. obtained massive X-gal staining of acinar cells 8 weeks after tamoxifen pulses were given during the postnatal period in Sox9$^{IRES-CreERT2}$; Rosa26R mice [19]. They also showed that duct cells accounted for a mere 1% of pancreatic endocrine cells at 8 weeks when tracing was initiated on the first day of life. These results clearly underline technical limitations of current lineage tracing approaches.

Neogenesis from ducts is influenced by the type and extent of pancreatic injury. This was highlighted in a recent study showing different regeneration depending on the affected cell type and the extent of diphtheria toxin-induced apoptosis [20]. When both acinar and islet cells were massively killed by diphtheria toxin expressed under the Pdx1 promoter, duct cells gave rise to both acinar and endocrine cells, recovery of 60% of the β-cell mass, and reversal of hyperglycemia, but when only acinar cells were killed by elastase-driven toxin, duct cells only gave rise to new acinar cells. Also, neogenesis from the ducts seems to happen by recapitulating pancreatic embryonic development as highlighted using a pancreatic duct ligation model in the mouse [21] and partial pancreatectomy in the rat [22, 23].

In the human pancreas, indirect evidence of neogenesis has been provided by showing cells coexpressing cytokeratin and insulin [24]. Many studies have demonstrated the presence of cells containing insulin within the ducts, either at autopsy [5] or in biopsy from organ donors [24, 25]. Currently, the general concept is that, after birth, neogenesis from ducts occurs mostly in the neonatal period and, as shown in rodents, can be stimulated in the regeneration following injury [17].

Stimulation of In Vivo Generation of New β-Cells

In rodents, growth factors such as betacellulin [26] or combinations of glucagon-like peptide (GLP)-1/gastrin [27] or epidermal growth factor/gastrin [28, 29] have shown a potential for increasing β-cell mass and reversing diabetes. Data from rodents showed that these factors stimulate β-cell replication and neogenesis, but whether this happens in humans is unknown [2]. Islet neogenesis associated protein-pentadecapeptide (INGAP-PP) generated interest after the report of its potential for stimulating neogenesis and reversing diabetes in streptozotocin (STZ)-treated mice [30]. However, INGAP-PP has met with equivocal results when studied in humans, with only isolated parameters being modestly improved (arginine-stimulated C-peptide secretion in the T1D trial, HbA1C levels in the T2D trial) [31, 32]. The therapeutic potential of such combinations or single agents in humans has not been explored in enough detail to draw firm conclusions.

Incretin therapy or GLP-1 receptor agonism has been considered to have potential for diabetes therapy after proof-of-concept studies were performed in T2D patients with administration of GLP-1 [33, 34]. Because of its short biological half-life, long-acting analogues (namely, exenatide and liraglutide) and inhibitors of the degrading enzyme DPPIV were developed that are now widely used for treatment of T2D [35]. These agents are likely to provide benefit for T2D patients through stimulation of insulin secretion (incretin effect), inhibition of glucagon release, delay of gastric emptying, and decrease of food intake. However, although GLP-1 receptor agonism has been shown to increase β-cell mass in rodents [36, 37], long-term data have yet to provide evidence for such increase in T2D patients [38].

β-Cell Replacement Therapy

(A) What Kind of Cells?

Embryonic Stem Cells. Embryonic stem cells (ESCs) have advantages over other potential sources because they are now readily available, are highly expandable, and can be differentiated to β-cells [39]. Many studies have demonstrated the derivation of Pdx1$^+$ or endocrine cells from ESCs, and some groups generated insulin or C-peptide-secreting cells [40–42]. Using a stepwise differentiation protocol mimicking pancreatic embryonic development, a team from Novocell (now Viacyte) drove ESCs toward an endocrine phenotype in vitro and obtained up to 12% insulin$^+$ cells [43]. When early pancreatic progenitors were transplanted into diabetic SCID mice, over time these cells became glucose-responsive and secreted large amounts of insulin, comparable to amounts released from human islets, leading to near normalization of blood glucose levels in the transplanted animals [44].

Improvements of current differentiation procedures are still mandatory to ensure their reproducibility. High-throughput screening of small molecules appears to be an attractive approach to identify new compounds promoting differentiation while being "clinical-friendly" [45, 46]. Additionally, three-dimensional (3D) organization of cell populations might foster β-cell differentiation [47], and studies are needed to evaluate at which state of differentiation cells would benefit from cell-to-cell interactions and which culture system (suspension culture, gels, scaffolds) would be most suitable for implementation to clinical situations. Lack of oxygen in the core of large 3D structures is a limitation, and a recent study used a size-controlled clustering protocol to derive endocrine precursors from human ESCs [48]. Whether extracellular matrices would give permissive signals for differentiation [49] and could be incorporated into a 3D structure without altering cross-talk between cells requires investigation.

In addition to ethical issues, clinical use of ESCs is still seriously hampered by the risk of in vivo teratoma formation [44, 50]. This risk is mainly associated with the transplantation of undifferentiated phenotypes, and efforts are now being concentrated on the selection of differentiated cells. Recent studies showed the possibility of sorting for ESC-derived endodermal cells using cell surface markers (CD49e$^+$CD141$^+$CD238$^+$ [51], EpCAM$^+$SSEA1$^-$SSEA3$^-$ [52], or CD24$^+$ selection [53]) without detectable teratoma formation $>$160 days post-transplantation. Investigations are now needed to determine how reliable the elimination of undifferentiated cells can be, as only a few cells are necessary for tumorogenesis. Also, differentiated cells may retain epigenetic traits of original cells, as has been described after reprogramming to pluripotency [54, 55], after epithelial-mesenchymal transition (EMT) of β-cells [56], and after transdifferentiation of hepatocytes into neurons [57].

Whether the differentiated products might revert to a less differentiated and potentially dangerous state is unknown.

Induced Pluripotent Stem Cells. Induced pluripotent stem cells (iPSCs) have the unique property of allowing the generation of autologous cells that might be useful for therapy [58]. The β-cell differentiation potential of iPSCs has been shown in vitro with demonstration of partial glucose-responsive C-peptide release [42, 59, 60]. Moreover, recent studies highlighted the potential of mouse [61] and rhesus monkey [62] iPSCs to reverse hyperglycemia after in vitro differentiation and transplantation in diabetic mouse models.

Although huge efforts are invested in iPSC research, hurdles concerning their clinical application are multiple: long, complex, and costly in vitro procedures; low reprogramming efficiency; risk of insertional mutagenesis and of permanent transgene genome integration; tumor formation; use of Klf4 and c-Myc oncogenic factors; and the need for animal feeder cells [63]. Safety issues have recently been raised because coding mutations [64] or epigenetic anomalies [65] were observed after reprogramming, and undefined limitations also exist as to how to induce iPSC differentiation without generating large numbers of undifferentiated cells.

