Review

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Focus: Induced Pluripotency & Cellular Reprogramming

Toward beta cell replacement for diabetes

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Abstract

The discovery of insulin more than 90 years ago introduced a life-saving treatment for patients with type 1 diabetes, and since then, significant progress has been made in clinical care for all forms of diabetes. However, no method of insulin delivery matches the ability of the human pancreas to reliably and automatically maintain glucose levels within a tight range. Transplantation of human islets or of an intact pancreas can in principle cure diabetes, but this approach is generally reserved for cases with simultaneous transplantation of a kidney, where immunosuppression is already a requirement. Recent advances in cell reprogramming and beta cell differentiation now allow the generation of personalized stem cells, providing an unlimited source of beta cells for research and for developing autologous cell therapies. In this review, we will discuss the utility of stem cell-derived beta cells to investigate the mechanisms of beta cell failure in diabetes, and the challenges to develop beta cell replacement therapies. These challenges include appropriate quality controls of the cells being used, the ability to generate beta cell grafts of stable cellular composition, and in the case of type 1 diabetes, protecting implanted cells from autoimmune destruction without compromising other aspects of the immune system or the functionality of the graft. Such novel treatments will need to match or exceed the relative safety and efficacy of available care for diabetes.

Keywords beta cells; cell replacement therapy; stem cells; type 1 diabetes
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Introduction

The treatment of type 1 diabetes (T1D) is one of the most important medical success stories of the 20th century. Most type 1 diabetics can now expect a life span that is largely equivalent to those of the non-diabetic population. Secondary complications such as retinopathy and nephropathy were initially prevalent, caused by inadequate glycemic control (DCCT-Research-Group, 1993). In the last decades, technical advancements such as the generation of human recombinant insulin, better blood glucose monitoring devices, and insulin pumps have improved the patients' ability to self-regulate their blood glucose levels and reduced the risks of hypoglycemia. A selection of insulin variants now also exist that are either short or long acting and can be taken in combination for tighter glycemic control. But the treatment is still cumbersome and requires careful monitoring and multiple insulin injections each day. Maintaining blood glucose within physiological range is difficult to achieve, especially in children and adolescents. And even with intensive insulin therapy, the accumulative incidence of retinopathy, nephropathy, and cardiovascular disease after 30 years of T1D remains considerable, at 21, 9 and 9%, respectively (Nathan *et al*, 2013), although these numbers are much lower than decades ago. Therefore, even with ongoing improvements in diabetes care, there is need for a cure.

Because T1D is caused by the loss of beta cells due to autoimmune-mediated destruction, a cure may require the use of replacement beta cells. Upon diagnosis, patients show an age-dependent loss of 40–85% of their beta cell mass (Klinke, 2008), and a small amount of beta cells remain for many years in the majority of T1D patients (Oram *et al*, 2014). In mice, beta cell mass can recover after ablating most cells with a toxin (Nir *et al*, 2007) or if the autoimmune reaction in NOD mice is halted after beta cell destruction has commenced (Nishio *et al*, 2006). But human beta cells may not be as competent to regenerate: Immune suppression and maintenance of euglycemia following pancreas transplantation could not restore normal function of the endogenous pancreas (Liu *et al*, 2009). Even in recent-onset type 1 diabetes, no increase in beta proliferation is seen (Butler *et al*, 2007). Further, therapies aimed at inhibiting the autoimmune reaction in recent-onset T1D are only able to transiently maintain C-peptide levels in the blood, but not restore normal levels (Herold *et al*, 2002; Keymeulen *et al*, 2005). A cure for patients with established T1D is thus likely going to involve the transplantation of functional beta cells. Pancreas transplantation or allogeneic transplantation of donor islets is indeed effective and can often achieve insulin independence in T1D patients. Such therapy is mostly applied for T1D subjects with severe complications such as renal failure, where pancreas transplant is performed in tandem with kidney transplantation (Merani & Shapiro, 2006; Gruessner, 2011). A shortage in donor organs and the potentially adverse side effects of systemic immunosuppression that these grafts require greatly limits the utility of this approach. In addition, beta cells within the graft may still be destroyed by an autoimmune reaction (Tyden *et al*, 1996; Vendrame *et al*, 2010). These same limitations to cell therapies for T1D persist even if other sources, such as stem

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cells, become available. Therefore, other forms of diabetes that are not mediated by autoimmunity may suitable initial targets to determine the efficacy and safety of beta cell transplants. These experimental treatments will need continuous evaluation against existing care options. A source of beta cells very similar or equivalent to the beta cells of the human pancreas is a necessary requirement for the development of such therapies. Such stem cell-derived beta cells are also required for basic research aimed at understanding beta cell development and the mechanisms of beta cell failure in diabetes. Therefore, research and clinical goals can often be aligned, and indeed, progress toward clinical application is currently being made in a basic research context.

Beta cells differentiate from human pluripotent stem cells

Pluripotent stem cells have the ability to differentiate into all cell and tissue types of the body, most notably shown in mice by the generation of chimeric animals. Directed differentiation of human pluripotent stem cells into various cell types *in vitro* has only recently become possible by applying developmental signals based on lessons learned from developmental biology in animals. Initial attempts to generate beta cells from human embryonic stem cells relied on spontaneous differentiation. By culturing embryonic bodies in stem cell media, Assady and colleagues reported the upregulation of insulin transcripts and occasional insulin-positive cells, but most cells were not of pancreatic origin (Assady *et al*, 2001). To improve differentiation efficiency into insulin-positive cells, developmental steps were sequentially targeted, progressing from definitive endoderm to primitive gut tube, through posterior foregut, pancreatic endoderm, pancreatic endocrine progenitors and, finally, to beta cells. High levels of activin A induce definitive endoderm formation during development (Thisse *et al*, 2000; Whitman, 2001; Tam *et al*, 2003; Kubo *et al*, 2004) and, together with Wnt3a, can induce definitive endoderm from human embryonic stem cells (D'Amour *et al*, 2005). Induction of posterior foregut endoderm, characterized by the expression of PDX1, FOXA2, and HNF1beta, can further be achieved by the activation of FGF and retinoic acid signaling pathways and inhibition of the sonic hedgehog signaling pathway (D'Amour *et al*, 2006; Mfopou *et al*, 2010). In the embryo, pancreatic endocrine cells develop from Ngn3-positive pancreatic endocrine progenitors. These Ngn3-positive cells are derived from pancreatic progenitors upon down-regulation of Notch signaling (Shih *et al*, 2012). To mimic this stage *in vitro,* γ-secretase inhibitors have been added to inhibit Notch signaling, resulting in the expression of Ngn3 (D'Amour *et al*, 2006; Mfopou *et al*, 2010). Following this step-wise induction, insulin-expressing cells could be derived, although the cells were not responsive to glucose stimulation (D'Amour *et al*, 2006). This was the first report with clear evidence that cells resembling beta cells could be derived, and a powerful demonstration that developmental knowledge could be employed to direct differentiation of human embryonic stem cells to a specific developmental fate.

