

Review Article

Current approaches in regenerative medicine for the treatment of diabetes: introducing CRISPR/CAS9 technology and the case for non-embryonic stem cell therapy

Lauren Coombe*, Aamir Kadri*, Jessica Ferrer Martinez*, Vivas Tatachar*, Gary Ian Gallicano

*Department of Biochemistry and Molecular & Cellular Biology, Georgetown University Medical Center, 3900 Reservoir Road NW, Washington DC 20007, USA. *Equal contributors.*

Received October 2, 2018; Accepted October 28, 2018; Epub December 1, 2018; Published December 10, 2018

Abstract: Type 1 diabetes mellitus (T1DM) is an autoimmune disorder in which the body destroys its pancreatic β cells. Since these cells are responsible for insulin production, dysfunction or destruction of these cells necessitates blood glucose control through exogenous insulin shots. Curative treatment involves pancreas transplantation, but due to the incidence of transplant rejection and complications associated with immunosuppression, alternatives are being explored. Despite facing clinical challenges and issues with public perception, the field of regenerative stem cell therapy shows great promise for the treatment of diabetes. The idea of harnessing pluripotency to derive cells and tissues with characteristics of choice is astounding but feasible, and this review seeks to determine which method of stem cell derivation is preferable for diabetes treatment. In this report, we outline the methods for deriving human embryonic stem cells (hESCs), induced pluripotent stem cells (iPSCs), and adult stem cells or progenitor cells to generate functional islet cells and related tissues. We discuss the specific uses and advantages of each method, and we comment on the ethics and public perceptions surrounding these methods and how they may affect the future of stem cell research. For the reasons outlined in this paper, we believe that non-embryonic stem cell lines, including iPSCs, somatic cell nuclear transfer lines, and adult tissue derived stem cells, offer the highest therapeutic potential for treating diabetes.

Keywords: Stem cell therapy, type 1 diabetes, beta cells, adult stem cells, induced pluripotent stem cells

Introduction

In the United States, over 30 million people are living with diabetes, and an additional 84 million people have been diagnosed with pre-diabetes. With nearly 10 percent of the population already having diabetes and 33 percent of Americans likely to develop it, the cost of this disease accounts for 1 in 4 U.S. healthcare dollars. Type 2 diabetes mellitus (T2DM) accounts for 90 to 95 percent of the diabetic patient population. These patients have elevated blood glucose levels because either the β cells of the pancreas do not make sufficient insulin or the tissues in the body cannot use insulin efficiently [1]. Without enough insulin or with insulin resistance, glucose stays in the blood and cannot enter the cells of the body.

Type 1 diabetes mellitus (T1DM) is an autoimmune disorder rather than a metabolic disorder, and the hyperglycemia in these patients results from the body's immune system attacking the insulin-producing β cells [2]. Currently, the Edmonton Protocol has benefitted some patients with poorly controlled T1DM. This procedure requires recovery of islet cells from cadavers, followed by transplantation into a recipient to replace the lost β cells. However, this method comes with the risk of graft rejection and requires significant immunosuppressive therapy after transplantation. Although transplantation has proven to be effective in managing some T1DM patients in the short-term, only 10 percent of patients remained insulin-independent five years after islet transplantation [3]. Transplantation is also severely

limited by donor availability: the demand far exceeds the supply of donor islet cells. Thus, other methodologies are being explored to expand β cell populations by either increasing β cell replication, decreasing β cell death, deriving new β cells from the appropriate progenitor cells, or combining some of these methods [1].

The frontier of stem cell science is vast and exciting, as there are virtually limitless proposed uses for their regenerative power-including β cell regeneration. Current research suggests that certain cell lines and derivation techniques are preferable in certain circumstances over others. Here, we 1) review emerging studies in stem cell therapy as they relate to the treatment of diabetes, and 2) present an argument on which method carries the most promise for this purpose.