The search for nonintegrative techniques has generated new possibilities, such as the use of synthetic modified mRNA for Klf4, c-Myc, Oct4, and Sox2 (KMOS) that appears to be a safer and more efficient strategy for reprogramming [66]. Another team described the successful reprogramming of mouse and human somatic cells using *miR302/367* microRNAs, with a twofold-enhanced efficiency compared with the KMOS strategy [67]. Even more efficient (100×) and faster reprogramming was obtained using nonintegrating episomal vectors on bone marrow and cord blood cells [68]. In parallel, the replacement of oncogenic factors in reprogramming protocols is important for safety. Accordingly, Yamanaka and coworkers recently reported the efficient generation of iPSCs by replacing c-Myc in the KMOS protocol by Glis1, a GLI-like transcription factor [69]. Further work is needed to confirm the reliability and safety of these techniques. With the aim of moving the iPSC field closer to clinical application, an intense effort is focused on the use of small molecules that may improve reprogramming efficiency [70] and even avoid the use of transcription factors [71].

Human Pancreatic Epithelial Cells. (1) Human Islets. Because islet donors are scarce, exploitation of human β-cells for therapy could be obtained by expanding the cells in vitro. Because epithelial cells have limited mitotic activity in vitro, an alternative way of forcing their expansion could be via a phenotype shift. Accordingly, human β-cells were shown to be able to proliferate in vitro after shifting toward a mesenchymal phenotype through EMT [72]. These mesenchymal-like cells appear to have been directly derived from original β-cells, as confirmed by lineage tracing experiments [73, 74] with human cells; however, mouse β-cells were shown not to be able to undergo EMT [75–77]. Moreover, it has not been convincingly shown that these mesenchymal-like cells can be differentiated into bona fide β-cells.

Islets, as well as the ducts, were recently shown to contain a population of pancreas-derived multipotent precursor (PMP) cells that are isolated under clonal conditions (at the rate of 2.6 sphere-producing colonies per 10,000 cells) and generate pancreatic and neural lineages in vitro [78]. PMPs derived from human islets were able to reverse diabetes in STZ-treated NOD-SCID mice [79]. This might represent another alternative use of islet preparations for treating diabetes.

(2) Duct Cells. Several studies showed the potential for differentiation of cells derived from the islet-depleted exocrine tissue [78, 80–82]. These studies all used relatively unselected populations, making identification of the starting material difficult and contamination of residual β-cells a possible explanation of the observed results. Yet clear demonstration of the β-cell differentiation of human duct cells has been provided on purified populations expressing CA19–9 antigen [83]. Although these cells are numerous in the pancreas and are easily purified, they lack sustained proliferation and tend to lose their phenotype in vitro [84, 85]. New techniques are thus needed to derive proliferating cells from the ducts that are able to differentiate into islet cells.

(3) Acinar Cells. Controversy exists concerning the in vivo potential for rodent acinar cells to differentiate into β-cells after injury [86, 87] since a report showing no acinar-to-β cell reprogramming after 70% pancreatectomy, PDL, or caerulein-induced pancreatitis [88]. A recent study shed new light on the potential of exocrine cells by showing their reprogramming into β-cells after injection of viral vectors carrying Pdx1, Ngn3, and MafA transcription factors into the mouse pancreas [89]. The differentiated products had many characteristics of bona fide β-cells with regard to expression profile and insulin content and could partially reverse the diabetic state. Although the in vitro expansion of human acinar cells has not yet been successful, these cells remain an attractive source for β-cell engineering, and their therapeutic potential will no doubt be fully explored.

(4) α-Cells. The potential of α-cells recently aroused much excitement after the unexpected demonstration of their differentiation into β-cells. Collombat et al. showed generation of insulin$^+$ cells and restoration of hyperglycemia in mice after overexpressing Pax4 in glucagon-expressing cells [90]. In addition, using a selective diphtheria toxin-mediated apoptosis model, Thorel et al. confirmed the differentiation potential of mouse α-cells [91]. After a β-cell ablation of $>$99%, 5%–10% of the α-cells had the capacity to reprogram into β-cells, which could then replicate and substantially increase β-cell mass, resulting in improved glycemic control in the mice. However, the combined ablation of β- and α-cells prevented the reprogramming [91]. Whether this plasticity might exist in humans is unknown but is suggested by the observation of insulin$^+$glucagon$^+$ cells in fibrotic pancreases [92]. However, such double-stained cells could be immature cells differentiating during neogenesis. The possibility of such reprogramming is supported by the in vitro demonstration of β-cell differentiation of an α-cell line using the small molecule BRD-7389 [93].

This new area of research holds potential promise as α-cells are preserved in T1D or T2D patients [92] and could be a source for β-cell mass expansion. Furthermore, α-cells could also be made available for cell therapy as they can probably be generated in vitro from human ESCs [94]. However, the findings on α- to β-cell differentiation still need confirmation and generalization because in vivo STZ-induced β-cell ablation does not seem to provoke α-cell replication nor β-cell regeneration in nonhuman primates [95].

Hepatocytes. The rationale for using hepatocytes arises from the liver's common endodermal origin with the pancreas. β-Cell

reprogramming of hepatocytes has been shown using Pdx1 overexpression, which led to the lowering of hyperglycemia in diabetic mice [96]. These results were confirmed by the same group [97] and others using Pdx1, NeuroD, and Ngn3 overexpression [98].

Even though the liver is a massive pool of reprogrammable cells, the use of hepatocytes ex vivo is made difficult by the shortage of donors, poor in vitro proliferation capacities, and rapid dedifferentiation of these cells [99]. Use of autologous cells via in situ reprogramming approaches should be evaluated, as autologous hepatocyte isolation would require surgical procedures with high comorbidity and should be considered only if a cure of diabetes is expected.

Porcine Islets. Pigs represent an unlimited supply of islets for transplantation, and the purity of islet preparations is usually higher in pigs than in humans [100]. Proof-of-concept of unmodified adult porcine islet xenotransplantation has been made in nonhuman primates using immunosuppression [101, 102], microencapsulation [103], or a subcutaneous macrodevice [104] without immunosuppression. The team of Elliott reported only modest changes with transplantation into nonhuman primates of microencapsulated porcine neonatal pancreatic cell clusters [105]. Importantly, they also showed no evidence of porcine endogenous retrovirus infection after a similar transplant in a human subject [106]. Preliminary data from phase I and IIa clinical trials showed a possible reduction of hypoglycemia unawareness after neonatal porcine islet xenotransplantation without immunosuppression in T1D patients, whereas daily insulin needs were only modestly reduced [107]. Although still experimental, porcine islet xenotransplantation is a promising area of investigation, and efforts currently focused on improvement of immunosuppressive regimens [100] might help overcome current limitations. Genetic modification is another way of making pig islets more suitable for transplantation, and transgenic animals are being evaluated for their potential to avoid rejection or even retrovirus infections [108].

Other. Multipotent mesenchymal stromal cells (MSCs) are easily isolated from many tissue sources, are highly expandable in vitro, are resistant to cryopreservation, and have the potential to differentiate into many different lineages [109]. Reversal of diabetes has been reported with human MSCs that differentiated into insulin$^+$ cells after transplantation into STZ-diabetic rats without immunosuppression [110]; however, these data need confirmation. MSCs seem to be able to enhance results of experimental islet transplantation, perhaps via paracrine effects, including the secretion of angiogenic cytokines and antiapoptotic factors, that can regulate endothelial and epithelial permeability, decrease inflammation, and enhance tissue repair [111, 112]. This is corroborated by reports showing amelioration of hyperglycemia after intravenous [113, 114] or intracardiac [115] infusion of MSCs in STZ-diabetic mice.