Other groups were initially not as successful when replicating these results using a variety of embryonic stem cell lines (Cho *et al*, 2008; Mfopou *et al*, 2010). Though PDX1-positive cells were obtained, the efficiency was low, and a high percentage of cells were positive for the liver markers alpha-fetoprotein and albumin, resembling hepatocytes instead of pancreatic cells (Mfopou *et al*, 2010; Nostro *et al*, 2011). During mouse development, before the onset of pancreatic lineage commitment, BMP4 favors liver development by inhibiting Pdx1 and by inducing albumin expression (Wandzioch & Zaret, 2009). Inhibition of BMP4 signaling allowed a shift from hepatocyte to the pancreatic lineage, characterized by the generation of 50–80% of PDX1-positive cells from several human embryonic stem cell lines (Mfopou *et al*, 2010; Nostro *et al*, 2011). Additional improvements were made to allow for the efficient differentiation of PDX1-positive cells into endocrine cells. Nostro and colleagues found that inhibition of the TGF-β pathway was required for endocrine lineage commitment, increased the expression of the endocrine lineage marker NGN3 and, when combined with BMP4 inhibition, resulted in 25% C-peptide-positive cells (Nostro *et al*, 2011). These additional manipulations of signaling pathways resulted in a protocol that was effective for many different stem cell lines.

Based on these studies, a number of groups have succeeded in generating insulin-producing cells with an efficiency of about 25% (Kelly *et al*, 2011; Nostro *et al*, 2011; Kunisada *et al*, 2012; Rezania *et al*, 2012, 2013; Schulz *et al*, 2012; Hua *et al*, 2013; Shang *et al*, 2014). However, none of them reported the derivation of glucose-responsive and monohormonal insulin-positive cells. Most of the cells co-expressed insulin and glucagon, and the secretion of insulin increased only marginally upon a glucose challenge. These studies failed to generate pancreatic progenitors expressing all transcription factors required for pancreatic development and beta cell function. A pancreatic progenitor cell is defined by coexpression of the transcription factors Pdx1, Nkx6.1, Nkx2.2, Ptf1a, and Sox9 in the embryonic pancreas epithelium, and the lack of one of these transcription factors can result in abnormal beta cell development (Gittes, 2009). For instance, pancreatic cells in homozygous Nkx6.1 mutant mice develop into NGN3-positive endocrine progenitors and insulin and glucagon co-expressing cells, but fail to form monohormonal insulin-expressing beta cells at embryonic day 18.5 (Sander *et al*, 2000). During mouse embryonic development, two waves of Ngn3 expression, initiated before and after Nkx6.1 expression, have a vastly different outcome on the phenotype of endocrine cells. The early wave of NGN3 expression occurs around mouse embryonic day 9.5 in the absence of Nkx6.1 and drives pancreatic cells into polyhormonal and glucagon-positive alpha cells. Only the second wave of NGN3 expression around embryonic day 12.5 in the presence of Nkx6.1 enables differentiation into monohormonal insulin-positive beta cells (Johansson *et al*, 2007). To generate NKX6.1-positive cells from human pluripotent stem cells, the BMP4 signaling pathway had to be more precisely controlled. In previously published studies, BMP4 was continuously inhibited with the intention to first induce the pancreatic lineage over the hepatocyte lineage and then to induce NGN3 expression (Nostro *et al*, 2011). However, PDX1-positive cells fail to upregulate NKX6.1 when BMP4 signaling is inhibited (Sui *et al*, 2013). Therefore, in order to generate NKX6.1-positive cells, BMP4 signaling should be transiently activated after onset of PDX1 expression. In addition, the protein kinase C (PKC) pathway also plays a role in the generation of NKX6.1-positive pancreatic progenitors. Rezania and colleagues demonstrated that activation of the PKC pathway induced NKX6.1 expression before the onset of NGN3 expression (Rezania *et al*, 2013). NGN3-expressing endocrine progenitors with or without NKX6.1 had

markedly different developmental potential after transplantation into mice. Though endocrine cells could be derived from both of these groups, only NKX6.1-positive progenitors gave rise to monohormonal insulin-expressing cells, while the NKX6.1 negative cells gave rise to polyhormonal and glucagon-positive cells. This maturation into monohormonal insulin-producing cells in mice takes several months and is poorly understood.

To generate functional monohormonal beta cells *in vitro*, Pagliuca and colleagues as well as Rezania and colleagues manipulated additional pathways implicated in the development of the pancreas (Pagliuca *et al*, 2014; Rezania *et al*, 2014). TGF-β inhibitor (ALK5 inhibitor) and the BMP4 inhibitor (LDN-193189), thyroid hormone, and a Notch signaling inhibitor were applied for the generation of beta cells from PDX1 and NKX6.1 double-positive pancreatic progenitors. It has previously been shown that three-dimensional cues are important for pancreatic development (Greggio *et al*, 2013), and in fact, both studies applied 3D culture systems in their differentiation process, starting with either pluripotent stem cells cultured in suspension, or aggregating cells at the pancreatic progenitor stage of differentiation. Approximately 50% insulin-positive cells could be obtained, and most insulin-positive cells coexpressed the beta cell transcription factors NKX6.1 and PDX1, but not glucagon, and increased secretion of C-peptide during incubation in high levels of glucose. The cells derived also responded to multiple sequential high-glucose challenges (Pagliuca *et al*, 2014). Rezania's study further evaluated the dynamics of insulin secretion to glucose stimulation with a perifusion system. In contrast to adult human islet cells, the cells displayed a slow response to glucose and did not return to the pre-treatment baseline, indicating that these cells are still slightly different from adult human beta cells. Nevertheless, these beta cells were functional and protected mice from diabetes after transplantation. Although differences remain between stem cell-derived beta cells and beta cells of the human pancreas, they are very similar.

The availability of stem cell-derived beta cells now allows the investigation of mechanisms of beta cell failure in a human system. Beta cells differentiated from stem cells of a diabetes patient carry all the genes responsible for the disease, allowing the study of how a particular genotype affects beta cell function.

Disease-specific stem cells and beta cells

Advances in stem cell technologies have made it possible to generate stem cells from adult-differentiated cells, including from patients with T1D, T2D, and various forms of diabetes caused by single-gene mutations (Fig 1) (Takahashi *et al*, 2007; Maehr *et al*, 2009; Ohmine *et al*, 2012; Hua *et al*, 2013; Teo *et al*, 2013; Shang *et al*, 2014; Yamada *et al*, 2014). Initial protocols to derive these stem cell lines used a retroviral delivery system of four embryonic transcription factors (Takahashi *et al*, 2007), which resulted in vector integration and low levels of continued expression of the transgenes, and the resulting cells sometimes had reduced differentiation potential (Koyanagi-Aoi *et al*, 2013). DNA viruses have since been replaced by modified mRNA (Warren *et al*, 2010) or Sendai virus (Ban *et al*, 2011), which cannot integrate into the genome. These readily accessible methods have made induced pluripotent stem cells (iPSCs) a popular choice for stem cell biologists. However, several reports have indicated that reprogramming by transcription factors can affect the genetic and epigenetic integrity of iPSCs, including loss of gene imprinting (Nishino *et al*, 2011), epigenetic memory (Bar-Nur *et al*, 2011; Kim *et al*, 2011), alterations in hydroxymethylation (Wang *et al*, 2013) and appearance of *de novo* coding mutations (Gore *et al*, 2011). These defects are not generally seen in embryonic stem cells (ESCs) derived from blastocysts, raising the question regarding their cause and origin.

