Autologous Pluripotent Stem Cell–Derived β-Like Cells for Diabetes Cellular Therapy

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Development of stem cell technologies for cell replacement therapy has progressed rapidly in recent years. Diabetes has long been seen as one of the first applications for stem cell–derived cells because of the loss of only a single cell type—the insulin-producing β-cell. Recent reports have detailed strategies that overcome prior hurdles to generate functional β-like cells from human pluripotent stem cells in vitro, including from human induced pluripotent stem cells (hiPSCs). Even with this accomplishment, addressing immunological barriers to transplantation remains a major challenge for the field. The development of clinically relevant hiPSC derivation methods from patients and demonstration that these cells can be differentiated into β-like cells presents a new opportunity to treat diabetes without immunosuppression or immunoprotective encapsulation or with only targeted protection from autoimmunity. This review focuses on the current status in generating and transplanting autologous β-cells for diabetes cell therapy, highlighting the unique advantages and challenges of this approach.

GENERATION OF β-CELLS FROM HUMAN PLURIPOTENT STEM CELLS

Diabetes is a group of diseases that affects hundreds of millions of people worldwide and is the result of the failure of insulin-producing β-cells to properly regulate blood glucose levels as a result of β-cell death, dysfunction, or inability to keep up with demand. Since the discovery of insulin in 1921, many patients are treated with administration of exogenous insulin or compounds to sensitize tissue to insulin or increase endogenous insulin secretion, but these interventions are imperfect surrogates for proper β-cell function in vivo, resulting in inconsistent blood glucose control and causing many patients to suffer from long-term complications (1). Over the past several decades, much attention has been paid to the development of methods to replace dead or dysfunctional tissue with new β-cells, particularly for type 1 diabetes (T1D), an autoimmune form of β-cell destruction, and for insulin-dependent type 2 diabetes (T2D) (2,3). A small number of patients have been transplanted with allogeneic human islets from cadaveric donors, which contain β-cells, and have remained insulin independent for years. Unfortunately, many factors limit this approach, particularly the scarcity and variability of isolated human islets, with patients often requiring islets from multiple donors to achieve normal blood glucose levels. The use of immunosuppressant drugs causes side effects for the patient, provides incomplete protection of the allogeneic cells, and, in the case of T1D, is necessary to control autoimmunity (4). Nonetheless, this clinical proof of concept shows that the replacement of functional tissue can be highly effective. The promise of cell replacement therapy for diabetes and the limitations of using allogeneic human islets have prompted much interest in a renewable source of β-cells.

Recently, several studies have reported generating β-like cells from human pluripotent stem cells (hPSCs) in vitro, including human embryonic stem cells (hESCs) (5–8), human induced pluripotent stem cells (hiPSCs) from donors without diabetes (ND) (5–7,9), hiPSCs from donors with T1D (9), and fibroblasts from ND donors (10) (Tables 1–3). hPSCs are developmentally immature cells that have the potential to become virtually any cell type found in the body (11). These pluripotent cells can be induced to undergo specific stages of differentiation by exposure to

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Table 1—Summary of reports on generating β-like cells: key in vitro characteristics

Report, year (ref.)	Cells used	Culture format	Percent β-like cells	NKX6-1?	PDX1?	MAFA?	GLIS3?	MNX1?
Rezania et al., 2014 (5)	ES, ND iPSC	Attachment/ALI	~40% (INS^+/$NKX6\text{-}1^+$)	Yes[a,b]	Yes[a,b]	Yes[a,b]	n.d.	n.d.
Pagliuca et al., 2014 (6)	ES, ND iPSC	Spinner flasks	33 ± 3% (CP^+/$NKX6\text{-}1^+$)	Yes[a]	Yes[a,b]	n.d.	Yes[b]	Yes[b]
Russ et al., 2015 (8)	ES	Low-adherence plates	17 ± 6% (CP^+/$NKX6\text{-}1^+$)	Yes[a,b]	Yes[a,b]	Yes[b]	n.d.	n.d.
Zhu et al., 2016 (10)	Fibroblast	Attachment/ALI	15% (CP^+/GCG^-/SST^-)	Yes[a,b]	Yes[a,b]	Yes[b]	n.d.	n.d.
Vegas et al., 2016 (7)	ES	Spinner flasks	~47% (CP^+/$NKX6\text{-}1^+$)	Yes[a]	n.d.	n.d.	n.d.	n.d.
Millman et al., 2016 (9)	T1D iPSC	Spinner flasks	24 ± 2% (CP^+/$NKX6\text{-}1^+$)	Yes[a]	Yes[a,b]	Yes[a]	Yes[b]	Yes[b]
Millman et al., 2016 (9)	ND iPSC	Spinner flasks	27 ± 2% (CP^+/$NKX6\text{-}1^+$)	Yes[a]	Yes[a,b]	Yes[a]	Yes[b]	Yes[b]

ALI, air-liquid interface; CP, C-peptide; ES, embryonic stem cells; GCG, glucagon; INS, insulin; iPSC, induced pluripotent stem cells; n.d., not determined; SST, somatostatin. [a]Detected by immunostaining. [b]Detected by microarray or PCR.