Following up on their previous work with very small embryonic-like stem cells, Ratajczak et al. recently published the isolation of these cells in mouse pancreas and their differentiation into β-cell-like derivatives [116]. Although interesting, these results need corroboration by other teams and careful analysis of the in vivo behavior and potential of the cells after transplantation.

(B) How Can the Immune Destruction of the Transplanted Cells Be Circumvented?

Encapsulation. Cell transplantation for treating diabetes is challenged by the host immune system via allo- and autoimmunity. Current immunosuppression protocols are still associated with numerous side effects that mitigate the benefits of cell therapy compared with conventional treatment. One alternative way of bypassing the immune system is to encapsulate the cells within a barrier allowing diffusion of glucose, other nutrients, and insulin but not of larger molecules, cells, or antibodies. This system has the theoretical advantage of precluding the need for immunosuppression and allowing the use of various cell types including porcine islets [117] or islet cells derived from stem/precursor cell sources. It also sequesters the cells, thus avoiding dissemination of potentially tumorogenic derivatives of genetically-modified cells.

Encapsulation can be configured using many different polymers, methods, and sizes [118]. Microcapsules have some advantages such as being small and easily placed into the peritoneal cavity. Their smaller size (conformal to 1,000 μm) improves surface-to-volume ratios and exchange of nutrients and molecules. Macroencapsulation is challenging because of the size of the devices that must be implanted, as well as the problems of slow nutrient delivery and delayed insulin secretory responses to stimuli. The first phase I trial with microencapsulated human islets in two T1D patients without immunosuppression showed a decrease in insulin daily needs and detectable C-peptide levels after 1 year of follow-up [119]. A more recent study demonstrated the safety of the technique in four T1D patients but detected only low levels of C-peptide secretion and no change in insulin requirements [120]. Ongoing trials should provide more information about the feasibility of this technique. Preliminary results from the team of Calafiore using intraperitoneal injection protocols showed possible reduction of insulin requirements and improvement of hypoglycemia unawareness and HbA1c levels [117].

The field of encapsulation is still struggling with a wide variety of issues, such as purity, stability, homogeneity, porosity, and biocompatibility of the materials [121]. Improvement in the efficacy of islet encapsulation has been obtained by combining a short-term or low-dose immunosuppression regimen with effects on survival, function, and capsular overgrowth being described [122]. Toso et al. showed a marked decrease of overgrowth of alginate microcapsules after portal vein infusion while using a short-term immunosuppression regimen [123]. Lee et al. evaluated transplants of rat islets with a combined therapy with cyclosporin A and PEGylation, a surface modification of islets using polyethylene glycol (PEG), and observed survival and glucose-responsiveness of the islets up to 100 days post-transplantation [124]. They confirmed the efficiency of PEGylation by showing survival and function of PEGylated rat islets in three of seven recipients 100 days post-transplantation [125]. Hypoxia remains a major concern with encapsulation. The survival and function of encapsulated islets might be improved after stimulation of local neovascularization. One approach that has been explored incorporated fibroblastic growth factor-1 into alginate microcapsules, which were then transplanted into rats [126].

Various approaches to macroencapsulation have emerged; for example, a planar pouch from TheraCyte is made of polytetrafluorethylene with an inner impermeable layer with 0.4-μm pores. This device allowed survival of tissue in allogeneic setting up to 1 year post-implantation [127, 128]. Recently, mouse islets

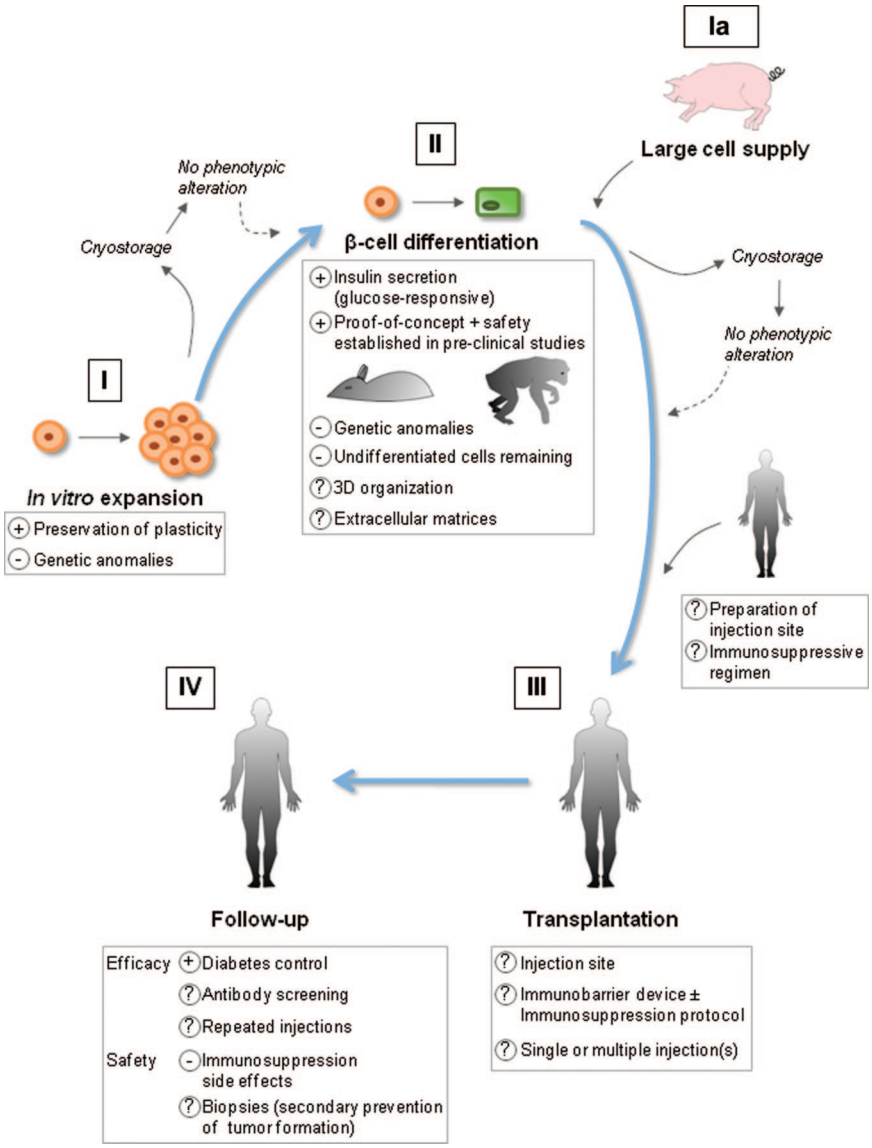

Figure 1. Requirements and remaining uncertainties concerning cell therapy for diabetes. Candidate cells for β-cell replacement therapy should be expandable in vitro as required for clinical application, with preservation of their plasticity and without generation of genetic anomalies (I). The cells might be usable when fresh or after cryostorage. Quality control after storage should allow selection of batches without phenotypic alteration. A reliable β-cell differentiation protocol should then generate glucose-responsive insulin-secreting cells in large amounts with no undifferentiated remaining cells (II). Whether current protocols would benefit from 3D cell organization or inclusion of extracellular matrices needs evaluation. Some partially differentiated phenotypes could be tolerated at this point, whereas generation of genetic anomalies by the differentiation protocol must be excluded. Proof-of-concept and safety assurance should be provided in small and probably large animal models before cells can be considered for clinical use. Differentiated products should ideally also be storable for future use. Cell sources allowing large tissue supply (e.g., porcine islets) might be immediately available for patients, but storage with cryostorage or long-term culture might be warranted to facilitate clinical application (Ia). Uncertainties remain concerning the preparation of the diabetic patient with regard to the infusion site or immunosuppressive regimen. Investigations are still mandatory to determine the ideal injection site, the immunoprotective protocol (immunobarrier device $\pm$ immunosuppression), and the amount of tissue needed (III). Besides the control of diabetes and monitoring of treatment side effects, clinical follow-up will be required to determine whether the patient is tolerating the graft, whether repeated injections are needed, or whether the graft contains cells with tumorogenic potential (IV). Desired, undesired, and unknown features are represented, respectively, by (+), (−), and (?). Abbreviation: 3D, three-dimensional.

and human fetal islet-like clusters macroencapsulated in the device were shown to survive and ameliorate diabetes after implantation into diabetic SCID mice, whereas adult human islets had poor survival in the same device [129].