It has recently become possible to generate patient-specific ESC by somatic cell nuclear transfer (SCNT) (Tachibana *et al*, 2013; Chung *et al*, 2014; Yamada *et al*, 2014). While reprogramming to iPSCs forces transformation by chemical means, reprogramming of a somatic cell in the oocyte cytosol follows a developmental path. Therefore, stem cells derived by nuclear transfer might be more similar to ES cells than iPSCs with regard to the above-described aberrations. But surprisingly, using isogenic NT-ESC and iPSC lines, the two derivation methods resulted in highly similar cell types with regard to gene expression and DNA methylation (Johannesson *et al*, 2014). And both NT-ESCs and iPSCs differed from ESCs with regard to the number of *de novo* coding mutations and imprinting aberrations: iPSCs and NT-ESCs showed an average of 10 *de novo* coding mutations, and 10% of imprinted regions showed changes in DNA methylation consistent with erosion of imprinting. Therefore, the somatic origin of the stem cell lines—as opposed to a germ line origin—and not the specific method of reprogramming is responsible for the elevated mutation rate and aberrations in imprinting. A functional comparison was not performed, and it remains possible that an iPSC-reprogramming more frequently generates outliers with decreased differentiation potential (Koyanagi-Aoi *et al*, 2013). However, functional studies are challenging to perform in the human system, as they cannot match the stringency of a chimera assay in the mouse system. We do, however, know that both methods of derivation result in stem cell lines that can differentiate into insulin-producing cells with the genotype of a type 1 diabetic (Maehr *et al*, 2009; Yamada *et al*, 2014).

Several studies have shown that beta cells can also be generated without the transition though the pluripotent state, by a process termed transdifferentiation (Fig 1C). For instance, Zhou and colleagues found that ectopic expression of Pdx1, MafA, and Ngn3 in pancreatic exocrine cells *in vivo* converted them into beta cells in mice (Zhou *et al*, 2008). Cells from other developmentally related lineages have also been transdifferentiated into beta cells. The same recipe of Pdx1, MafA, and Ngn3 overexpressed in either mouse or human intestinal crypt cells results in clusters of insulin-positive cells (Chen *et al*, 2014). Transformation from gut cells to beta cells also occurs when the transcription factor Foxo1 is conditionally deleted in the mouse gut (Talchai *et al*, 2012). Furthermore, gut organoids derived from human pluripotent stem cells with conditional inhibition of Foxo1 form C-peptide-positive cells, though with low efficiency (Bouchi *et al*, 2014). The authors speculated that if such cells could be generated *in vivo*, in the human gut, they might be able to evade the autoimmune response in T1D, as gut-derived cells are intrinsically short-lived and regenerate continuously.

These various methods of generating stem cells and beta cells (Fig 1) now allow generation of patient-specific beta cells, providing a tool for studying the molecular and cellular mechanisms of beta

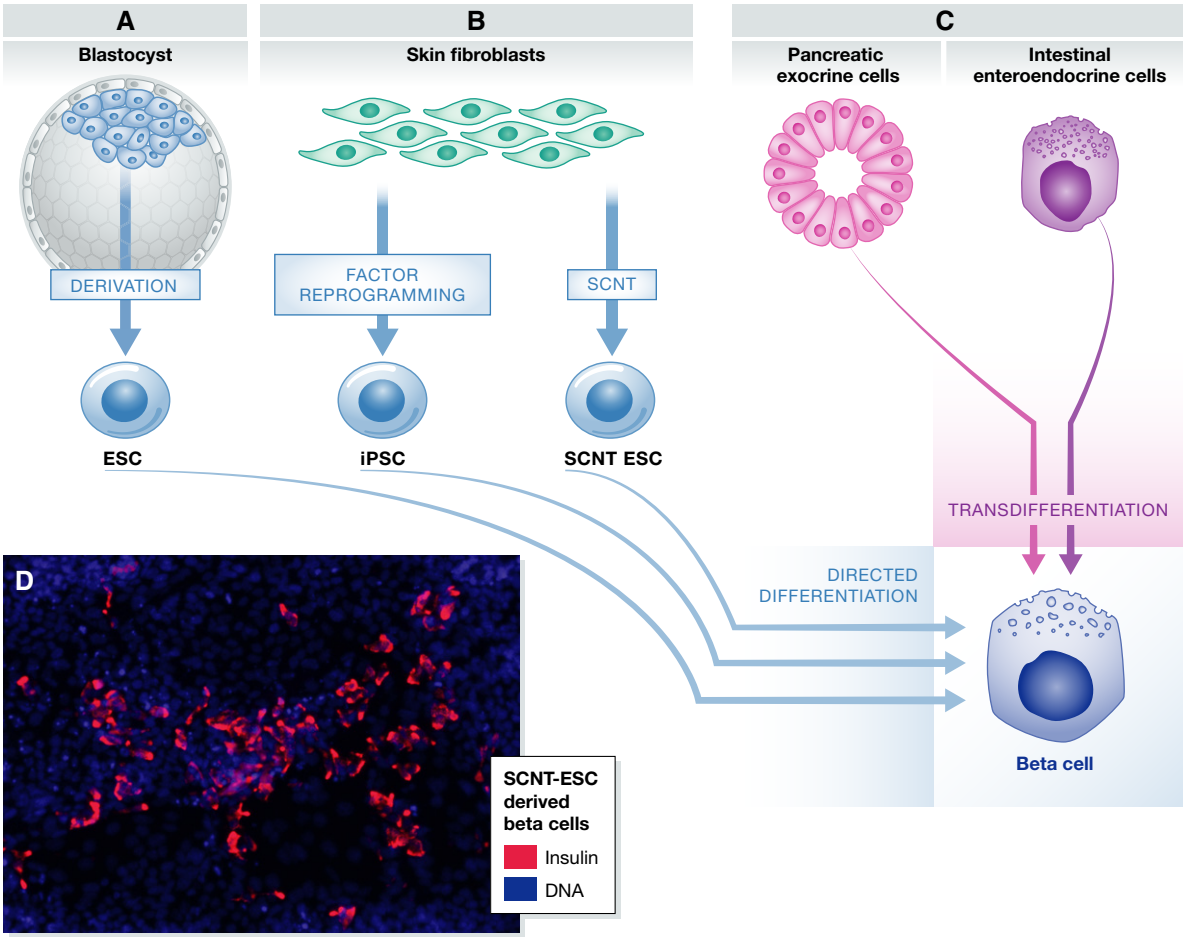

Figure 1. Different strategies for making beta cells.
(A) Beta cells can be generated by differentiation of pluripotent stem cells derived from IVF blastocysts (ESCs). (B) Autologous stem cells with the genotype of a patient can be derived by reprogramming of somatic cells via somatic cell nuclear transfer (SCNT-ESCs) or factor-mediated reprogramming (iPSCs). (C) Alternatively, pancreatic exocrine cells or intestinal enteroendocrine cells can be trans-differentiated into beta cells by ectopic expression or elimination of specific transcription factors. (D) An example of SCNT-ESC-derived beta cells; insulin is stained in red and DNA in blue.

cell failure in diabetes, or for experimental testing of cell replacement therapies.

Monogenic diabetes as an entry point for disease modeling and cell replacement

The generation of patient-specific stem cells and their differentiation into specific cell types provides a powerful tool to model disease-specific phenotypes (Robinton & Daley, 2012). As a proof of principle in diabetes, Hua and colleagues generated iPSCs and beta cells from patients with maturity onset diabetes of the young (MODY) 2 (Hua *et al*, 2013). MODYs are forms of diabetes caused by mutations in genes involved in the function and/or the development of beta cells. In MODY2, this mutation is located in the glucokinase (GCK) gene (for review, see (Vaxillaire & Froguel, 2008). GCK catalyzes a rate-limiting step in glycolysis in beta cells. Reduced GCK levels due to a heterozygous or homozygous mutation result in decreased glycolysis and lower ATP levels, and thereby in reduced insulin secretion. iPSC-derived beta cells indeed showed decreased insulin secretion upon a glucose stimulus. Gene correction of the GCK mutation reversed this phenotype and restored glucose responsiveness to the levels of cells from healthy controls. These phenotypes conformed according to our expectations and showed the utility of patient-specific beta cells for modeling diabetes.