defined combinations of growth factors and small molecules to activate and inhibit signaling pathways to mimic normal human pancreatic development (Fig. 1). Earlier work with hPSCs identified the pathways necessary to effectively specify definitive endoderm, gut tube, and pancreatic progenitor fates (12,13), which were later built upon and refined (14–17) (reviewed by Pagliuca and Melton [18]). Pancreatic progenitors are proliferative and are multipotent, having the ability to generate the acinar, endocrine, and ductal compartments of the pancreas, and are commonly identified through coexpression of PDX1 and NKX6-1. Earlier reports demonstrated that, upon transplantation into rodents, a portion of these cells will spontaneously differentiate into β-like cells through an unknown mechanism (17,19), a phenomenon that is the basis for a clinical trial being conducted by ViaCyte, Inc. (ClinicalTrials.gov identifier: NCT02239354). This trial uses a macroencapsulated allogeneic hESC-derived pancreatic progenitor product known as VC-01.

An alternative strategy to pancreatic progenitor transplantation would be the transplantation of in vitro differentiated β-cells. This path has been stymied by the significant challenge of discovering methods and signaling pathways to induce β-cell differentiation. However, recently several groups have discovered strategies to generate endocrine and β-like cells in vitro from pancreatic progenitors. These protocols typically use signaling with thyroid hormone, retinoic acid, and EGF and inhibiting of γ-secretase, TGF-β, Shh, Axl, and BMP. There are several notable differences that exist among the protocols. Rezania et al. (5) describe the importance of the Axl inhibitor R428 in achieving certain β-cell features, which is not necessary in most other reports. Russ et al. (8) are notable for achieving their results with far fewer factors and in a shorter total differentiation time than the other protocols. Zhu et al. (10) use a Ca^{2+} channel agonist BayK-8644, which is not used by the other protocols. Rezania et al. (5) and Zhu et al. (10) perform the first several differentiation stages in attachment culture, then create clusters on an air-liquid interface or in suspension culture, respectively, whereas other protocols perform the entire differentiation in suspension culture. Despite the differences, most methods primarily involve modulation of thyroid hormone, γ-secretase, and TGF-β signaling.

These in vitro derived β-like cells share features with adult β-cells from cadaveric islet donors, making stem cell–derived β-like cells a promising renewable cell source for diabetes cell replacement therapy (Table 1). They function both in vitro and in vivo, responding to elevated glucose by increased cytoplasmic Ca^{2+} concentrations and increased secretion of insulin and C-peptide. This insulin is packaged into secretory granules that can be observed with electron microscopy. However, insulin secretion per cell is low compared with cadaveric human islets, 2–3× in vitro and 4–5× in vivo lower according to Pagliuca et al. (6), and proper dynamic secretion profiles, including first- and second-phase response to glucose and pulsatile insulin secretion, have not yet been reported. Even with these limitations, transplanted β-like cells from hPSCs are able to control

blood glucose in several different models of diabetes in mice (5–7,9,10). Furthermore, by global transcriptional profiling, β-like cells from hPSCs are more similar to primary adult β-cells than fetal β-cells. Key transcription factors NKX6-1, PDX1, MAFA, GLIS3, and MNX1 are expressed by these β-like cells, although expression levels of some genes are not identical to cadaveric adult β-cells, such as MAFA and GLIS3. Even with these critical features, there are several important limitations to note in addition to the before-mentioned differences in function and gene expression compared with adult β-cells. The published in vivo studies have demonstrated that β-like cells persist for up to half a year, but actual longevity of these cells after transplantation has not yet been established, in part because of limitations of animal life span. In unpublished studies in immunocompromised mice, we have observed continued function of transplanted β-like cells for over a year and expression of Ki67 in an exceedingly small fraction of insulin-expressing cells, suggesting the potential for long-term graft function.

Additionally, although β-like cells are transplanted along with other cell types, the composition of these cellular populations differs significantly from that in human islets. In particular, while glucagon-expressing and somatostatin-expressing cells are present in β-like cell preparations, the functional and maturation status of these cells is unclear. These other islet endocrine cells are typically found in a smaller proportion to β-like cells than what is observed in human islets. Finally, an additional concern for hPSC-derived β-like cells, compared with cadaveric islets, is the potential for transplanted cells to contain residual undifferentiated hPSCs that can form teratomas (20). Studies generating β-like cells have not noted tumor formation after short-term transplantation, but a longer study with a sufficiently large number of replicate animals has not yet been reported for evaluating this risk. Protocols that are highly efficient at generating β-like cells may be sufficient to avoid this risk. Alternatively, sorting to keep cells that express pancreatic cell surface markers (21) or to remove cells that express hPSC surface markers (22), introducing an inducible suicide gene as a safeguard to kill the entire graft if teratomas arise (23), or establishing media formulations that destroy residual hPSCs (24) but not β-like cells could be explored as needed.