The healing potential of stem cell therapy revolves around the idea of pluripotency, a term used to describe a cell's ability to differentiate into any cell type within the embryo that will eventually be found in the adult human body [4]. Harnessing the pluripotency of these cells, followed by carefully controlled differentiation into specific tissue types, provides unlimited applications for disease treatments or cures, including deriving pancreatic β cells to replace cells implicated in T1DM. In the case of human embryonic stem cells (hESCs), a human fertilized embryo is arrested in its pluripotent stage, at which point it can be manipulated to form tissues of choice. The process for deriving hESCs has almost become routine: fertilized embryos produced from *in vitro* fertilization (IVF) are grown into the blastocyst stage; from the blastocyst, cells from the inner cell mass (ICM) are extracted, as these are the only cells of the blastocyst that are pluripotent; and then, cells from the ICM, which may now be called "stem cells", are placed in a nutritive medium. By manipulating the stem cells' surrounding environment, one can determine their fate [4].

Human embryonic stem cells compared to induced pluripotent stem cells (iPSCs)

Aside from obtaining embryonic stem cells in their pluripotent stage, another method of obtaining pluripotent cells is by deriving Induced Pluripotent Stem Cells (iPSCs or iPS cells). An iPSC is produced by inducing pluripotency in an already differentiated, mature adult cell. This

method differs from hESC development in that we are essentially reverse-engineering a pluripotent cell from one that has already differentiated, whereas hESCs are embryonic cells that we arrest at the pluripotent stage before inducing differentiation into a tissue of choice. One study provides a step-by-step protocol for deriving iPSCs from human pancreatic cells using the CRISPR-Cas9 system [5]. This method is enticing for a number of reasons:

1. Specifically, in the case of diabetes research, a proposed therapy is to build a pool of pancreatic islet cells to transplant into a patient with T1DM or an otherwise reduced β cell density and/or function. If the end goal is to create working islet cells to be transplanted into a pancreas, beginning with a pancreatic cell can, perhaps, give us an iPSC that retains certain qualities from its pancreatic heritage that would make for easier and more effective reprogramming.
2. Many current methods for iPSC creation require the use of viruses as a method of DNA reconstruction and/or delivery. The method outlined in this article promises reduced incidence of viral transgenes or exogenous factors being integrated into the final iPSC through this process. It is reasonable to surmise that having fewer exogenous factors could reduce the incidence of antigen production and transplant rejection.
3. The CRISPR-Cas9 system is inexpensive, user-friendly, and intuitive. This system brings the science of genetic engineering into the hands of many more people, from world-renowned stem cell researchers to undergraduate lab assistants. Put simply, the sheer volume of iPSCs that can be created and investigated using this method makes it an extremely desirable option that maximizes the potential and reach of stem cell research.

However, does ease of access to iPSC technology warrant its use over hESCs? The Reznick group sought to answer this question when they compared their human embryonic "S7" cells to an iPSC line. In normal human physiology, pancreatic β cells are self-regulating, meaning that they secrete insulin in response to glucose, and subsequently, shut off at the appropriate time to prevent hypoglycemia. Trying to mimic this natural function has proven difficult with all stem cell therapies, especially when the cells are *in vitro*. When injected into mouse models, the Reznick group's S7 insulin-

secreting cells displayed a positive sensitivity to glucose. Although its responsiveness to glucose was slower than that of normal human β cells, the S7s displayed swift diabetes reversal. In fact, the embryo-developed S7s reversed diabetes in mice approximately four times faster than iPSCs from pancreatic progenitors. According to this study, the iPSC method also was less efficient than the hESC model at creating a pool of usable insulin-producing cells. In other words, iPSCs were not able to produce as many insulin-producing cells than their hESC counterparts [6]. However, since both cell types were comparable in terms of whether or not they reversed diabetes in mice, using them in tandem would perhaps be an alternative option, considering the novelty of stem cell studies in general.

A different group of researchers successfully generated billions of functional human pancreatic β cells (SC- β cells) using iPSCs and hESCs together. These SC- β cells exhibited glucose-sensitive insulin secretion *in vitro* and were comparable in function to cadaveric islet cells and human β cells. When injected into mice, the SC- β cells from both hESC and hiPSC cell lines secreted insulin directly into the host's bloodstream and showed increased human insulin secretion after a glucose challenge [7]. The ability to produce large amounts of SC- β cells *in vitro* overcomes one of the major limitations in human β cell replication; however, the challenge of successfully implanting these cells and preventing graft immunogenicity remains.