To gain novel insight into the mechanisms of beta cell failure in a less well understood form of diabetes, iPSC-derived beta cells were generated from subjects with Wolfram syndrome. Wolfram syndrome is caused by homozygous mutations in wolframin (Inoue *et al*, 1998), an endoplasmic reticulum (ER) resident protein that had been implicated in regulating protein folding stress (Fonseca *et al*, 2005). Beta cells with wolframin mutations showed signs of elevated ER stress under basal conditions and rapidly lost the ability to secrete insulin when additional protein folding stress was induced (Shang *et al*, 2014). Interestingly, this phenotype could be reversed using chemical chaperones, highlighting the potential of this model for drug screening. Similar strategies should allow investigating whether beta cell intrinsic defects contribute to T2D and perhaps also to T1D. While many of these experiments were performed

in vitro, stem cell-derived beta cells can also be transplanted into immunocompromised mice. Kroon and colleagues, and more recently other groups, have shown that mice can be protected from diabetes using stem cell-derived human beta cells (Kroon *et al*, 2008; Pagliuca *et al*, 2014; Rezania *et al*, 2014). This will allow testing of beta cell function under physiologically relevant circumstances, including under conditions of obesity, insulin resistance, aging or other forms of environmental stress. Transplantation studies will also allow continuous optimization and functional testing of grafts for cell replacement therapies.

All forms of diabetes are caused by insufficient insulin relative to metabolic need, caused by either decreased ability to produce insulin in the pancreas, or by insulin resistance in the periphery. Therefore, all forms of diabetes might benefit from the transplantation of insulin-producing cells. In the case of T1D, the transplanted beta cells will need to be protected from the immune system as they will likely be subject to the same autoimmune destruction that originally eliminated the native beta cells (Fig 2A) (Vendrame *et al*, 2010). Unlike in T1D, beta cells from individuals with monogenic diabetes do not need to be protected from autoimmune destruction and may therefore be more suitable initial subjects for cell replacement. These monogenetic forms of diabetes could serve as a stepping stone toward cell replacement for T1D. For instance, MODY3 (monogenetic diabetes of the young 3) is one of the most frequent forms, accounting for an estimated 1% of all diabetes cases (Steck & Winter, 2011), and is caused by mutation of the HNF1alpha (hepatocyte nuclear factor 1 alpha) gene. MODY3 subjects can be treated with sulfonylureas, agents that stimulate insulin secretion by modulating the activity of a potassium channel on the beta cell. However, most subjects ultimately become insulin-dependent and are treated like T1D subjects (Thanabalasingham & Owen, 2011). Transplanted beta cells with a corrected allele should be fully functional, and not be subject to an autoimmune response.

Engineering functional pancreatic grafts

For successful transplantation of stem cell-derived beta cells, they have to be prepared and transplanted in a way that supports long-term function of the graft and ensures the safety of the host.

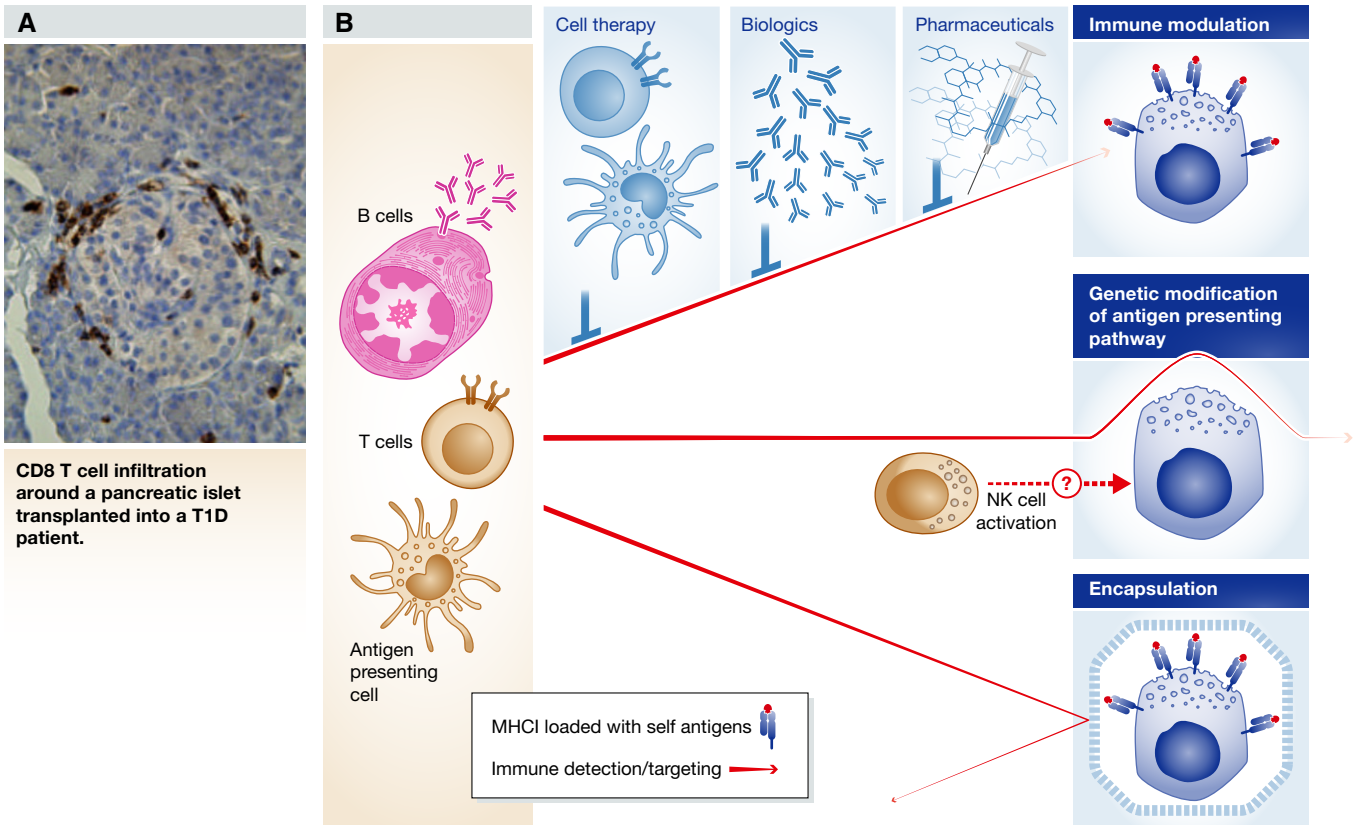

Figure 2. Avoiding autoimmune destruction of grafted beta cells.
(A) Reoccurrence of autoimmunity after pancreas transplantation into a T1D patient indicated by infiltration of $CD8^+$ T cells around islets in the graft (Vendrame *et al*, 2010). (B) There are several potential ways of protecting transplanted beta cells from immune destruction. First, the immune system can be modified either pharmaceutically, with biologics, or by transplantation of regulatory T cells (Tregs) or tolerogenic dendritic cells (DCs). Second, the beta cells can be genetically modified to reduce immunogenicity prior to transplantation, though this could cause the activation of natural killer (NK) cells. Finally, beta cells can be encapsulated in a way that keeps the immune system at bay while allowing for the exchange of nutrients and oxygen. The red arrows indicate detection and targeting of beta cells by the immune system, and the characteristics of the arrows indicate from top to bottom: reduced intensity of the immune response, inability to detect beta cell antigens and inability to penetrate encapsulation devices. Transplanting autologous beta cells into patients with non-autoimmune forms of diabetes would not require such protection.