PATHWAYS TO THE CLINIC

With the discoveries of how to generate functional β-like cells from hPSCs, attention has now turned to how best to transplant these cells into patients. Two primary challenges are present: *1)* how to manufacture sufficiently pure and potent cells at scale for clinical use and *2)* how to protect the cells from immunorejection following transplantation into a patient. Fortunately for the stem cell technology field, scientists have been focused on the latter for several decades. Leading strategies for preventing immunorejection of allogeneic cadaveric islets include immunosuppression and macro- or microencapsulation, with examples of each of these approaches reaching clinical trials (reviewed by Tomei

Table 2—Summary of reports on generating β-like cells: key in vivo characteristics

Report, year (ref.)	In vitro secretion per cell	In vitro stimulation index	In vivo concentration[a]	In vivo stimulation index[a]	Diabetes reversal/ prevention?
Rezania et al., 2014 (5)	n.d.	1.4–3.3[b] (CP)	0.5–2.5 ng/mL; 167–833 pmol/L (CP; 2 weeks; 1.25 × 10^6 cells)[c]	∼1.4 (2 weeks)	Yes (40 days[a])
Pagliuca et al., 2014 (6)	1.6 ± 0.2 μIU/10^3 cells (INS)	2.2 ± 0.3[b] (INS)	8.4 ± 1.8 μIU/mL (INS; 2 weeks; 5 × 10^6 cells)[c]	1.7 ± 0.2[b] (2 weeks)	Yes (18 days[a])
Russ et al., 2015 (8)	n.d.	1.8 ± 0.9 (CP)	0.12 ± 0.08 ng/mL; 40 ± 28 pmol/L (CP; 7–10 days; 5 × 10^6 cells)[c]	∼1.3 (7–10 days)	No
Zhu et al., 2016 (10)	n.d.	2.0 ± 0.4[b] (INS)	0.04–1.2 ng/mL; 14–400 pmol/L (CP; 2 months; 5 × 10^6 cells)[c]	2.2[b] (2 months)	Yes[d]
Vegas et al., 2016 (7)	1.7–2.1 μIU/10^3 cells (INS)	1.7–2.0 (INS)	0.72 ± 0.06 ng/mL; 240 ± 20 pmol/L (CP; 3 weeks; 250 cell clusters)[e]	n.d.	Yes (7 days[a])
Millman et al., 2016 (9): T1D iPSC	2.0 ± 0.4 μIU/10^3 cells (INS)	1.9 ± 0.5[b] (INS)	6.1 ± 1.0 μIU/mL (INS; 2 weeks; 5 × 10^6 cells)[c]	1.4 ± 0.3[b] (2 weeks)	Yes[d]
Millman et al., 2016 (9): ND iPSC	1.9 ± 0.3 μIU/10^3 cells (INS)	2.2 ± 0.5[b] (INS)	7.4 ± 0.9 μIU/mL (INS; 2 weeks; 5 × 10^6 cells)[c]	1.5 ± 0.2[b] (2 weeks)	Yes[d]

CP, C-peptide; INS, insulin; iPSC, induced pluripotent stem cells; n.d., not determined. [a]Earliest time point shown. [b]Statistically significant. [c]After glucose injection. [d]Diabetes induced 2–4 months after transplantation. [e]After 1-h fast.

Table 3—Summary of reports on generating β-like cells: other notable features

Report, year (ref.)	Other notable features
Rezania et al., 2014 (5)	Insulin granules, cytosolic calcium flux, reduced C-peptide after insulin injection in vivo
Pagliuca et al., 2014 (6)	Insulin granules, cytosolic calcium flux, multiple sequential responses to glucose in vitro, global gene expression
Russ et al., 2015 (8)	Insulin granules
Zhu et al., 2016 (10)	Expansion of early progenitors
Vegas et al., 2016 (7)	Diabetes reversal in immunocompetent mouse with encapsulation, multiple sequential responses to glucose in vitro
Millman et al., 2016 (9): T1D iPSC	Insulin granules, multiple sequential responses to glucose in vitro, global gene expression
Millman et al., 2016 (9): ND iPSC	Insulin granules, multiple sequential responses to glucose in vitro, global gene expression

iPSC, induced pluripotent stem cells.

et al. [25] and Scharp and Marchetti [26]). Additional approaches for islet transplant, including inducing immune tolerance and genetically engineering cells, have not yet been tested in humans but may provide a path forward in the future. The potential transplantation of autologous cells may remove this immunoprotection challenge but introduces other unique hurdles, as discussed below.