The case for adult stem cells in the creation of insulin-producing cells (IPCs)

The use of hESCs is controversial from an ethical standpoint, and their use poses tumorigenic risks, both of which limit the potential of hESCs as a sustainable treatment option for T1DM. iPSCs are also tumorigenic and are more prone to genetic mutations because of the methods in which they are induced. That said, a potentially more attractive option may be utilizing adult tissue-derived stem cells to produce β cells (Table 1).

Research has shown that human progenitor cells taken from fetal pancreata can be used to generate functional insulin-producing cells (IPCs) *in vitro*, and after implantation into mice, these cells resolve and maintain normoglycemia [8]. If intestinal progenitor cells could be

induced *in vitro* to differentiate into β cells, maintain their functionality in a T1DM patient, and evade an autoimmune attack, it is reasonable to assume that this method could cure T1DM. One of the main challenges in this process is inducing intestinal cell-to- β cell differentiation, which involves an in-depth understanding of the complex molecular signaling pathways involved in β cell development.

One breakthrough study illustrated a successful method of generating IPCs from intestinal progenitor cells in mice by knocking out *FOX-O1*, a transcription factor that is fundamental for β cell growth in the developing pancreas. Researchers in this group identified several key factors and pathways that were affected downstream in the signaling pathway by investigating the effects of *FOX-O1* ablation. Knocking out *FOX-O1* led to the activation of Wnt signaling, which prevented *Neurog3* from differentiating into enteroendocrine cells. Activation of Wnt signaling also led to increased levels of Amino-terminal enhancer of split (*Aes*), which was critical for generating IPCs by repressing Notch signaling and *HES-1* expression (both of these are involved in suppressing pancreatic development) [9]. The IPCs generated from *FOX-O1* ablation were capable of insulin secretion in response to glucose levels *in vivo*, showing a promising strategy for further research into T1DM. Additionally, the mice were capable of regenerating these IPCs after STZ-induced diabetes. This finding provides evidence for the potential regeneration of β cells and takes a step in the right direction towards the development of a cure for T1DM.

Adipose tissue-derived stem cells (ADSCs or ASCs) have also shown promise for therapeutic potential in treating T1DM. Chandra's group developed a novel protocol for differentiating ASCs into islet-like cell aggregates (ICAs) that is remarkably efficient, producing 57-71% *PDX-1+* cells (a key regulator of pancreatic development and β cell differentiation) and 47-51% C-peptide-positive cells (a key marker in determining diabetes). These ICAs demonstrated *in vitro* and *in vivo* functionality: two weeks after the injection of ICAs into diabetic mice, normoglycemia was restored and maintained for 1 month [10].

Additionally, a separate group successfully treated STZ-induced diabetes in mice through the intravenous transplantation of ASCs that

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Table 1. Summary of the potential uses, advantages, and disadvantages of various types of stem cells

Source	Potential Use(s)	Advantages	Disadvantages
Human Embryonic Stem Cell (hESC)	<ul style="list-style-type: none"> Insulin-producing cells Transplantable device containing hESCs Interspecies organogenesis 	<ul style="list-style-type: none"> hESC-derived pancreatic cells differentiate <i>in vivo</i> to β cells Lesser incidence of viral transgene incorporation Reverse diabetes in mice faster than iPSCs More efficient than iPSCs at producing insulin-producing cells 	<ul style="list-style-type: none"> Ethical concerns over derivation process Lack of public support Embryo supply is limited by legislation
Induced Pluripotent Stem Cell (iPSC)	<ul style="list-style-type: none"> Insulin-producing cells Blastocyst complementation to produce functional pancreatic islet cells <i>in vivo</i> Interspecies organogenesis 	<ul style="list-style-type: none"> Exogenic iPSCs can generate functional pancreata CRISPR-Cas9 method can be used to reduce time and cost of chimeric organ generation Excludes risk of teratoma development that is of concern with islet cells developed <i>in vitro</i> As effective as hESCs in reversing diabetes in mice (though slower) Avoids the embryo controversy 	<ul style="list-style-type: none"> Immune tolerance needs to improve before the technique can be used in humans Less efficient than hESCs at generating insulin-producing cells Possibility of viral transgene incorporation
Intestinal Progenitor Cell (IPC)	<ul style="list-style-type: none"> Insulin-producing cells 	<ul style="list-style-type: none"> Shares common germ cell origin with pancreas-might make β-cell creation easier 	<ul style="list-style-type: none"> Need to control multiple transcription factors in order for transplanted IPCs to demonstrate glucose-related insulin secretion
Adipose Tissue Derived Stem Cells (ADSC or ASC)	<ul style="list-style-type: none"> Hormone producing islet-like cell aggregates 	<ul style="list-style-type: none"> Stem cells from adipose tissue are relatively easy to obtain High yield of functional cells from ASCs using established protocol 	<ul style="list-style-type: none"> Method still needs further investigation in larger animals