Immunomodulation. Current aims of immunotherapy are to prevent the onset of T1D or reverse autoimmunity to preserve residual β-cell function and maintain endogenous insulin production. Reports have shown some preservation of insulin secretion in new-onset diabetes using vaccination with glutamic acid decarboxylase or anti-CD3 antibodies [130], for periods extending up to 5 years post-treatment. However, two phase III clinical trials using anti-CD3 monoclonal antibodies (teplizumab [131] and otelixizumab [132]) failed to meet their primary endpoints. Other antibodies, such as anti-CD20 rituximab, or antigen-specific agents, such as GAD65 and DiaPep277, are currently being tested [130]. Whether beneficial

effects on autoimmunity can be obtained with similar procedures in patients with long-standing T1D is unknown, and near total β-cell depletion will likely require interventions to enhance β-cell regeneration.

Classical immunosuppression regimens are still associated with lifelong side effects that represent an unwanted additional burden for unstable diabetic patients [133]. This is further complicated by the fact that routinely used calcineurin inhibitors are toxic for both kidneys and β-cells. Cell-specific immunomodulation, regulatory T-cells, or plasmapheresis might prove useful as alternative or adjuvant therapies. For instance, the anti-T-cell antibody alemtuzumab has recently been suggested to be important for achieving a 50% insulin independence at 5 years post-islet transplantation when associated with tacrolimus and mycophenolate mofetil [133]. Posselt et al. showed the possibility to obtain insulin independence 515 days after a single islet transplant with a diabetogenic and nephrotoxic drug-free protocol including costimulation blockade with belatacept, coupled with sirolimus, mycophenolate mofetil, and thymoglobulin [134]. Ongoing clinical trials will help determine efficacy and safety of these new treatment combinations.

Because of their immunotolerigenic properties, MSCs have been regarded as a potential tool to modulate autoimmunity in T1D [135]. In the NOD mouse model, serial congenic MSCs infusions decreased hyperglycemia after recent onset of diabetes, which was associated with modulation of diabetogenic cytokine, effector T-cell, and antigen-presenting cell profiles [136]. Another potential mechanism might be the induction of IL10-secreting regulatory T-cells by the infused MSCs [137]. With these properties and their potential availability in large numbers for autologous procedures, MSCs may provide benefits in various ways for diabetes.

With the aim of developing a radical approach to wipe out autoimmunity against β-cells, Voltarelli and coworkers designed a nonmyeloablative autologous stem cell transplantation protocol [138]. Among 23 T1D patients included in the study, 12 were insulin-independent for a mean of 31 months and 8 were receiving a low-dose insulin regimen (0.1–0.3 IU/kg) for up to 58 months. However, the burden of the procedure and the worrisome reported side effects (bilateral pneumonia, endocrine dysfunction, and oligospermia) can be expected to limit the clinical applicability of this approach.

Finally, encapsulation and/or immunomodulation will be mandatory either in auto- or heterologous transplantation protocols, with the aim of alleviating the assaults of auto- and alloimmunity. With recent developments in tissue-engineering techniques, the possibility of deriving β-cells from a patient's tissue without invasive surgery may become real and would offer the advantage of repeated procedures in the case of graft failure.

(C) Where to Transplant?

The optimal site for transplantation has not been determined. Islets were first transplanted into the peritoneal cavity [139]. When injected into the peritoneal cavity, encapsulated syngeneic and allogenic islets reversed diabetes in Balb/c and NOD mice for up to 350 days after transplantation [140]. However, the peritoneum does not allow easy access to the grafts as islets can disperse throughout the cavity or form a sediment on the pelvic floor. Portal vein injection, which has been used for the initial clinical trials, is associated with the risk of portal hypertension and portal vein thrombosis and has been suggested to activate the innate immune system and instant blood-mediated inflammatory reaction [141] that probably contributes to some degree of cell death. The kidney subcapsular space provides an alternative microenvironment and allows relatively easy graft retrieval. However, one study concluded that porcine islet xenotransplantation in this site in primates had poor graft survival [142].

The omental pouch, which is accessible and potentially reconstructed, appears to provide good blood supply. Survival of microencapsulated syngeneic islets in the omental pouch has been shown up to 400 days post-transplantation in NOD mice [143]. Furthermore, a report concluded that transplanted islets functioned better in the omentum than under the kidney capsule [144]. The gastric submucosal space is another potential site because it provides good oxygenization of the grafts because of its vascularization [145] and is accessible via endoscopy for transplantation and subsequent biopsy.

The subcutaneous site, although easy to access, is associated with mechanical strain, is thought to be immunologically hostile, and has poor vascularization; however, this might be ameliorated by prevascularization techniques [146]. Even the pancreas, the natural home of islets, holds some theoretical attractions [147], but there are major concerns about the risk of pancreatitis.

(D) Requirements for Translational Studies

Before new sources of cells can be considered for therapy, they must fulfill some prerequisites (Fig. 1). In vitro, these include being sufficient in number to provide a clinically relevant mass, cryoresistant to allow good manufacturing practice-compliant cell banking, and genetically stable with no signs of genomic alteration after expansion [148]. Besides being able to engraft, survive, and function long-term, transplanted cells must have minimal neoplastic potential. Cell localization at the time of infusion can be followed by tracing cells with a radioactive label, as was recently shown during infusion of MSCs in cirrhotic patients [149]. This technique may be useful for in vivo procedures in preclinical studies to obtain reassurance about the noninvasiveness of the transplanted cells [150]. A detailed checklist of desirable features will be helpful to evaluate whether a cell type can be an adequate candidate for β-cell replacement therapy [151].

Conclusion

For T1D, β-cell replacement therapy is a two-pronged problem that requires (a) replacing the β-cells and (b) controlling the autoimmunity and allorejection. There is reason to be optimistic that sufficient numbers of β-cells for transplantation will someday be available. Therapy with cells derived from stem cells has gained attention with high levels of differentiation obtained with ESCs and iPSCs. However, safety is a critical issue with these cell types and might delay their clinical applications. New insights on progenitor or somatic cell differentiation have opened the door for investigation, but in vitro evaluation is necessary to understand their potential for therapy. Stimulating replication or neoformation of β-cells within the pancreas could be a less invasive procedure and of high clinical value. New compounds being tested may be helpful in increasing β-cell mass. Progress is also being made with parallel developments of new immunosuppressive regimens, the quest to induce tolerance, and new approaches to encapsulation.