Transplantation of cadaveric islets using immunosuppression has been the most common and successful approach to date. The Edmonton trial in 2000 generated significant excitement by demonstrating that insulin independence could be achieved in seven patients with T1D through islet transplantation combined with steroid-free immunosuppression (27). In this process, islets are isolated from an allogeneic cadaveric pancreas and infused through the portal vein into the liver. More recently, a multi-institutional effort called the Collaborative Islet Transplant Registry (CITR, www.citregistry.org) has moved immunosuppression-based islet transplants into phase 3 clinical trials in the U.S. (28,29). These data will hopefully result in approval of a biologics license application that would enable broader patient access and reimbursement for this therapy in the U.S. Although this approach has provided clinically meaningful results to many patients with severe T1D, including insulin independence for more than 6 years in the best cases, the ultimate goal is to provide β-cell replacement without the need for lifelong immunosuppression. Micro- and macroencapsulation strategies aim to provide a physical barrier with sufficient permselectivity to allow nutrient and insulin exchange while simultaneously preventing components of the immune system from destroying the transplanted islets (26). This approach has the significant advantage of theoretically being able to prevent both allogeneic rejection and autoimmune attack. For example, Living Cell Technologies and others have tested alginate-based microencapsulation approaches in clinical trials with small patient cohorts with preliminary results suggesting the potential for long-term survival and function. Retrievable, permselective membrane-based macroencapsulation devices are being developed by companies including Beta-O2 Technologies, Sernova Corp., ViaCyte, Defymed, and Semma Therapeutics (reviewed by Scharp and Marchetti [26]). These approaches all face challenges, including nutrient limitations due to lack of tissue integration/vascularization and the prospect of fibrosis encasing the foreign material (reviewed by Colton [30]). Innovative device designs and materials are being developed to overcome these hurdles to enable clinical success of encapsulated β-cells or islets.

Biological approaches provide a potential alternative to the engineering solutions for preventing allogeneic rejection and autoimmune attack but have their own critical challenges for clinical translation. Costimulation blockade with CTLA4-Ig and anti-CD40L monoclonal antibodies prevents rejection of hESC-derived pancreatic progenitors in immune-competent mice and mice humanized with peripheral blood mononuclear cells (PBMCs) (31). Genetic engineering of a universal cell line is being pioneered by companies like Universal Cells by replacing the class I human leukocyte antigen (HLA) locus with HLA-E or HLA-G to mimic pregnancy-based tolerance of allogeneic tissue, without triggering natural killer cell–based killing, and by deleting transcription factors required for class II HLA expression, although proof of effectiveness in humans has yet to be demonstrated (32). Additional editing approaches have taken inspiration from the cancer field by introducing molecules like PD-L1 and CTLA4, which can induce immune tolerance (33). The challenges of these approaches include demonstrating the effectiveness of the strategies in animal models that poorly predict human outcomes and establishing safety of a cell population that could theoretically harbor viruses or malignant tissue from immune destruction. A further issue is that these models are studying allo- or xenogeneic rejection and not directly studying human autoimmunity. It remains to be seen if a biological approach that prevents allo- or xenogeneic rejection would also prevent autoimmunity.

STRATEGIES FOR GENERATING AUTOLOGOUS β-CELLS FROM hiPSCS

Alternatively, technologies like hiPSCs provide the opportunity to generate patient-specific cell lines from which matched, autologous tissue could be manufactured (Fig. 2). In the case of diabetes, these patient-specific hiPSC lines could be differentiated into β-like cells for autologous transplantation. These cells are generated by reprogramming somatic cells via gene overexpression into a pluripotent state. hiPSCs have been derived from several different patient populations with diabetes, including T2D (34), cystic fibrosis–related diabetes (35), maturity-onset