Table 2. Summary of molecules and/or signaling pathways discussed in this paper and their involvement in stem cell generation, synthesis of insulin-producing cells, or interspecies organogenesis of pancreata

Molecule	Method	Effect
<i>PDX-1</i> β -cell Transcription Factor	<ul style="list-style-type: none"> Knock-out <i>PDX-1</i> in subject receiving implantation 	<ul style="list-style-type: none"> Establishes niche for iPSC implantation for organogenesis
<i>MIXL-1</i> Transcription Factor and Mesoderm Marker	<ul style="list-style-type: none"> Overexpress <i>MIXL-1</i> in subject receiving implantation 	<ul style="list-style-type: none"> Prevents stem cells from differentiating into mesoderm, thereby preventing formation of undesired organs
<i>HES-1</i> Transcription Factor for Biliary Development	<ul style="list-style-type: none"> Overexpress <i>HES-1</i> under the <i>PDX-1</i> promoter Decrease <i>HES-1</i> expression (via <i>FOX-O1</i> ablation and repression of Notch signaling) 	<ul style="list-style-type: none"> Inhibits pancreatic tissue development to establish niche for iPSC implantation in organogenesis Removes <i>HES-1</i>'s inhibition of pancreatic tissue development
CTLA4Ig Immunoglobulin	<ul style="list-style-type: none"> Administration of CTLA4Ig into subject receiving transplantation 	<ul style="list-style-type: none"> Induces immunological tolerance to xenogeneic organ transplantation
Anti-CD154 Antibody	<ul style="list-style-type: none"> Administration of anti-CD154 into subject receiving transplantation 	<ul style="list-style-type: none"> Induces immunological tolerance to xenogeneic organ transplantation
<i>FOX-O1</i> Transcription Factor	<ul style="list-style-type: none"> Knock-out <i>FOX-O1</i> in gut epithelial cells 	<ul style="list-style-type: none"> Expands population of gut progenitor cells
Notch Signaling Cascade	<ul style="list-style-type: none"> Suppress Notch signaling pathway in gut progenitor cells (via <i>FOX-O1</i> ablation) 	<ul style="list-style-type: none"> Decreases expression of <i>HES-1</i>, enabling the development of gut progenitor cells into pancreatic tissue
Wnt Signaling Cascade	<ul style="list-style-type: none"> Activate Wnt signaling pathway in gut progenitor cells (via <i>FOX-O1</i> ablation) 	<ul style="list-style-type: none"> Increases levels of <i>Aes</i>
<i>Aes</i> Transcriptional co-repressor	<ul style="list-style-type: none"> Increase levels of <i>Aes</i> expression in gut progenitor cells (via <i>FOX-O1</i> ablation and activation of Wnt signaling) 	<ul style="list-style-type: none"> Induces the differentiation of gut progenitor cells into IPCs Represses Notch signaling and decreases <i>HES-1</i> expression