References

1 Atkinson MA, Bluestone JA, Eisenbarth GS et al. How does type 1 diabetes develop? The notion of homicide or beta-cell suicide revisited. Diabetes 2011;60:1370–1379.

2 Halban PA, German MS, Kahn SE et al. Current status of islet cell replacement and regeneration therapy. J Clin Endocrinol Metab 2010; 95:1034–1043.

3 Desgraz R, Bonal C, Herrera PL. Beta-cell regeneration: The pancreatic intrinsic faculty. Trends Endocrinol Metab 2011;22:34–43.

4 Dor Y, Brown J, Martinez OI et al. Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. Nature 2004;429:41–46.

5 Meier JJ, Butler AE, Saisho Y et al. Beta-cell replication is the primary mechanism subserving the postnatal expansion of beta-cell mass in humans. Diabetes 2008;57: 1584–1594.

6 Willcox A, Richardson SJ, Bone AJ et al. Evidence of increased islet cell proliferation in patients with recent-onset type 1 diabetes. Diabetologia 2010;53:2020–2028.

7 Meier JJ, Bhushan A, Butler AE et al. Sustained beta cell apoptosis in patients with long-standing type 1 diabetes: Indirect evidence for islet regeneration? Diabetologia 2005;48: 2221–2228.

8 Pipeleers D, Ling Z. Pancreatic beta cells in insulin-dependent diabetes. Diabetes Metab Rev 1992;8:209–227.

9 Keenan HA, Sun JK, Levine J et al. Residual insulin production and pancreatic ss-cell turnover after 50 years of diabetes: Joslin Medalist Study. Diabetes 2010;59:2846–2853.

10 Butler AE, Janson J, Bonner-Weir S et al. Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. Diabetes 2003;52:102–110.

11 Rahier J, Guiot Y, Goebbels RM et al. Pancreatic beta-cell mass in European subjects with type 2 diabetes. Diabetes Obes Metab 2008;10 Suppl 4:32–42.

12 Perl S, Kushner JA, Buchholz BA et al. Significant human beta-cell turnover is limited to the first three decades of life as determined by in vivo thymidine analog incorporation and radiocarbon dating. J Clin Endocrinol Metab 2010;95:E234–239.

13 In't Veld P, De Munck N, Van Belle K et al. Beta-cell replication is increased in donor organs from young patients after prolonged life support. Diabetes 2010;59:1702–1708.

14 Jovanovic L, Knopp RH, Brown Z et al. Declining insulin requirement in the late first trimester of diabetic pregnancy. Diabetes Care 2001;24:1130–1136.

15 Van Assche FA, Aerts L, De Prins F. A morphological study of the endocrine pancreas in human pregnancy. Br J Obstet Gynaecol 1978; 85:818–820.

16 Butler AE, Cao-Minh L, Galasso R et al. Adaptive changes in pancreatic beta cell fractional area and beta cell turnover in human pregnancy. Diabetologia 2010;53:2167–2176.

17 Bonner-Weir S, Li WC, Ouziel-Yahalom L et al. Beta-cell growth and regeneration: Replication is only part of the story. Diabetes 2010; 59:2340–2348.

18 Kopp JL, Dubois CL, Schaffer AE et al. Sox9+ ductal cells are multipotent progenitors throughout development but do not produce new endocrine cells in the normal or injured adult pancreas. Development 2011;138:653–665.

19 Furuyama K, Kawaguchi Y, Akiyama H et al. Continuous cell supply from a Sox9-expressing progenitor zone in adult liver, exocrine pancreas and intestine. Nat Genet 2011;43:34–41.

20 Criscimanna A, Speicher JA, Houshmand G et al. Duct cells contribute to regeneration of endocrine and acinar cells following pancreatic damage in adult mice. Gastroenterology 2011; 141:1451–1462.

21 Xu X, D'Hoker J, Stange G et al. Beta cells can be generated from endogenous progenitors in injured adult mouse pancreas. Cell 2008;132:197–207.

22 Bonner-Weir S, Baxter LA, Schuppin GT et al. A second pathway for regeneration of adult exocrine and endocrine pancreas. A possible recapitulation of embryonic development. Diabetes 1993;42:1715–1720.

23 Li WC, Rukstalis JM, Nishimura W et al. Activation of pancreatic-duct-derived progenitor cells during pancreas regeneration in adult rats. J Cell Sci 2010;123:2792–2802.

24 Martin-Pagola A, Sisino G, Allende G et al. Insulin protein and proliferation in ductal cells in the transplanted pancreas of patients with type 1 diabetes and recurrence of autoimmunity. Diabetologia 2008;51:1803–1813.

25 Reers C, Erbel S, Esposito I et al. Impaired islet turnover in human donor pancreata with aging. Eur J Endocrinol 2009;160:185–191.

26 Yamamoto K, Miyagawa J, Waguri M et al. Recombinant human betacellulin promotes the neogenesis of beta-cells and ameliorates glucose intolerance in mice with diabetes induced by selective alloxan perfusion. Diabetes 2000;49:2021–2027.

27 Suarez-Pinzon WL, Power RF, Yan Y et al. Combination therapy with glucagon-like peptide-1 and gastrin restores normoglycemia in diabetic NOD mice. Diabetes 2008;57:3281–3288.

28 Brand SJ, Tagerud S, Lambert P et al. Pharmacological treatment of chronic diabetes by stimulating pancreatic beta-cell regeneration with systemic co-administration of EGF and gastrin. Pharmacol Toxicol 2002;91:414–420.

29 Suarez-Pinzon WL, Yan Y, Power R et al. Combination therapy with epidermal growth factor and gastrin increases beta-cell mass and reverses hyperglycemia in diabetic NOD mice. Diabetes 2005;54:2596–2601.

30 Rosenberg L, Lipsett M, Yoon JW et al. A pentadecapeptide fragment of islet neogenesis-associated protein increases beta-cell mass and reverses diabetes in C57BL/6J mice. Ann Surg 2004;240:875–884.

31 Fleming A, Rosenberg L. Prospects and challenges for islet regeneration as a treatment for diabetes: A review of islet neogenesis associated protein. J Diabetes Sci Technol 2007;1:231–244.

32 Dungan KM, Buse JB, Ratner RE. Effects of therapy in type 1 and type 2 diabetes mellitus with a peptide derived from islet neogenesis associated protein (INGAP). Diabetes Metab Res Rev 2009;25:558–565.

33 Nauck MA, Kleine N, Orskov C et al. Normalization of fasting hyperglycaemia by exogenous glucagon-like peptide 1 (7–36 amide) in type 2 (non-insulin-dependent) diabetic patients. Diabetologia 1993;36:741–744.

34 Rachman J, Barrow BA, Levy JC et al. Near-normalisation of diurnal glucose concentrations by continuous administration of glucagon-like peptide-1 (GLP-1) in subjects with NIDDM. Diabetologia 1997;40:205–211.

35 Garber AJ. Long-acting glucagon-like peptide 1 receptor agonists: A review of their efficacy and tolerability. Diabetes Care 2011;34 Suppl 2:S279–S284.