Proper integration of the transplanted cells into the host tissue has to be promoted while retaining the ability to remove the grafted cells, should they show abnormal function. These aims present a formidable challenge that require innovative tissue engineering and, for T1D, novel ways to modify the immune system or the immunological properties of beta cells. The main goal of tissue engineering is to generate functional tissue. Though we can now generate many cell types from human pluripotent stem cells, these cell types are often immature and do not reach the functionality of tissues *in vivo*. The lack of appropriate tissue structure is likely part of the under-performance of stem cell-derived products. All cell types and organs in the human body are organized in specific structures composed of multiple cell types. Interactions between different cell types and vascularization, as well as the 3D arrangement, of the cells play an important role in tissue reconstruction (Badylak *et al*, 2011; Goh *et al*, 2013). The function of tissues such as the heart or the kidney is highly dependent upon accurate 3D structure, which cannot be achieved by spontaneous or directed differentiation of pluripotent stem cells. Engineering of insulin-producing beta cell constructs is unlikely to require such degree of complexity: human islets can effectively regulate blood glucose levels in isolation from their pancreatic environment, such as after infusion through the portal vein into the liver (Rodriguez Rilo *et al*, 2003). In addition, clusters of beta cells that differentiate from human stem cells appear to self-organize into spheres that resemble human islets (Pagliuca *et al*, 2014). Nevertheless, tissue engineering can likely provide an effective method of delivery and improve survival upon transplantation, and the ability to retrieve transplanted cells and immunoprotection through encapsulation.

Encapsulation of transplanted cells

Encapsulation serves a dual function of protecting the graft from immune destruction and protecting the host from the graft (Fig 2B). Immune protection is required when non-autologous cells are used for transplantation or if autologous cells are transplanted into an autoimmune environment. This can be achieved by blocking the cellular response via physical isolation of the cells using semi-permeable membranes or scaffolds. This approach reduces the need for immunosuppression and has recently been reviewed in detail elsewhere (Sakata *et al*, 2012; Qi, 2014). Encapsulation also prevents cells from escaping the location of the graft, and allows for removal if needed. This is particularly relevant, as uncontrolled differentiation and growth, so-called teratomas, have often been observed in mice grafted with stem cell-derived pancreatic precursors (Kroon *et al*, 2008; Kelly *et al*, 2011). Teratoma formation may be preventable by generating grafts consisting of a pure population of mature beta cells, either by using cell purification (Kelly *et al*, 2011) or by improving differentiation methods.

The material used for encapsulation has stringent requirements to maintain long-term function of the graft. The chosen biomaterial needs to be biocompatible and promote the survival of the islet cells. At the same time, the material properties need to permit bi-directional diffusion of glucose, hormones, proteins, and cellular waste produced by the islet cells while allowing the influx of nutrients and oxygen from surrounding tissues. The chosen biomaterial also needs to be inert, in order to avoid triggering a host tissue response and fibrous encapsulation that can adversely affect the long-term functionality of the transplant. Example of biomaterials that have been explored for the encapsulation of islet cells includes alginate (Yang *et al*, 1997; Lanza *et al*, 1999b), polysulfone (Lembert *et al*, 2005), polyvinyl alcohol (Qi *et al*, 2004), polyacrylonitrile–polyvinyl chloride (Scharp *et al*, 1994), and poly(ethylene glycol) (Weber *et al*, 2008). However, this approach requires efficient and reproducible micro-encapsulation protocols, and some cells may die within the encapsulated material without the possibility of being cleared or removed. Some reports have shown micro-encapsulated beta cells remain viable up to 6 months after implantation (Orlando *et al*, 2014), but it remains unclear how long the survival of these cells can be extended and thus how often the encapsulation device would need to be exchanged. An additional limitation of this approach is that at least one dimension of the graft must be small to allow efficient diffusion as blood vessels cannot permeate the device, restricting the ability to construct 3D tissues.

Vascularization of transplanted cells

One limitation of current islet transplantation approaches is the poor survival of the transplanted cells. Direct transplantation of cells into a tissue cavity mostly relies on diffusion of oxygen and nutrients from the nearest capillary bed for survival of the cells (Qi, 2014). To improve this, a vascularized cavity can be prepared within the recipient prior to delivery of the beta cells. For example, Sernova Corp's approach is to subcutaneously implant a "pouch" with inert rods as place holders into the patient. Once the material has enough surrounding vasculature, the rods are removed and the islet cells are then injected into the pouch where they remain protected but close enough to the vasculature for nutrient exchange to take place. Details of this approach have only been available via public disclosure from the company's Web site (sernova.com). Finally, beta cells can be transplanted in scaffolds that have been engineered to slowly release growth factors that promote neovascularization (Phelps *et al*, 2013). Although this would not reduce hypoxia immediately post-transplantation, it can speed up vascular integration of the graft.

Decellularized organs as a scaffold for tissue engineering

One of the advantages of using synthetic polymers to encapsulate or deliver islet cells is the reproducibility of the manufacturing process and the precise control over material properties. However, synthetic materials lack the bioactivity and ultrastructural composition of native tissues. Previous reports have shown that synthetic biomaterials, alone or in combination with extracellular matrix (ECM) proteins, can enhance islet survival and function *in vitro* highlighting the importance of the composition and structure of the chosen material for optimal beta cell function. For example, islet function was enhanced by mimicking the islet extracellular matrix via the combination of ECM proteins such as collagen type I, collagen type IV, fibrinogen, fibronectin, laminin, and vitronectin with a synthetic component such as poly(ethylene glycol) hydrogel (Cheng *et al*, 2011). However, the recapitulation of the native ultrastructure of the

pancreas, which is likely to play an important role during islet cell differentiation and maturation, has not been achieved *in vitro*.

One exciting new area of research is the use of ECM as a bio-inductive scaffold for functional and phenotypical maintenance of cells. Pancreatic islets cells have been shown to prefer a variety of ECM-derived substrates such as endothelial cell-derived matrix, fibrin, Matrigel, and collagen when compared to synthetic components (Cheng *et al*, 2011; Daoud *et al*, 2011; Krishnamurthy *et al*, 2011). For instance, porcine and rat islet cells seeded on Matrigel displayed improved beta cell function and islet cluster-like morphology when compared to plastic (Hayek *et al*, 1989; Vukicevic *et al*, 1992; Lucas-Clerc *et al*, 1993; Perfetti *et al*, 1996; Nagata *et al*, 2001; Oberg-Welsh, 2001). Greggio and colleagues also showed that mouse pancreatic progenitors could be cultured and differentiated in 3D hydrogels containing ECM components such as laminin (Greggio *et al*, 2013) and by combining 3D hydrogels with Wnt induction. Huch and colleagues showed primary mouse progenitors could be expanded indefinitely (Huch *et al*, 2013). In other words, the ECM not only serves as a scaffolding material for cells to attach, grow, and populate, but it also provides cues and signals to the cells that help maintain phenotype and function.