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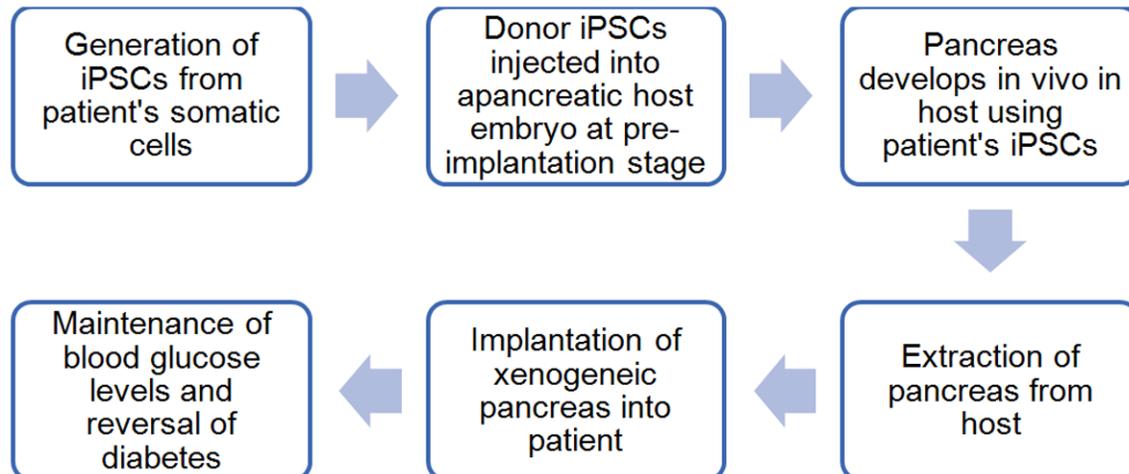


Figure 1. This figure shows the treatment potential of diabetes in humans by interspecies blastocyst complementation. Ideally, the end result is normalization of blood glucose levels and complete reversal of diabetes through generation of insulin secreting β -cells derived from the patient's own tissues.

were transfected with *PDX-1*. These *PDX-1*-ASCs were successfully engrafted into the pancreata of diabetic mice, where they developed β cell functionality *in vivo* and supported the notion that ASC-derived β cells have regenerative properties [11] (Table 2).

Interspecies organogenesis and transplantation: combination hESC, iPSC, and SCNT therapy

Advancements in the field of regenerative medicine have taken steps towards harnessing the power of hESCs, iPSCs, and somatic cell nuclear transfer (SCNT) lines for the treatment of diabetes. One successful method, termed blastocyst complementation, delivers donor stem cells into blastocysts at the pre-implantation stage to produce exogenous pancreata. This method prevents the formation of the organ's associated vasculature and lymphatics, thereby avoiding immunological challenges that can arise when trying to match donor cells to host structures [12].

A landmark study demonstrated the effectiveness of this method by producing functional mouse-rat chimeric pancreata using mouse iPSCs. To establish a developmental niche for the implantation of mouse iPSCs, mutant rats were created by knocking out the *PDX-1* gene, which is responsible for pancreatic development. After injecting mouse iPSCs, the pancreata within mutant rats produced functional

islet cells that normalized and maintained host blood glucose levels for over 370 days and effectively reversed streptozotocin (STZ)-induced diabetes, all without the use of long-term immunosuppression [13].

Another group utilized the aforementioned CRISPR-Cas9 system to generate *PDX-1*-deficient organisms through blastocyst complementation more quickly and efficiently [14]. This breakthrough had profound implications for reducing the time and cost of developing chimeric organs and offered a potential solution for the current global shortage of donor organs. Most importantly, this study demonstrated that human iPSCs could integrate successfully into post-implantation pig embryos, develop functional pancreata, and stabilize and maintain blood glucose levels when subjected to a glucose tolerance test [14]. Similarly, another groundbreaking study in apancreatic cloned pigs used blastocyst complementation to generate xenogeneic pancreata *in vivo* [12]. Together, the Matsunari group and the Wu group's studies provide evidence that researchers could generate a functional organ from exogenic iPSCs. These results offer considerable evidence for the potential of producing mass quantities of pig progeny for organogenesis with iPSCs. Additionally, the process of SCNT (the transfer of a nucleus from a somatic cell into a germ cell that has had its nucleus removed to generate a viable, chimeric embryo) has shown great promise towards becoming a