36 Xu G, Stoffers DA, Habener JF et al. Exendin-4 stimulates both beta-cell replication and neogenesis, resulting in increased beta-cell mass and improved glucose tolerance in diabetic rats. Diabetes 1999;48:2270–2276.

37 Sturis J, Gotfredsen CF, Romer J et al. GLP-1 derivative liraglutide in rats with beta-cell deficiencies: Influence of metabolic state on beta-cell mass dynamics. Br J Pharmacol 2003;140:123–132.

38 Garber AJ. Incretin effects on beta-cell function, replication, and mass: The human perspective. Diabetes Care 2011;34 Suppl 2:S258–S263.

39 Mfopou JK, Chen B, Sui L et al. Recent advances and prospects in the differentiation of pancreatic cells from human embryonic stem cells. Diabetes 2010;59:2094–2101.

40 Soria B, Roche E, Berna G et al. Insulin-secreting cells derived from embryonic stem cells normalize glycemia in streptozotocin-induced diabetic mice. Diabetes 2000;49:157–162.

41 Mao GH, Chen GA, Bai HY et al. The reversal of hyperglycaemia in diabetic mice using PLGA scaffolds seeded with islet-like cells derived from human embryonic stem cells. Biomaterials 2009;30:1706–1714.

42 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;19:429–438.

43 D'Amour KA, Bang AG, Eliazer S et al. Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. Nat Biotechnol 2006;24:1392–1401.

44 Kroon E, Martinson LA, Kadoya K et al. Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. Nat Biotechnol 2008;26:443–452.

45 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;4:348–358.

46 Chen S, Borowiak M, Fox JL et al. A small molecule that directs differentiation of human ESCs into the pancreatic lineage. Nat Chem Biol 2009;5:258–265.

47 Chung WS, Stainier DY. Intra-endodermal interactions are required for pancreatic beta cell induction. Dev Cell 2008;14:582–593.

48 Van Hoof D, Mendelsohn AD, Seerke R et al. Differentiation of human embryonic stem cells into pancreatic endoderm in patterned

size-controlled clusters. Stem Cell Res 2011;6:276–285.

49 Higuchi Y, Shiraki N, Yamane K et al. Synthesized basement membranes direct the differentiation of mouse embryonic stem cells into pancreatic lineages. J Cell Sci 2010;123:2733–2742.

50 Fujikawa T, Oh SH, Pi L et al. Teratoma formation leads to failure of treatment for type I diabetes using embryonic stem cell-derived insulin-producing cells. Am J Pathol 2005;166:1781–1791.

51 Wang P, Rodriguez RT, Wang J et al. Targeting SOX17 in human embryonic stem cells creates unique strategies for isolating and analyzing developing endoderm. Cell Stem Cell 2011;8:335–346.

52 Kahan B, Magliocca J, Merriam F et al. Elimination of tumorigenic stem cells from differentiated progeny and selection of definitive endoderm reveals a Pdx1+ foregut endoderm stem cell lineage. Stem Cell Res 2011;6:143–157.

53 Jiang W, Sui X, Zhang D et al. CD24: A novel surface marker for PDX1-positive pancreatic progenitors derived from human embryonic stem cells. STEM CELLS 2011;29:609–617.

54 Polo JM, Liu S, Figueroa ME et al. Cell type of origin influences the molecular and functional properties of mouse induced pluripotent stem cells. Nat Biotechnol 2010;28:848–855.

55 Bar-Nur O, Russ HA, Efrat S et al. Epigenetic memory and preferential lineage-specific differentiation in induced pluripotent stem cells derived from human pancreatic islet beta cells. Cell Stem Cell 2011;9:17–23.

56 Mutskov V, Raaka BM, Felsenfeld G et al. The human insulin gene displays transcriptionally active epigenetic marks in islet-derived mesenchymal precursor cells in the absence of insulin expression. STEM CELLS 2007;25:3223–3233.

57 Marro S, Pang ZP, Yang N et al. Direct lineage conversion of terminally differentiated hepatocytes to functional neurons. Cell Stem Cell 2011;9:374–382.

58 Takahashi K, Tanabe K, Ohnuki M et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007;131:861–872.

59 Tateishi K, He J, Taranova O et al. Generation of insulin-secreting islet-like clusters from human skin fibroblasts. J Biol Chem 2008;283:31601–31607.

60 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;106:15768–15773.

61 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;107:13426–13431.

62 Zhu FF, Zhang PB, Zhang DH et al. Generation of pancreatic insulin-producing cells from rhesus monkey induced pluripotent stem cells. Diabetologia 2011;54:2325–2336.

63 Liu SP, Fu RH, Huang YC et al. Induced pluripotent stem (iPS) cell research overview. Cell Transplant 2011;20:15–19.

64 Gore A, Li Z, Fung HL et al. Somatic coding mutations in human induced pluripotent stem cells. Nature 2011;471:63–67.

65 Laurent LC, Ulitsky I, Slavin I et al. Dynamic changes in the copy number of pluripotency and cell proliferation genes in human ESCs and iPSCs during reprogramming and time in culture. Cell Stem Cell 2011;8:106–118.

66 Warren L, Manos PD, Ahfeldt T et al. Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. Cell Stem Cell 2010;7:618–630.

67 Anokye-Danso F, Trivedi CM, Juhr D et al. Highly efficient miRNA-mediated reprogramming of mouse and human somatic cells to pluripotency. Cell Stem Cell 2011;8:376–388.

68 Hu K, Yu J, Suknuntha K et al. Efficient generation of transgene-free induced pluripotent stem cells from normal and neoplastic bone marrow and cord blood mononuclear cells. Blood 2011;117:e109–e119.

69 Maekawa M, Yamaguchi K, Nakamura T et al. Direct reprogramming of somatic cells is promoted by maternal transcription factor Glis1. Nature 2011;474:225–229.

70 Feng B, Ng JH, Heng JC et al. Molecules that promote or enhance reprogramming of somatic cells to induced pluripotent stem cells. Cell Stem Cell 2009;4:301–312.

71 Zhu S, Li W, Zhou H et al. Reprogramming of human primary somatic cells by OCT4 and chemical compounds. Cell Stem Cell 2010;7:651–655.

72 Gershengorn MC, Hardikar AA, Wei C et al. Epithelial-to-mesenchymal transition generates proliferative human islet precursor cells. Science 2004;306:2261–2264.

73 Russ HA, Ravassard P, Kerr-Conte J et al. Epithelial-mesenchymal transition in cells expanded in vitro from lineage-traced adult human pancreatic beta cells. Plos One 2009;4:e6417.

74 Russ HA, Bar Y, Ravassard P et al. In vitro proliferation of cells derived from adult human beta-cells revealed by cell-lineage tracing. Diabetes 2008;57:1575–1583.

75 Chase LG, Ulloa-Montoya F, Kidder BL et al. Islet-derived fibroblast-like cells are not derived via epithelial-mesenchymal transition from Pdx-1 or insulin-positive cells. Diabetes 2007;56:3–7.

76 Atouf F, Park CH, Pechhold K et al. No evidence for mouse pancreatic beta-cell epithelial-mesenchymal transition in vitro. Diabetes 2007;56:699–702.