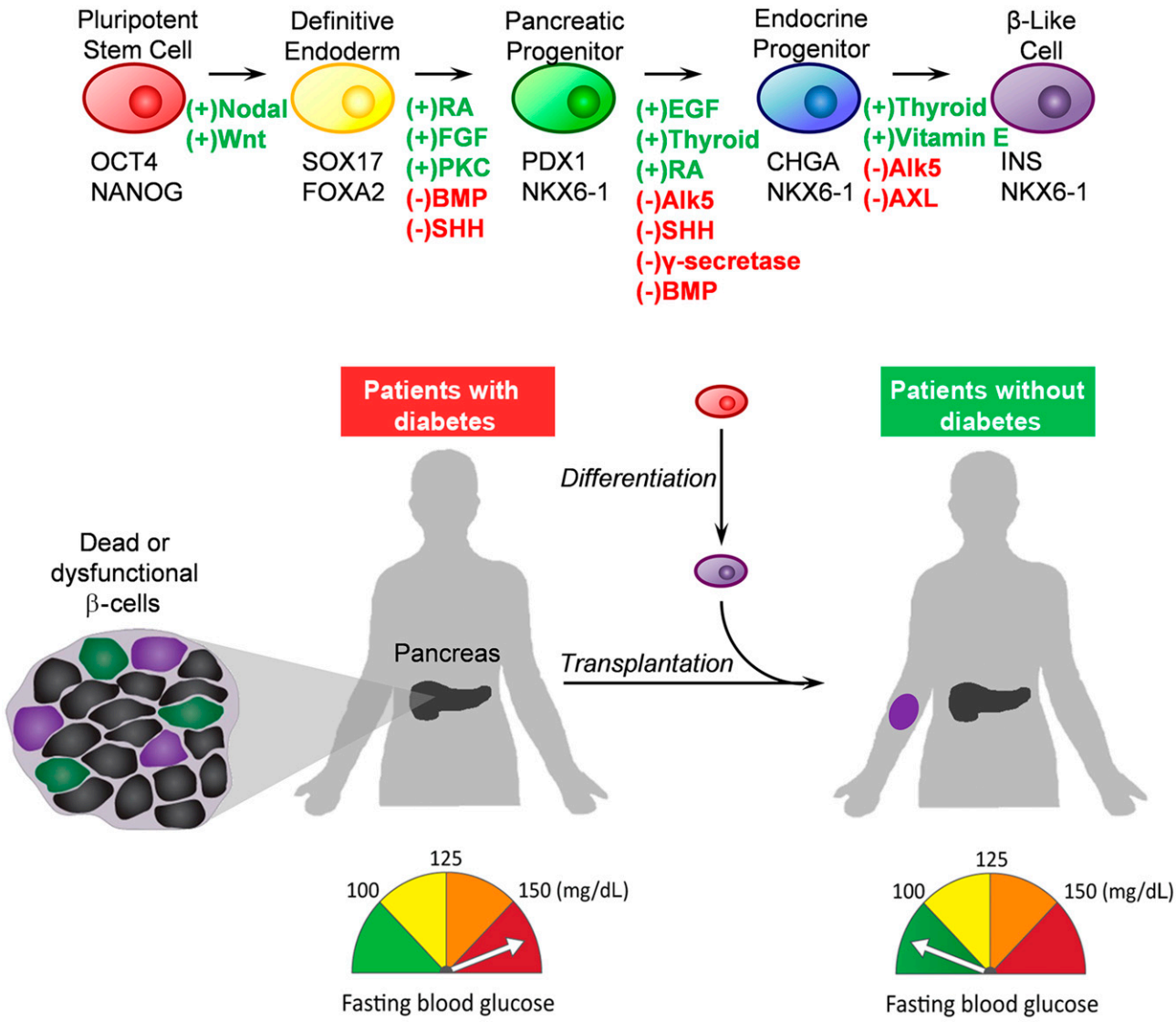

Figure 1—Directed differentiation of hPSCs into β-like cells. Top: Schematic of signaling pathways that are activated [green, (+)] or inhibited [red, (-)] during stepwise differentiation of hPSCs to β-like cells that are shared by at least two differentiation protocols. Major gene expression markers for each cell type are also indicated. Bottom: Overview schematic for cellular therapy to control blood glucose in patients with diabetes. RA, retinoic acid.

diabetes of the young (36), Wolfram syndrome (37), and T1D (38). β-Like cells have been differentiated from ND hiPSCs without obvious differences observed compared with hESC-derived cells (5,6). More recently, β-like cells from T1D patient-derived hiPSCs have been generated and compared with hiPSCs from ND donors (9). In this study, hiPSCs from three T1D and three ND donors were studied, and each donor line was able to differentiate into β-like cells. T1D and ND β-like cells were indistinguishable from each other in terms of in vitro and in vivo function and general gene expression, even protecting mice from alloxan-induced diabetes. Although analysis of more patient lines and assays will likely reveal some differences and autologous β-like cells would still be subject to autoimmune attack in T1D, these data provide the first direct evidence that β-like cells from patients with diabetes may be valuable for cell replacement therapy.

A major assumption in the field has been that transplanted differentiated cells from autologous hiPSCs will not provoke an immune response. This has been a controversial topic, prompted by a report from Zhao et al. (39) showing mouse induced pluripotent stem cells (miPSCs) but not mouse embryonic stem cells transplanted into syngeneic mice provoked an immune response. However, this immune response was against the resulting teratomas formed following transplantation of the undifferentiated cells—an approach that differs significantly from the transplantation of differentiated cells, as would be used in cell replacement therapy. More recently, the same group reported a cell type–dependent