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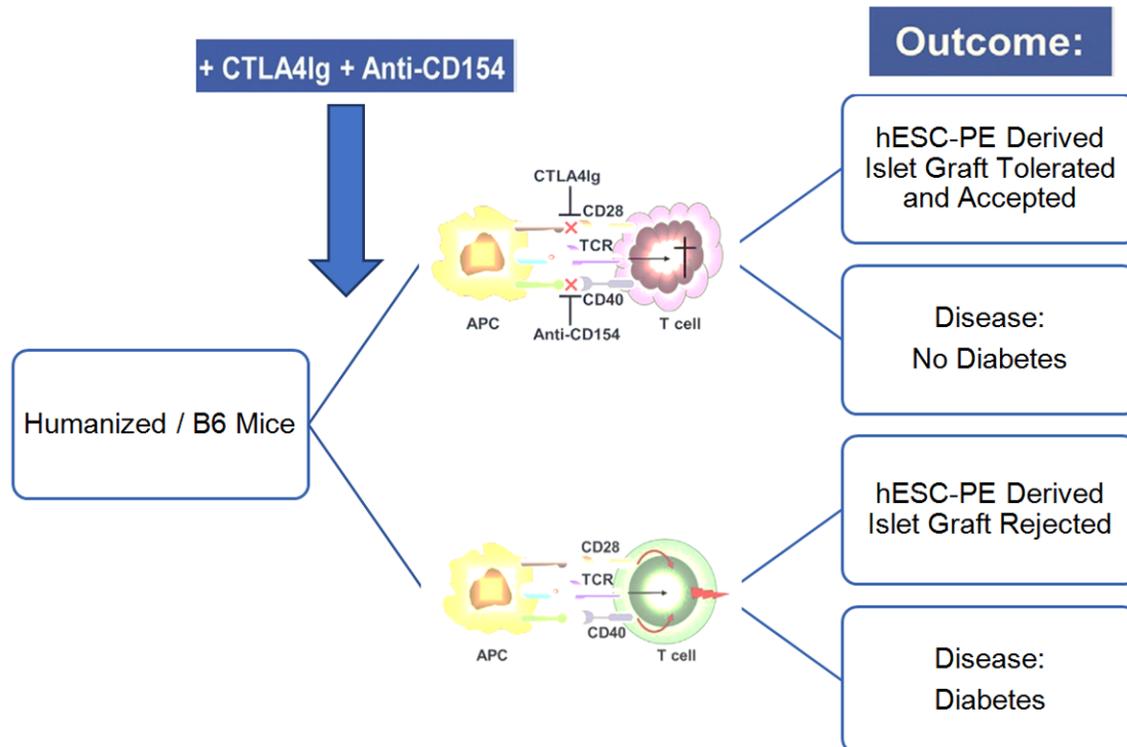


Figure 2. This figure shows a graphical representation adapted from the Szot group's study. This study demonstrated that blocking the T-cell costimulatory pathway in both B6 and humanized mice prevents rejection of a xenogeneic pancreas. As a result, grafts developed into mature structures capable of producing human insulin and regulating and maintaining blood glucose levels [16]. When combined with the techniques discussed in this paper, this method offers a potential long-term solution for the treatment of diabetes. Note: hESC-PE indicates human embryonic stem cell derived pancreatic endoderm.

standardized treatment option when coupled with human iPSCs. Most importantly, generating organs in chimeric pigs offers a potential solution to the ethical dilemmas surrounding human embryo destruction to obtain stem cells [12].

Collectively, these studies provide ample evidence for the therapeutic potential of the xenogeneic generation of transplantable organs containing tissues derived from an individual's cells (Figure 1). The first two studies utilized a rat model system, which offers a comprehensive understanding of the molecular factors underlying interspecies chimerism with iPSCs. The third and fourth studies showed proof-of-principle for production of a functional organ in an organism closer in size to humans. Together, these studies highlighted the ability to develop normal, donor-specific organs in chimeric hosts when provided with the proper embryonic niche in which to inject iPSCs, which was the absence of a pancreas through genetic manipulation [13].

Along with direct implantation of pancreata, a transplantable device containing hESC-derived pancreatic cells that differentiate *in vivo* to β cells offers an optimistic cure for diabetes [15]. Due to its use of host tissues, this device reduces the need for immunosuppression. Further, since vasculogenesis occurs around the device, the β cells inside the device are in direct contact with the blood and begin to secrete insulin in response to changes in blood glucose levels. Currently, this device is undergoing human clinical trials, but the preliminary results already show excellent potential for treating diabetes [15]. When combined with the advances in chimeric organ formation, this device could help researchers move towards developing a sustainable treatment plan and a potential cure for diabetics worldwide.