77 Weinberg N, Ouziel-Yahalom L, Knoller S et al. Lineage tracing evidence for in vitro dedifferentiation but rare proliferation of mouse pancreatic beta-cells. Diabetes 2007;56:1299–1304.

78 Seaberg RM, Smukler SR, Kieffer TJ et al. Clonal identification of multipotent precursors from adult mouse pancreas that generate neural and pancreatic lineages. Nat Biotechnol 2004;22:1115–1124.

79 Smukler SR, Arntfield ME, Razavi R et al. The adult mouse and human pancreas contain rare multipotent stem cells that express insulin. Cell Stem Cell 2011;8:281–293.

80 Bonner-Weir S, Taneja M, Weir GC et al. In vitro cultivation of human islets from expanded ductal tissue. Proc Natl Acad Sci U S A 2000;97:7999–8004.

81 Gao R, Ustinov J, Pulkkinen MA et al. Characterization of endocrine progenitor cells and critical factors for their differentiation in human adult pancreatic cell culture. Diabetes 2003;52:2007–2015.

82 Hao E, Tyrberg B, Itkin-Ansari P et al. Beta-cell differentiation from nonendocrine epithelial cells of the adult human pancreas. Nat Med 2006;12:310–316.

83 Yatoh S, Dodge R, Akashi T et al. Differentiation of affinity-purified human pancreatic duct cells to beta-cells. Diabetes 2007;56:1802–1809.

84 Baertschiger RM, Bosco D, Morel P et al. Mesenchymal stem cells derived from human exocrine pancreas express transcription factors implicated in beta-cell development. Pancreas 2008;37:75–84.

85 Seeberger KL, Dufour JM, Shapiro AM et al. Expansion of mesenchymal stem cells from human pancreatic ductal epithelium. Lab Invest 2006;86:141–153.

86 Bertelli E, Bendayan M. Intermediate endocrine-acinar pancreatic cells in duct ligation conditions. Am J Physiol 1997;273:C1641–C1649.

87 Hesselson D, Anderson RM, Stainier DY. Suppression of Ptf1a activity induces acinar-to-endocrine conversion. Curr Biol 2011;21:712–717.

88 Desai BM, Oliver-Krasinski J, De Leon DD et al. Preexisting pancreatic acinar cells contribute to acinar cell, but not islet beta cell, regeneration. J Clin Invest 2007;117:971–977.

89 Zhou Q, Brown J, Kanarek A et al. In vivo reprogramming of adult pancreatic exocrine cells to beta-cells. Nature 2008;455:627–632.

90 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;138:449–462.

91 Thorel F, Nepote V, Avril I et al. Conversion of adult pancreatic alpha-cells to beta-cells after extreme beta-cell loss. Nature 2010;464:1149–1154.

92 Gianani R. Beta cell regeneration in human pancreas. Semin Immunopathol 2011;33:23–27.

93 Fomina-Yadlin D, Kubicek S, Walpita D et al. Small-molecule inducers of insulin expression in pancreatic alpha-cells. Proc Natl Acad Sci U S A 2010;107:15099–15104.

94 Rezania A, Riedel MJ, Wideman RD et al. Production of functional glucagon-secreting alpha-cells from human embryonic stem cells. Diabetes 2011;60:239–247.

95 Saisho Y, Manesso E, Butler AE et al. Ongoing beta-cell turnover in adult nonhuman primates is not adaptively increased in streptozotocin-induced diabetes. Diabetes 2011;60:848–856.

96 Ferber S, Halkin A, Cohen H et al. Pancreatic and duodenal homeobox gene 1 induces expression of insulin genes in liver and ameliorates streptozotocin-induced hyperglycemia. Nat Med 2000;6:568–572.

97 Sapir T, Shternhall K, Meivar-Levy I et al. Cell-replacement therapy for diabetes: Generating functional insulin-producing tissue from

adult human liver cells. Proc Natl Acad Sci U S A 2005;102:7964–7969.

98 Kaneto H, Nakatani Y, Miyatsuka T et al. PDX-1/VP16 fusion protein, together with NeuroD or Ngn3, markedly induces insulin gene transcription and ameliorates glucose tolerance. Diabetes 2005;54:1009–1022.

99 Rowe C, Goldring CE, Kitteringham NR et al. Network analysis of primary hepatocyte dedifferentiation using a shotgun proteomics approach. J Proteome Res 2010;9: 2658–2668.

100 Marigliano M, Bertera S, Grupillo M et al. Pig-to-nonhuman primates pancreatic islet xenotransplantation: An overview. Curr Diab Rep 2011;11:402–412.

101 Hering BJ, Wijkstrom M, Graham ML et al. Prolonged diabetes reversal after intraportal xenotransplantation of wild-type porcine islets in immunosuppressed nonhuman primates. Nat Med 2006;12:301–303.

102 Cardona K, Milas Z, Strobert E et al. Engraftment of adult porcine islet xenografts in diabetic nonhuman primates through targeting of costimulation pathways. Am J Transplant 2007;7:2260–2268.

103 Sun Y, Ma X, Zhou D et al. Normalization of diabetes in spontaneously diabetic cynomologus monkeys by xenografts of microencapsulated porcine islets without immunosuppression. J Clin Invest 1996;98:1417–1422.

104 Dufrane D, Goebbels RM, Gianello P. Alginate macroencapsulation of pig islets allows correction of streptozotocin-induced diabetes in primates up to 6 months without immunosuppression. Transplantation 2010;90: 1054–1062.

105 Elliott RB, Escobar L, Tan PL et al. Intraperitoneal alginate-encapsulated neonatal porcine islets in a placebo-controlled study with 16 diabetic cynomolgus primates. Transplant Proc 2005;37:3505–3508.

106 Elliott RB, Escobar L, Tan PL et al. Live encapsulated porcine islets from a type 1 diabetic patient 9.5 yr after xenotransplantation. Xenotransplantation 2007;14:157–161.

107 Elliott RB, Limited LCT. Microencapsulated Neonatal Porcine Islet Implants Alleviate Unaware Hypoglycaemia without Immune Suppression, 13th World Congress of IPITA, the International Pancreas and Islet Transplant Association, Prague, Czech Republic, 2011.

108 Klymiuk N, Aigner B, Brem G et al. Genetic modification of pigs as organ donors for xenotransplantation. Mol Reprod Dev 2010; 77:209–221.

109 Salem HK, Thiemermann C. Mesenchymal stromal cells: Current understanding and clinical status. Stem Cells 2010;28:585–596.

110 Chao KC, Chao KF, Fu YS et al. Islet-like clusters derived from mesenchymal stem cells in Wharton's Jelly of the human umbilical cord for transplantation to control type 1 diabetes. PLoS One 2008;3:e1451.

111 Hocking AM, Gibran NS. Mesenchymal stem cells: Paracrine signaling and differentiation during cutaneous wound repair. Exp Cell Res 2010;316:2213–2219.

112 Lee JW, Fang X, Krasnodembskaya A et al. Concise review: Mesenchymal stem cells for acute lung injury: Role of paracrine soluble factors. Stem Cells 2011;29:913–919.

113 Bell GI, Broughton HC, Levac KD et al. Transplanted human bone marrow progenitor subtypes stimulate endogenous islet regeneration and revascularization. Stem Cells Dev 2011 [Epub ahead of print].