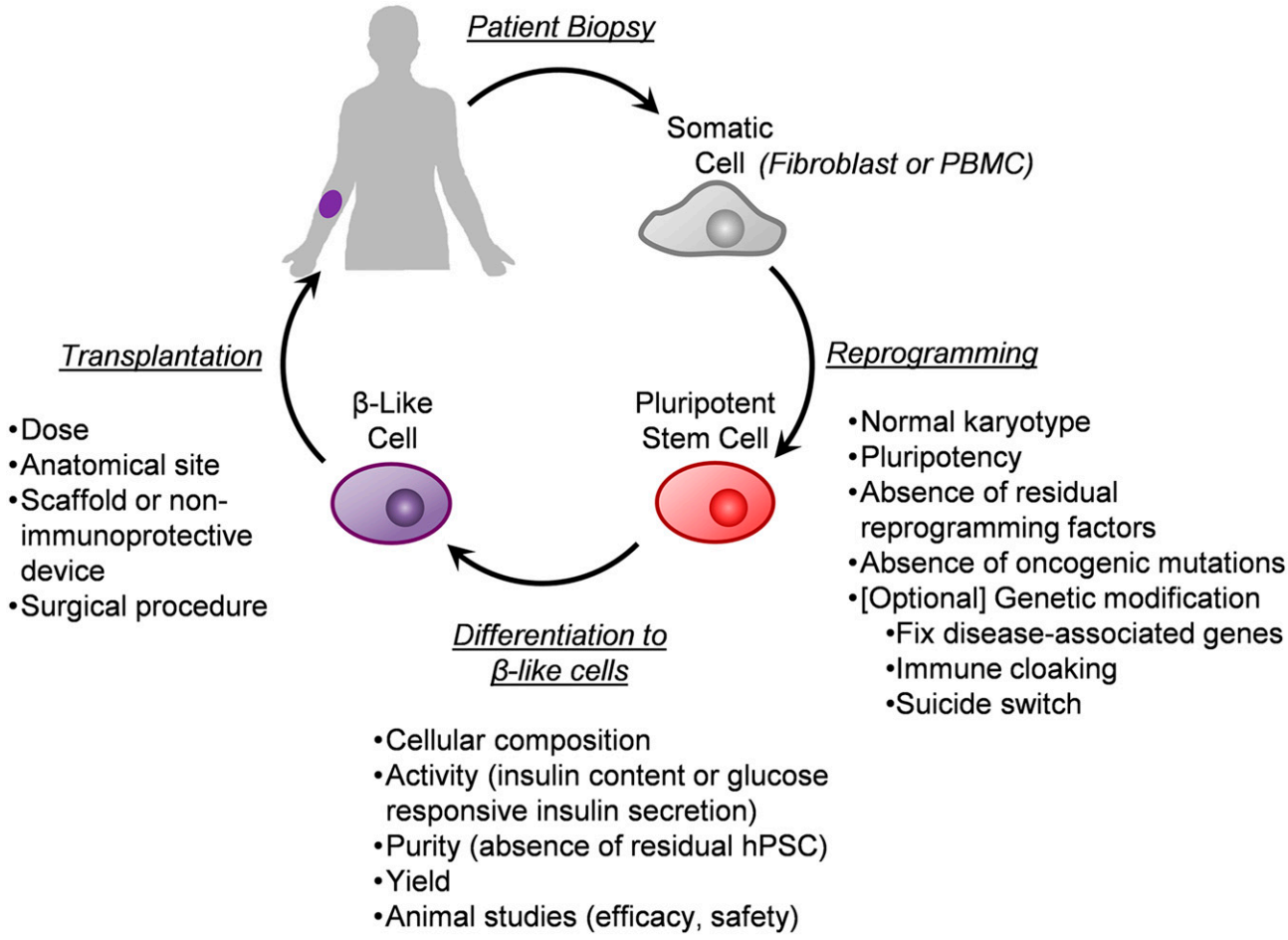

Figure 2—Overview of derivation of autologous β-like cells. Somatic cells—for example, fibroblasts or PBMCs—are harvested from the patient and reprogrammed into an hiPSC by gene overexpression. hiPSCs undergo quality control testing, are genetically modified to meet patient need, and are expanded to a therapeutic dose and then differentiated into β-like cells in vitro. Several additional quality control metrics are assessed, including measurements of purity, glucose responsiveness, and confirmation of the cellular preparation being free from malignant cells, such as residual hPSCs. β-Like cells that pass all quality control measures can then be transplanted into patients.

immune response using hiPSC-derived donor-matched humanized mice that had an immune system constructed from fetal liver, $CD34^+$ cells, and thymus (40). hiPSC-derived smooth muscle but not retinal pigment epithelial (RPE) cells were highly immunogenic, which the authors attributed to abnormal expression of antigens on the smooth muscle cells (40). Guha et al. (41) differentiated miPSCs into cells from all three germ layers and observed little evidence of immune rejection when transplanted into syngeneic mice. In nonhuman primates, autologous induced pluripotent stem cells differentiated into neurons provoked minimal immune response and allogeneic cells a stronger one (42). The cause for these discrepancies and the true immunogenicity of autologous hiPSCs is currently unclear. A possible cause is the quality of the differentiation procedures used. Less efficient protocols could contain residual undifferentiated or partially differentiated cells within mature, differentiated populations that provoke an immune response, or the presence of xenogeneic material in the in vitro differentiation culture, which is often not controlled in these types of studies, could be carried over upon transplantation. The ultimate test will be the transplantation of autologous hiPSC-derived tissues into patients with appropriate monitoring for immune activation or sensitization.

Alternatives sources of autologous cells for generating β-like cells include direct reprogramming from a somatic cell and somatic cell nuclear transfer (SCNT). To date, direct conversion of a human somatic cell into a β-like cell has not been reported, although human cells expressing pancreatic markers, including insulin, have been specified and significant progress directly reprogramming mouse cells to β-like cells has been made (reviewed by Cavelti-Weder et al. [43]). Zhu et al. (10) recently published a procedure starting with fibroblasts by using a combination of short-term overexpression of OCT4, SOX2, KLF4, and a short hairpin against p53 followed by a directed differentiation protocol to produce endoderm, gut tube, pancreatic progenitors, and finally β-like cells. An interesting feature of these cells is the ability to proliferate and expand intermediate cell

types before final differentiation into β-like cells, which only very slowly proliferate. Furthermore, avoiding the need to generate full hiPSCs as an intermediate cell type could potentially avoid teratoma risk and save some time in manufacturing if high-efficiency, high-yield protocols can be developed. Alternatively, SCNT has been used to make hPSCs from T1D patients, which differentiated into cells that express pancreatic markers (44). Since SCNT produces hPSCs that are very similar to hESCs and hiPSCs, existing protocols to generate β-like cells would likely succeed with these cells, but given the enormous technical complexity of SCNT, it is currently unlikely that this methodology will overtake hiPSCs as a candidate cell source for autologous cell replacement therapies.