Since recreating the exact composition and structure of the pancreas is currently not possible, a logical alternative is to use native pancreatic tissue. The ECM contains multiple proteinaceous and non-proteinaceous components specifically tailored to a given tissue (Badylak *et al*, 2009). By harnessing the inherent information found in the pancreatic ECM, islet cells can be provided with a more native-like environment (Brizzi *et al*, 2012). A recent study described a method to isolate porcine pancreatic ECM via perfusion of detergents through an intact pancreas. The resulting scaffold material was a completely decellularized pancreas that can support the attachment and growth of islet and adult stem cells. The pancreatic ECM was shown to help up-regulate markers such as insulin secretion, further supporting their use as potential scaffolding material over synthetics (Mirmalek-Sani *et al*, 2013). This approach can be further improved by protecting the native vasculature during the decellularization process in order to maintain a patent vascular network that could be used to re-perfuse the pancreatic tissue. Understanding the appropriate ECM ultrastructure needed to maintain beta cell function can also improve emerging techniques such as bioprinting where both cells and ECM can be printed into three-dimensional units (Mendelsohn *et al*, 2010).

Though we know that bioengineering solutions such as encapsulation devices can protect transplanted cells from an immune response, it is not clear how long these devices will function. Therefore, modifications of the immune system in T1D, or of the immunological properties of the beta cells, would be desirable. Such approach might allow the use of autologous stem cell-derived beta cells and thereby harness the therapeutic potential of reprogramming technologies.

Modification of the immune system to restore tolerance

Although autologous stem cell-derived beta cells would circumvent issues of allogeneic rejection, the underlying autoimmunity still represents a threat to the newly generated and implanted beta cells. Among the many genetic predispositions associated with T1D, a great majority affects the immune system and its ability to remain tolerant to self. In fact, many of these susceptibility genes are shared with other autoimmune and autoinflammatory disorders such as celiac disease, rheumatoid arthritis, vitiligo, and Crohn's disease (www.T1Dbase.org). Long-term restoration of immune tolerance is paramount to the survival of residual endogenous or implanted islets. Two types of approaches have been evaluated or implemented to achieve this goal. The first type aims to eliminate parts of the immune system, such as T or B cells, regardless of whether the targeted immune cells play a role in the autoimmune process or not. This type of approach is quite effective for as long as it is applied, but patients become vulnerable to infections and certain cancers, and autoimmunity usually resurges after treatment interruption. Considering the safety and efficacy of current diabetes care, this is not a viable long-term solution for most T1D subjects. The second type of approach aims to specifically eliminate or tame the immune cells that are responsible for the destruction of the beta cells by exploiting their beta cell antigen specificity. More recently, emphasis has been placed on combination therapies aimed at tackling the disease at multiple levels, by crippling specific immune cells, quenching inflammation and targeting antigen-specific T cells for reprogramming. The evaluation of all these approaches has been made possible thanks to useful animal models, the non-obese diabetic (NOD) mouse, and to a lesser extent the BioBreeding (BB) rat, both of which spontaneously develop T1D and share many of the disease susceptibility genes associated with the human disease. New humanized versions of these animal models, which are reconstituted with a human immune system by transplantation of fetal thymic tissue together with human bone marrow-derived hematopoietic progenitor cells, are being developed and perfected (Greiner *et al*, 2011; Kalscheuer *et al*, 2012). Recent advancements in deriving thymic tissue from stem cells could potentially allow the generation of animal models containing immune systems that are fully patient-specific (Parent *et al*, 2013; Sun *et al*, 2013), which would greatly facilitate the testing and translation of new therapeutic strategies as well as the assessment of the function of transplanted human islets *in vivo*. The potential use of autologous stem cell-derived thymic tissue to induce tolerance in T1D patients is, however, less straightforward. First, the loss of central tolerance seen in autoimmune patients may be caused by genetically encoded traits that will also be present in the stem cell-derived thymic tissue. Second, the T-cell pool in adults is largely composed of memory T cells, and it is unlikely that restoration of central tolerance would be sufficient to offset a weak peripheral tolerance characterized by dysfunctional regulatory T cells (Tregs).

Systemic approaches

Immunosuppressive drugs such as cyclosporine are among the most potent in reversing disease in new-onset patients, but they have no durable effect if the treatment is interrupted (Staeva *et al*, 2013). Long-term treatment with these drugs is not tolerable due to their toxicity and the serious risk of developing opportunistic infections and tumors from weakened immune surveillance. Patients who undergo islet transplantation are subject to even stronger immunosuppression to compensate for the more aggressive allogeneic response against donor islets. Current treatments follow the Edmonton

Protocol, which involves rapamycin, FK-506, and anti-CD25, or variants thereof that may include anti-thymocyte globulin (ATG), anti-TNF, anti-CD52 or CTLA4-Ig (Bruni *et al*, 2014). The use of autologous stem cell-derived beta cells is likely to require treatments that are more in line with those assigned to new-onset T1D patients as described below.

The use of biologics (biomolecules such as antibodies, soluble Ig-fusion receptors, and soluble decoy receptors) offers greater specificity by targeting particular pathways (e.g. blocking costimulation with CTLA4-Ig) or immune cell populations (e.g. non-depleting anti-CD3 or depleting ATG for T cells; anti-CD20 for B cells). Despite showing promising efficacy in animal models, most of these biologics had no or only transient effect on the disease in humans, although prolonged effect after cessation of treatment has been observed in isolated cases, with preservation of C-peptide for up to 2 years (Staeva *et al*, 2013). These biologics did not induce durable tolerance on their own, but they were generally better tolerated than immunosuppressive small molecule drugs, although complications such as cytokine release syndrome or Epstein–Barr virus reactivation have been observed. Targeting proinflammatory pathways with biologics has proven beneficial in a number of major autoimmune diseases, but anti-TNF and anti-IL1β have shown no or poor efficacy on their own in T1D, suggesting that simply blocking inflammation is insufficient to restore immune regulation in this disease (Staeva *et al*, 2013). Alpha-1-antitrypsin, another anti-inflammatory candidate with immunoregulatory properties, is under clinical evaluation (Lewis, 2012). Resolving inflammation may still constitute a prerequisite before re-establishing tolerance, and these anti-inflammatory drugs may provide benefit as part of combination therapies.

Cell-based therapies offer an additional path to modifying the immune system, potentially in a safer and more targeted manner. Immune cells used for cell therapy demonstrate unmatched specificity, adaptability, and homing properties and typically consist of natural regulators of the immune system that have been isolated, expanded, boosted, and/or modified (Barcala Tabarrozzi *et al*, 2013; Fischbach *et al*, 2013). Autologous polyclonal Tregs that are re-infused after *in vitro* expansion are currently being tested in the clinics (Thompson *et al*, 2012), and the suppressive function of endogenous Tregs may be improved with low-dose interleukin-2 (Hartemann *et al*, 2013). Recombinant human IGF-1 has also recently been shown to stimulate regulatory T cells *in vivo* and prevent the onset of diabetes in NOD mice (Bilbao *et al*, 2014). Enforcing expression of particular T-cell receptors in Tregs for recognition of relevant specific antigens is a way to personalize this approach, in a manner analogous to the adoptive cell therapies that are now revolutionizing the treatment of cancer (Brusko *et al*, 2010). Autologous tolerogenic dendritic cells (DCs) can also promote tolerance by inducing Tregs as well as other protective cell types such as Th2 cells and regulatory B cells (Barcala Tabarrozzi *et al*, 2013; Creusot *et al*, 2014). Tolerogenic antigen-presenting cells may be obtained by silencing immunogenic pathways or overexpressing tolerogenic molecules (Giannoukakis *et al*, 2011; Creusot *et al*, 2014), and the choice of tolerogenic pathways to be bolstered may be adapted to individual patients.

These approaches may be well tolerated, but their efficacy in treating T1D remains to be firmly demonstrated. Thus far, the above strategies did not involve antigen specificity, which represents the next step toward achieving a more targeted therapy.