114 Ezquer F, Ezquer M, Simon V et al. The antidiabetic effect of MSCs is not impaired by insulin prophylaxis and is not improved by a second dose of cells. PLoS One 2011;6: e16566.

115 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: 17438–17443.

116 Ratajczak MZ, Liu R, Ratajczak J et al. The role of pluripotent embryonic-like stem cells residing in adult tissues in regeneration and longevity. Differentiation 2011;81:153–161.

117 Basta G, Calafiore R. Immunoisolation of pancreatic islet grafts with no recipient's immunosuppression: Actual and future perspectives. Curr Diab Rep 2011;11:384–391.

118 Hernández RM, Orive G, Murua A et al. Microcapsules and microcarriers for in situ cell delivery. Adv Drug Deliv Rev 2010; 62:711–730.

119 Calafiore R, Basta G, Luca G et al. Microencapsulated pancreatic islet allografts into nonimmunosuppressed patients with type 1 diabetes: First two cases. Diabetes Care 2006; 29:137–138.

120 Tuch BE, Keogh GW, Williams LJ et al. Safety and viability of microencapsulated human islets transplanted into diabetic humans. Diabetes Care 2009;32:1887–1889.

121 O'Sullivan ES, Vegas A, Anderson DG et al. Islets transplanted in immunoisolation devices: A review of the progress and the challenges that remain. Endocr Rev 2011 [Epub ahead of print].

122 Fort A, Fort N, Ricordi C et al. Biohybrid devices and encapsulation technologies for engineering a bioartificial pancreas. Cell Transplant 2008;17:997–1003.

123 Toso C, Mathe Z, Morel P et al. Effect of microcapsule composition and short-term immunosuppression on intraportal biocompatibility. Cell Transplant 2005;14:159–167.

124 Lee DY, Park SJ, Nam JH et al. A combination therapy of PEGylation and immunosuppressive agent for successful islet transplantation. J Control Release 2006;110:290–295.

125 Lee DY, Park SJ, Lee S et al. Highly poly(ethylene) glycolylated islets improve long-term islet allograft survival without immunosuppressive medication. Tissue Eng 2007;13: 2133–2141.

126 Moya ML, Garfinkel MR, Liu X et al. Fibroblast growth factor-1 (FGF-1) loaded microbeads enhance local capillary neovascularization. J Surg Res 2010;160:208–212.

127 Tibell A, Rafael E, Wennberg L et al. Survival of macroencapsulated allogeneic parathyroid tissue one year after transplantation in nonimmunosuppressed humans. Cell Transplant 2001;10:591–599.

128 Rafael E, Wu GS, Hultenby K et al. Improved survival of macroencapsulated islets of Langerhans by preimplantation of the immunoisolating device: A morphometric study. Cell Transplant 2003;12:407–412.

129 Lee SH, Hao E, Savinov AY et al. Human beta-cell precursors mature into functional insulin-producing cells in an immunoisolation device: Implications for diabetes cell therapies. Transplantation 2009;87:983–991.

130 Waldron-Lynch F, Herold KC. Immunomodulatory therapy to preserve pancreatic beta-cell function in type 1 diabetes. Nat Rev Drug Discov 2011;10:439–452.

131 Sherry N, Hagopian W, Ludvigsson J et al. Teplizumab for treatment of type 1 diabetes (Protege study): 1-year results from a randomised, placebo-controlled trial. Lancet 2011;378:487–497.

132 GlaxoSmithKline and Tolerx Announce Phase III DEFEND-1 Study of Otelixizumab in Type 1 Diabetes Did Not Meet Its Primary Endpoint. http://www.gsk.com/media/pressreleases/2011/2011_pressrelease_10039.htm. Accessed March 11, 2011.

133 Shapiro AM. State of the art of clinical islet transplantation and novel protocols of immunosuppression. Curr Diab Rep 2011;11: 345–354.

134 Posselt AM, Szot GL, Frassetto LA et al. Islet transplantation in type 1 diabetic patients using calcineurin inhibitor-free immunosuppressive protocols based on T-cell adhesion or costimulation blockade. Transplantation 2010; 90:1595–1601.

135 Abdi R, Fiorina P, Adra CN et al. Immunomodulation by mesenchymal stem cells: a potential therapeutic strategy for type 1 diabetes. Diabetes 2008;57:1759–1767.

136 Jurewicz M, Yang S, Augello A et al. Congenic mesenchymal stem cell therapy reverses hyperglycemia in experimental type 1 diabetes. Diabetes 2010;59:3139–3147.

137 Madec AM, Mallone R, Afonso G et al. Mesenchymal stem cells protect NOD mice from diabetes by inducing regulatory T cells. Diabetologia 2009;52:1391–1399.

138 Couri CE, Oliveira MC, Stracieri AB et al. C-peptide levels and insulin independence following autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus. JAMA 2009; 301:1573–1579.

139 Ballinger WF, Lacy PE. Transplantation of intact pancreatic islets in rats. Surgery 1972; 72:175–186.

140 Duvivier-Kali VF, Omer A, Parent RJ et al. Complete protection of islets against allorejection and autoimmunity by a simple barium-alginate membrane. Diabetes 2001;50:1698–1705.

141 Bennet W, Sundberg B, Groth CG et al. Incompatibility between human blood and isolated islets of Langerhans: A finding with implications for clinical intraportal islet transplantation? Diabetes 1999;48:1907–1914.

142 Dufrane D, Goebbels RM, Saliez A et al. Six-month survival of microencapsulated pig islets and alginate biocompatibility in primates: Proof of concept. Transplantation 2006;81: 1345–1353.

143 Kobayashi T, Aomatsu Y, Iwata H et al. Survival of microencapsulated islets at 400 days posttransplantation in the omental pouch of NOD mice. Cell Transplant 2006;15:359–365.

144 Guan J, Behme MT, Zucker P et al. Glucose turnover and insulin sensitivity in rats with pancreatic islet transplants. Diabetes 1998;47:1020–1026.

145 Echeverri GJ, McGrath K, Bottino R et al. Endoscopic gastric submucosal transplantation of islets (ENDO-STI): Technique and initial results in diabetic pigs. Am J Transplant 2009; 9:2485–2496.

146 Wang W, Gu Y, Tabata Y et al. Reversal of diabetes in mice by xenotransplantation of a bioartificial pancreas in a prevascularized subcutaneous site. Transplantation 2002;73:122–129.

147 Lau J, Mattsson G, Carlsson C et al. Implantation site-dependent dysfunction of transplanted pancreatic islets. Diabetes 2007; 56:1544–1550.

148 Prockop DJ, Olson SD. Clinical trials with adult stem/progenitor cells for tissue repair: Let's not overlook some essential precautions. Blood 2007;109:3147–3151.

149 Gholamrezanezhad A, Mirpour S, Bagheri M et al. In vivo tracking of (111)In-oxine labeled mesenchymal stem cells following infusion in patients with advanced cirrhosis. Nucl Med Biol 2011;38:961–967.

150 Cai W, Zhang Y, Kamp TJ. Imaging of induced pluripotent stem cells: From cellular reprogramming to transplantation. Am J Nucl Med Mol Imaging 2011;1:18–28.

151 Naujok O, Burns C, Jones PM et al. Insulin-producing surrogate beta-cells from embryonic stem cells: Are we there yet? Mol Ther 2011;19:1759–1768.