Although these studies provide evidence for reversing diabetes in mouse models, researchers need to address several limitations before developing advantageous therapies for humans. Yamaguchi's group acknowledged

that two of their *PDX-1* mutant rats with mouse-derived pancreatic cells developed diabetes-like symptoms, asserting that improved regulation of peripheral tolerance is needed to improve the technique for use in humans and avoid graft rejection [13]. Wu's group showed that addressing the species-specific differences in epiblast and trophectoderm development and choosing organisms evolutionarily closer to humans are the most important aspects to confront before the technology can be useful in humans [14]. They also asserted that providing a selective advantage for the differentiation of donor iPSCs instead of host cells and generating a diverse set of iPSCs are necessary for effectiveness in humans. Similarly, Matsunari's group argued that efforts first need to focus on establishing a xenogeneic system between pigs and cows before embarking on human attempts. Moreover, they noted that careful cell fate control must be implemented to prevent unwanted organ formation, which addresses a prominent ethical concern with stem cell technology [12].

Despite these limitations, the future potential of interspecies organogenesis for the treatment of diabetes shows exceptional promise. Researchers have developed the ability to induce immunological tolerance to xenogeneic implantation by blocking the T-cell costimulatory pathway through the administration of CTLA4Ig to suppress T-cell activation and anti-CD154 antibody to reduce antibody affinity (Figure 2) [16]. This technique played an integral role in reversing diabetes in a humanized mouse model *in vivo*. By inducing immunological tolerance and showing evidence of reversing diabetes, the prospect of interspecies organogenesis for transplantation in humans becomes more viable. When combined with the techniques discussed in this section, it is reasonable to envision several different options for sustainable combination therapies for patients with diabetes. These therapies could reduce medical costs and provide alternative treatments that can ultimately increase the quality of life for diabetes patients.

Overall, these studies provide considerable evidence to show that xenogeneic organs can function normally in long-term maintenance of blood glucose levels while avoiding the immunological concerns of graft versus host disease. By showing therapeutic potential without

consequences, researchers have taken steps towards overcoming a significant barrier facing transplantation of interspecies organogenesis-derived pancreata and have improved the long-term outlook for diabetes treatment options.

The role of C-peptide: an important consideration for preventing pathophysiological problems associated with diabetes

C-peptide is an important molecule that has been shown to improve diabetes-induced abnormalities of the peripheral nerves and kidneys when injected in combination with insulin [17]. Typically, pro-insulin is cleaved to insulin and C-peptide in human pancreatic β cells. These molecules are secreted in equimolar amounts, making C-peptide a useful marker in determining whether a patient has Type 1 or Type 2 diabetes mellitus. Additionally, new findings likely suggest a beneficial role of C-peptide in many physiological abnormalities associated with diabetes.

For example, one issue with diabetes is hyperglycemia, which results in the activation of NADPH oxidase, resulting in the formation and accumulation of reactive oxygen species (ROS) [18]. When in circulation, ROS contribute to inflammation via the increased expression of cell-adhesion molecules, chemokines, and cytokines. C-peptide has been shown to inhibit NADPH oxidase and stimulate AMPK α , which further inhibits the formation of ROS [17]. Additionally, the pro-apoptotic enzyme, *Transglutaminase-2*, is overexpressed in diabetic patients; however, C-peptide administration has been shown to block the activation of this enzyme, most likely through alleviating ROS in circulation. Together, these findings also indicate in diabetic patients an increased likelihood of forming atherosclerotic lesions, which C-peptide has been shown to inhibit as well. C-peptide also blocks the nuclear factor K- β pathway, further mediating inflammation and providing cytoprotective effects [17].

In T1DM, diminished activity of the sodium-potassium pump has been shown, which leads to a variety of neuronal, cardiovascular, and renal abnormalities [18]. In a double-blind, placebo-controlled experiment conducted on diabetic patients who had the disease for ten years, C-peptide administration over a period of eight months resulted in the nearly total pre-

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vention of both axoglial disjunction and paranodal demyelination. Development of nerve conduction velocity deficits, as well as loss of vibration sensation, are quite common as well. However, administration of C-peptide via subcutaneous infusions prevented the development of such complications. Moreover, C-peptide was shown to improve left ventricular efficiency in diabetic patients, characterized by an increase in both ejection fraction and stroke volume [19]. C-peptide binding ultimately results in the activation of the MAPK pathway, an essential mediator of nitric oxide synthase [17]. Thus, pathophysiological problems are prevented through better perfusion via endothelial nitric oxide synthase activation coupled with C-peptide's ability to increase sodium-potassium ATPase activity. Together, these effects contribute to improved parasympathetic nervous activity [19].