Antigen-specific approaches

Overall, antigen-specific therapy involves retraining the immune system to ignore particular self-antigens, in a way similar to desensitization methods used to rectify allergic reactions to particular allergens. Administration of islet antigens (proinsulin, GAD65, HSP60 p277) systematically (intravenous peptides, DNA vaccines) or via the mucosal surface (oral, nasal) has been an extensively studied method of tolerance induction (Coppieters *et al*, 2013). This has been tested not only to treat overt disease (after onset), but also as an attempt to prevent disease by re-establishing tolerance in high-risk subjects. Successes in animal models have prompted numerous trials in humans that have failed to demonstrate meaningful preservation of beta cell function, despite the therapy being well tolerated and evidence of immune responses reflecting some level of tolerance induction (Coppieters *et al*, 2013). The relative lack of success of these approaches may be partly explained by epitope spreading, which corresponds to a diversification of the cellular and humoral immune responses to an increasingly large number of epitopes from the target tissue (Di Lorenzo *et al*, 2007). Once a large number of antigens have been targeted by T cells and antibodies, inducing tolerance to one single antigen may appear preposterous, yet this is what clinical trials have been limited to so far. Thus, targeting multiple antigens after attenuating the local inflammation may represent a sensible strategy that will have to be assessed. Efficient induction of tolerance to these antigens requires presentation by tolerogenic antigen-presenting cells (APCs). When exposed to apoptotic cells, APCs produce TGF-β, which enhances their tolerogenic effect (Hugues *et al*, 2002; Chatenoud, 2014). Accordingly, spleen or blood cells chemically coupled with specific antigens induce an effective tolerance to these antigens, whereby specific T cells become unresponsive or regulatory (Prasad *et al*, 2012). The clinical evaluation of this approach, which has already passed phase I safety appraisal for treatment of multiple sclerosis, may soon be extended to T1D patients (Lutterotti *et al*, 2013). Furthermore, an antigen-specific dimension can easily be added to the cell-based therapies described in the previous section, whereby antigen-specific T-cell receptors can be expressed in Tregs or relevant antigens expressed in tolerogenic antigen-presenting cells in order to exclusively target diabetogenic T cells.

Combinatory approaches

Although many monotherapies have demonstrated adequate efficacy in animal models, it has become clear that long-term inhibition of autoimmune diabetogenic responses in humans will require a combination of different approaches. T1D patients are heterogeneous in their genetic makeup, the environmental conditions in which they live and their age of disease onset. This heterogeneity reflects how a complex combination of genetic and environmental factors differentially modulates several tolerogenic pathways (Todd, 2010). Thus, the same disease outcome can be reached through varying defects that impact the induction and maintenance of tolerance. One type of therapy targeting a particular biological pathway or antigen is therefore unlikely to work to the same extent across all patients. Combination and/or individualized therapies are increasingly considered as necessary to overcome this patient heterogeneity.

Combination therapy allows us to tackle multiple issues, not only addressing the inherent defects that led to impaired tolerance induction in the first place, but also extinguishing the persistent inflammation that interferes with homeostasis of the immune system and contributes to improper signals being conveyed to T cells upon recognition of their antigens (Roep *et al*, 2010; Barthson *et al*, 2011).

We now have access to a plethora of new therapies, which have shown limited efficacy on their own, but could become effective when different types of approaches are combined. The three main types of therapies include the following: (i) T- and/or B-cell targeting in order to "reset" the immune system, (ii) modulation of the environment from pro- to anti-inflammatory, and (iii) provision of self-antigens to induce antigen-specific deletion (elimination by apoptosis) or anergy (state of unresponsiveness to antigen) in autoreactive T cells or induce/boost antigen-specific Tregs. Particular combinations have already been recommended for prioritization based on preclinical testing, possible synergy, as well as drug safety and availability (Matthews *et al*, 2010). Some suggested strategies may accomplish all of the above. For instance, as massive cell depletion leads to apoptosis and release of anti-inflammatory TGF-β, the depletion of $CD8^+$ T cells and B cells followed by provision of specific antigens can enhance the differentiation of remaining $CD4^+$ T cells into Tregs that are protective in mouse models of T1D and multiple sclerosis (Kasagi *et al*, 2014). Cell-based therapies also offer multiple levels of action. For example, tolerogenic APCs may be manipulated to express antigens, anti-inflammatory cytokines, and inhibitory ligands and can be targeted to specific sites of relevance (Creusot *et al*, 2014). More aggressive approaches such as non-myeloablative bone marrow transplantation have proved efficient in establishing long-term tolerance and preserving beta cell function (Barcala Tabarrozzi *et al*, 2013); however, the current conditioning regimen, involving immunosuppression and low-dose irradiation, makes it unattractive. Alternative conditioning regimens for bone marrow transplantation are under development, which may benefit both bone marrow and islet transplantation, for tolerance induction and islet replacement, respectively.

Despite limited clinical success of immunological therapies for T1D, much has been learnt in the past decade about their mechanism of action, which will help us formulate the safest and most efficient treatment going forward (Herold *et al*, 2013). Such treatments will most likely combine multiple approaches to eliminate pre-existing diabetogenic T cells (and possibly antibody-secreting B cells), promote peripheral tolerance with tolerogenic antigen-presenting cells and antigen-specific regulatory T cells, and normalize inflammation durably but locally, so that normal immune responses to pathogens may continue to be elicited as needed. The use of autologous stem cell-derived beta cells, combined with effective tolerance induction, could potentially allow T1D patients who have lost all their islets to live a normal life again. As autologous stem cells can provide an inexhaustible source of beta cells, it may be possible to first administer some of these beta cells in an apoptotic form to boost antigen-specific tolerance in conjunction with other aforementioned regimens before implanting live cells. Such use of apoptotic beta cells for treating new-onset diabetes or people with autoantibodies at risk of developing the disease may be associated with less risk than using living cells for cell transplantation.

Modification of beta cells to escape immune attack

As an alternative to the systemic modification of the immune system, immunological protection may be more readily achieved locally by modifying antigenic properties of the beta cells. The development of specific designer nucleases allows editing of the genome of stem cells without a "genetic footprint" and with high specificity. Zinc finger nucleases and transcription activator-like effector nucleases (TALENs) and more recently clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (CAS9) systems mediate site-specific DNA cleavage and induce homologous recombination when provided a repair template (reviewed elsewhere Kim & Kim, 2014). Several reports show that genetic manipulation can be used to reduce the immunological vulnerability of cells. For instance, it has been shown that zinc finger-mediated disruption of human leukocyte antigen A (HLA-A) locus reduces the immunogenicity of various cell types toward cytotoxic lymphocytes (CTLs) (Torikai *et al*, 2013). As HLA is an essential component of the antigen presentation mechanism, this result is not surprising, but the downside is that the complete ablation of HLA expression usually causes cells to be targeted by natural killer (NK) cells (Ljunggren & Karre, 1990). This can potentially be avoided by reducing, instead of eliminating, HLA expression using short hairpin RNAs (shRNA) (Haga *et al*, 2006). Viruses commonly target the antigen presentation pathway to reduce their immune detection for instance by inhibiting the transporter associated with antigen-processing (TAP) transporter (Ressing *et al*, 2013). Wiertz and colleagues utilize this concept by overexpressing cytomegalovirus-encoded TAP inhibitors in human islet cells and showed that this reduced their detection by CTLs *in vitro* and after transplantation into mice (Zaldumbide *et al*, 2013). In an alternative approach, Shieh and colleagues overexpressed a chimeric molecule that selectively binds and activates cytotoxic T-lymphocyte-associated protein 4 (CTLA4) in NOD beta cells. CTLA4 is a receptor expressed on T cells that inhibits their function upon activation, and the transgenic expression of this chimeric agonist prevented the onset of diabetes in NOD mice and prolonged graft survival upon transplantation (Shieh *et al*, 2009). Protection of islets has also been achieved by overexpression of secreted immunoregulatory factors such as IL-4 (Gallichan *et al*, 1998), TGF-β (Suarez-Pinzon *et al*, 2002), and IL-35 (Bettini *et al*, 2012). These are examples of how mouse beta cells can be genetically altered to modulate their own immunological microenvironment. Whether these strategies would also be effective in human system is unknown. A potential caveat for this approach is that the immune system is thought to play an important role in suppressing the growth of tumor cell (reviewed in: Swann & Smyth, 2007), and inhibiting of the antigen presentation pathway has been shown to increase the tumorigenicity of cancer cells (Johnsen *et al*, 1999). Although the genetic changes discussed above do not induce tumorigenic transformation by themselves, they could plausibly increase the risk of tumor growth.