TARGET PATIENT POPULATIONS

Several critical and unique challenges face development of autologous hPSC-derived therapies for diabetes, starting with appropriate patient selection. Diabetes results from a variety of underlying causes, meaning multiple potential target patient populations exist for autologous therapy. Most straightforward conceptually are patients that have received a pancreatectomy because of chronic pancreatitis and with underlying β-cell autoimmunity. Insulin-dependent T2D may also be relatively simple; however, insulin resistance and requirements in addition to other metabolic concerns (i.e., gluco- and lipotoxicity) are critical considerations for achieving meaningful clinical impact. Proof of concept for T2D was shown by Bruin et al. (45) transplanting diet-induced diabetic and obese mice with pancreatic progenitors from hESCs and demonstrating improved glucose tolerance. Combination of this cellular therapy with an antidiabetes drug resulted in greater improvements in glycemic control compared with drug treatment alone. Several monogeneic forms of diabetes (46), including cystic fibrosis–related diabetes, maturity-onset diabetes of the young, Wolfram syndrome, and neonatal diabetes, are also potentially good candidates. With the advent of gene editing methods with high efficiency and fidelity, such as CRISPR/Cas9, the genetic defect can be fixed in patient-derived hiPSCs or, as has been recently reported, simultaneously with reprogramming of somatic cells to hiPSCs (47). Fixing the underlying genetic defect would ensure that the autologous β-like cells do not fail, as occurred with the original endogenous β-cells. This approach may also have value in polygenic forms of diabetes, including T2D and T1D, to improve graft function, longevity, and response to stress. A genetic safety switch could also be introduced to kill the cells if any safety issues are encountered, for example, by overexpression of caspase-9 upon drug treatment (23). However, genetic engineering of the cells introduces an additional challenge that must be justified to support the additional complexity for clinical manufacturing and regulatory approval.

T1D is the form of diabetes that receives the most attention in the literature for cell replacement therapy and may ultimately be treatable with a combination of autologous β-like cells and immunotherapies to halt the autoimmune attack. Therapies to address the autoimmunity are currently under development, including by modulating T regulatory cells. Bluestone et al. (48) reported persistent C-peptide levels for over 2 years in a phase 1 clinical trial with a small cohort of patients with recent-onset T1D by transplantation of patient-derived T regulatory cells that had been expanded in vitro. In humanized mice, human T regulatory cells can be stimulated with insulin mimetopes (49). In nonhumanized mice, mouse T regulatory cells can be modulated to tolerate specific antigens by binding antigens to the surface of erythrocytes, which die by eryptosis in vivo, inducing tolerance to the antigen (50). T regulatory cells can also be derived from miPSCs, and those cells have been shown to suppress autoimmunity in a mouse arthritis model (51). These immune therapies may correct the autoimmune problem and prevent further β-cell loss, but as T1D diagnosis usually occurs after the patient has lost most or all of their β-cell mass and β-cells have very limited proliferative capability, only a combination therapy that also replaces the destroyed tissue will restore normal glycemic control.

MANUFACTURING AND PRECLINICAL STRATEGIES FOR AN AUTOLOGOUS APPROACH

After a patient with diabetes has been selected, an autologous hiPSC line (or equivalent) needs to be generated. Fibroblasts have been the most common starting material for research hiPSC lines, but PBMCs are increasingly popular because of the ease of procurement. Viral vectors are also commonly used in research and are efficient but are disfavored clinically. Nonintegrating RNA-based Sendai virus may prove a suitable method clinically if materials are sourced and lines are assayed adequately. Recently, a process was described to generate hiPSCs under current good manufacturing practice using a nonintegrative, nonviral episomal-based technology to overexpress OCT4, SOX2, KLF4, c-MYC, and LIN28. This approach was used to create cell banks of hiPSCs that express pluripotency markers, maintain normal karyotype, and differentiate into all three germ layers (52). However, unintentional plasmid integration continues to be a concern, necessitating development of sensitive assays for their detection. Transfection with modified RNA has the advantage of involving no exogenous DNA and generating colonies faster, but it is laborious, has variable efficiency, and has not been successful with all starting somatic cell types (53). Standards to help achieve consistency in hPSC products, primarily focused on development of reference materials, were recently proposed from a number of experienced investigators in the field (54). However, no formal guidance from the U.S. Food and Drug Administration (FDA) is available yet to guide development of pluripotent or, specifically, hiPSC lines.

In all cases, current scientific practice would indicate that multiple hiPSC clones should be selected at the start to

ensure good-quality starting material that will pass quality control checkpoints, including karyotype testing, evaluation of pluripotency, absence of exogenous reprogramming material, absence of oncogenic mutations, and efficient differentiation capacity. Derivation protocols must also be robust enough to overcome potential donor differences and validated with a large number of hiPSC lines derived from different donors. Although costs are currently high for manufacturing each hiPSC line, this cost is likely to continually fall in coming years, particularly with the adoption of more automated processes (55).

Importantly, autologous hiPSCs are already being used in a first clinical trial. Differentiated RPE cells were transplanted into a patient in Japan in September 2014 (56). The hiPSC line was derived by episomal reprogramming of dermal fibroblasts from the patient, and no safety concerns have been reported to date. However, a second patient has not yet been transplanted for a variety of reasons, including identification of a genomic mutation in the derived autologous hiPSCs (57). Nonetheless, this trial demonstrates the feasibility of clinical development of an autologous hiPSC-derived cell replacement product and highlights the importance of robust quality control.

The next manufacturing challenges include establishment of robust manufacturing protocols across multiple starting cell lines. Patient-to-patient variation in hiPSCs exists because of differences in genetic background, which influences hiPSC gene expression and methylation state (58,59) and likely influences differentiation to β-like cells. Some well-established protocols, such as that for generating RPE cells for the treatment of macular degeneration or cardiomyocytes for disease modeling or transplantation, now work well across multiple starting lines (60,61). New protocols for generating β-like cells will likely require some additional process development to reproducibly generate clinically suitable cells from multiple patients. Key considerations include ensuring composition (number of β-like cells and other pancreatic cell types), purity (absence of residual hPSCs or other cells with malignant potential), and activity (insulin release per cell) standards. Assay development related to these product characteristics will be critical to support release of cell material expected to be both safe and efficacious. Demonstration of the characterization of each of these features (composition, purity, and activity), the reproducibility of the manufacturing process, and consistent pharmacology and toxicology results in animal studies showing safety and efficacy will be required before FDA approval to initiate first-in-human clinical trials.

Patient-specific product development and manufacturing is one of the main bottlenecks and cost drivers of autologous cell therapies. Although development of protocols that are highly reproducible across cell lines is a specific challenge for autologous programs, one manufacturing advantage is that the scale required for a single patient is relatively small, on the order of 10^8–10^9 cells for diabetes. This has already been achieved in academic settings (6,7,9) and would require minimal to no scale-up format changes. In contrast, allogeneic programs necessitate significant scale-up bioreactors or other systems to support very large production scales ($>10^{11}$ cells), of which, limited manufacturing infrastructure for pluripotent cell expansion or differentiation currently exists. However, individualized manufacturing at small scale does not lend itself to the economies of scale that can ultimately reduce the cost of allogeneic cell therapies far below that of autologous therapies.

Finally, development of autologous cell products for diabetes cell therapy requires careful design of preclinical animal studies. Two primary strategies are possible to enable first entry into clinic. First, cells from each patient can be tested in animals to individually establish safety and efficacy profiles. In all cases, transplantation site and surgical procedure in the animal should mimic as closely as possible the envisioned human procedure and sites in order to provide appropriate data for FDA review in an investigational new drug application to begin a first clinical trial. Transplantation site possibilities for islets include the portal vein, the omentum, intraperitoneal, intramuscular, or subcutaneous (discussed by Merani et al. [62]). Patient dosing could be tailored based on patient medical characteristics, like insulin demand and sensitivity, combined with an algorithm that takes into account the insulin release profile in the animal studies. However, this approach is laborious and expensive, particularly if individual investigational new drug–enabling studies are required. Second, an alternative strategy involves developing a set manufacturing process, including in-process controls and release criteria, which is then uniformly applied across all patient lines. In this approach, cells from a subset of patient lines would be manufactured and released according to this process and tested through animal studies. Subsequent lines manufactured with the same process and released according to the same assays and criteria could then theoretically be used clinically without further preclinical animal testing. This approach requires far more up-front development work in establishing robust processes, assays, and preclinical data sets but will ultimately be the path required for application of autologous therapies to a large number of patients in a cost-effective manner.

FUTURE OUTLOOK

Diabetes cell replacement therapy will hopefully become reality over the next several years for an increasing number of patients, with the potential to greatly improve health and quality of life. A number of potential paths to achieving this goal have been made feasible by recent discoveries. Current stem cell technology now allows for β-like cells to be generated from hiPSCs derived from patients with diabetes, opening the possibility of autologous cell therapies. This approach provides the first path to clinic for a cell therapy that does not require systemic immunosuppression or development of an effective immunoprotective encapsulation device. Further development of robust hiPSC and β-like cell protocols and establishment of efficient methods of manufacturing and

testing clinical grade cells will be the next steps. However, if these challenges can be addressed, autologous hiPSC-derived β-like cells could provide pivotal proof of concept in humans that stem cell–derived β-like cells can transform the treatment options for patients with diabetes.

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