Discussion

The human body is made of cells, whose function or survival is affected in many incurable and chronic diseases, including diabetes. Drugs, such as insulin, can partially substitute for a cellular

deficiency, but generally results in disease management, rather than a cure and a life-long dependence on health care. Therefore, basic research and medicine must learn how to use cells, and preferably the patient's own cells to cure disease (Kind & Colman, 1999; Lanza *et al*, 1999a). The generation of personalized pluripotent stem cells by either nuclear transfer or factor-mediated reprogramming, and the development of improved protocols for differentiation into beta cells (Pagliuca *et al*, 2014; Rezania *et al*, 2014), has moved us closer than ever to this goal. However, not only questions regarding feasibility, but chiefly economic considerations have thus far stalled significant efforts: Production and quality control costs for personalized stem cells would be enormous, as opposed to a single cell line that can be mass-produced. Therefore, companies such as Viacyte are developing a beta cell product based on a single embryonic stem cell line that will be encapsulated for protection from both auto- and allo-immunity. Others proposed the development of haplotype-matched array of stem cell lines to reduce allo-immunity (Turner *et al*, 2013). However, if patients were able to make a choice, they would likely choose their own cells as a means to restore insulin independence.

Here, we reviewed available cell sources, the methods of delivery, as well as potential avenues of immune protection toward a cell replacement therapy for type 1 and non-immune-mediated forms of diabetes. Several sources of autologous beta cells may be considered. Patient-specific stem cells with the genotype of a type 1 diabetic can be generated either by transcription factors (Maehr *et al*, 2009), or by nuclear transfer (Yamada *et al*, 2014), and both methods can give rise to insulin-producing beta cells. Cells generated by either method have gene expression and DNA methylation patterns that are essentially indistinguishable from embryonic stem cell lines derived from IVF embryos, but unlike their IVF counterparts, both methods result in an increased rate of *de novo* coding mutations and occasional loss of imprinting. Many of these mutations are likely pre-existing in the somatic cell used for reprogramming. This may not disqualify them for clinical use, though appropriate quality controls, such as DNA sequencing to exclude cells with damaging mutations, will likely be required. The group of Nissim Benvinisti recently showed that cells derived by transdifferentiation show fewer chromosomal abnormalities than cells differentiated from stem cells (Weissbein *et al*, 2014). These findings were made in the nervous system, but they may also apply to other cell types. This unexpected finding indicates that either clonal cell expansion or the transition through the pluripotent state increases the frequency of chromosomal errors. Though pluripotent stem cells often show karyotype abnormalities, they can be grown clonally, allowing the generation of a well-characterized cell population prior to differentiation. The generation of beta cells by trans-differentiation is not as amenable to quality controls: though beta cells can be made by induction from other somatic cells, each cell can represent a separate reprogramming event, cell populations will likely be heterogeneous, and not all cells can be analyzed. Such uncertainties regarding their specific cellular identity will not prevent their use in research, but presents challenges for the development of therapeutic applications.

A prerequisite for developing cell replacement therapies for diabetes is the ability to generate stem cell-derived beta cells that are functionally equivalent to human islet cells. Previous beta cell differentiation protocols resulted in either immature or polyhormonal beta-like cells that were minimally responsive to glucose *in vitro*. Recent improvements now yield monohormonal and glucose-responsive beta cells that, when transplanted into diabetic mice, restore normoglycemia. This points to their therapeutic potential, but more proximal applications of these cells are in basic research. By generating stem cell-derived beta cells from subjects with various forms of diabetes, genetically encoded defects in beta cell function can be identified and targeted. Monogenetic forms of diabetes caused by defined lesions in genes implicated in beta cell function have thus far been most tractable in this approach (Hua *et al*, 2013; Shang *et al*, 2014). Humanized models of T1D (immunodeficient mice hosting a human immune system) are also being developed (Brehm *et al*, 2012; Kalscheuer *et al*, 2012). These models may be important to test strategies that can prevent or stop immunity to self on human cells.

One of the main caveats for transplanting stem cell-derived beta cells into T1D patients is recurrence of autoimmunity. Though effective treatments in suppressing autoimmunity are available, the risk that such interventions might compromise the ability of the immune system to fend off infectious diseases and cancer does not compare well to the risks of continued standard diabetes care. Encapsulation of beta cells can in principle avoid the use of immune therapies, but life-long function of such capsules is unlikely. Therefore, a more proximal path to developing a personalized cell replacement therapy for diabetes may be on the forms of diabetes that are not immune mediated, including diabetes caused by single-gene mutations. Though monogenetic forms of diabetes are not as common, they can serve as a stepping stone toward cell therapy for type 1 and perhaps type 2 diabetes. Stem cell-based models of these monogenetic forms of diabetes will allow functional testing of cells with the mutation, and upon gene correction. Such functional testing will not only need to be performed on diabetic mice, but also on an animal model more similar in size and physiology to humans, such as the monkey or the pig. Unfortunately, immune-compromised swine that do not reject transplanted human cells have a life span of only a few months (Basel *et al*, 2012; Lee *et al*, 2014). Inducing tolerance to human cells in an immune competent animal or improved methods to allow long-term survival will be critical to advance this goal.

In parallel, immune modulatory approaches targeting the autoimmune reaction may be optimized with the goal to halt and possibly revert new-onset T1D, and to restore tolerance to autologous beta cells in established T1D. Although a number of individual therapies have shown efficacy in animal models, they have not yet been translated into human patients, and few specifically target autoimmunity without affecting other aspects of the immune system. The vital importance of the immune system for fighting infectious diseases and preventing tumor formation requires that immune therapies are specific in suppressing immunity to beta cells only. Another potential approach to protect transplanted beta cells from autoimmune destruction is to genetically modify the beta cells themselves to become less immunogenic and escape immune detection and rejection. Although the use of gene editing technologies requires additional quality controls, there is precedent for genetic modification of somatic cells in gene therapy. However, the *in vivo* behavior of cells that are invisible to the immune system needs further study.

The use of stem cell-derived beta cells will need to exceed the benefit to risk ratio of current diabetes treatments. As current

care, centered around glycemic control through insulin administration, is relatively safe and effective, the bar is set very high for any new approach. Though questions regarding the path of translation and the economic viability of such treatments remain, they may have a transformative effect on medicine, replacing pharmacological care, with the use of a patient's own cells to restore normal physiology.

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Conflict of interest

The authors declare that they have no conflict of interest.

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