Renal dysfunction is also a common issue with diabetic patients. In animal studies, C-peptide administration diminished the expansion of the mesangial matrix by inhibiting *TGF- β 1* gene expression in the podocyte cell line [20]. C-peptide, via activation of nuclear factor κ -B regulated genes, also protects against inflammatory damage to the renal tubules by decreasing glomerular hyperfiltration, restoring the glomerular filtration rate (GFR), and reducing urinary albumin excretion [17]. C-peptide was hypothesized to restore the GFR by constricting the afferent and dilating the efferent capillaries, which ultimately allows for greater reabsorption within peritubular capillaries [21].

The therapeutic possibilities surrounding C-peptide are quite promising for diabetic patients. C-peptide's role in preventing the formation of ROS makes it a promising endogenous antioxidant, while its role in suppressing immune responses and pathological inflammation in diabetes makes it a vital cytoprotective molecule in preventing many pathophysiological problems associated with diabetes. Therefore, the ability of a stem cell-derived β cell to secrete C-peptide should be considered in addition to its ability to secrete insulin.

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Thus far, we have discussed the various methods of stem cell derivation with regard to their efficacy as tools in diabetes treatment. We have also detailed the specific strengths and

uses for each cell line. However, the argument over which cell line to use and when remains a point of controversy within the field of stem cell science. All stem cell sources discussed thus far have documented success, but it is imperative to evaluate the risks of each method when considering their therapeutic potential in a clinical setting.

Pluripotent cells inherently carry the risk of tumorigenesis when transplanted into host tissue. iPSCs are increasingly being considered as comparable to hESCs in their potential research and clinical uses. However, there are critical differences between iPSCs and hESCs that may limit the use of iPSCs in a clinical setting. hESCs have been shown to acquire genetic mutations at any point during their differentiation into somatic cells [22]. iPSCs, however, are susceptible to incorporating more genetic mutations into their genome because they must first be reprogrammed into pluripotency through a modification process that compromises the integrity of the genome [22]. After reprogramming, they are subject to the same fate of hESCs and may integrate additional mutations into their genome during their differentiation into somatic cells. This principle alone certainly warrants the additional protocols for quality testing of iPSCs, as compared to hESCs or adult stem cells. There is also research that suggests that stem cells derived via the SCNT method are more closely related to hESCs [23].

For these reasons, adult stem cells and adult progenitor cells should be at the forefront of regenerative therapy in the clinic for patients with T1DM. In this review, we have examined methods of deriving pancreatic β cells using intestinal progenitor cells and adipocytes as the source of a multipotent cell population. In addition to these cell lines, progenitor cells in the human liver and pancreatic cells have also proven effective in generating β cells [24]. These cell lines have been proven to be capable of developing into functional human-like β cells both *in vitro* and *in vivo* and should be considered excellent candidates for clinical trials regarding stem cell therapy in patients with T1DM.

Concluding remarks

In the hunt for diabetes treatments and solutions to obstacles such as whole organ supply, transplant rejection, persistent insulin resis-

tance, and other pathophysiological issues associated with diabetes, significant attention has been given to stem cells and their potential for regenerative therapy. hESC creation involves a fertilized human embryo that is allowed to proliferate into the pluripotent blastocyst stage (around day five post-fertilization), at which point further development is arrested, and ICM cells are extracted [25]. From this stage, adding various growth factors and/or transgenes- or otherwise manipulating the environment surrounding the ICM cells-enables us to create self-proliferating cell lines and various tissue types. For therapeutic purposes, immunosuppressants may still be necessary, although scaffolding devices using hESCs are being explored to address this problem [15]. In mouse studies, hESC-derived therapies reversed diabetes approximately four times faster than iPSC-derived therapies. hESCs were also shown to produce insulin-producing cells more efficiently than their iPSC counterparts [6]. However, hESC use also comes with a great deal of political and social controversy since it must destroy embryos to harvest pluripotent cells.

Alternatively, iPSCs are derived by inducing pluripotency in already differentiated, fully mature adult cells that do not require destruction of an embryo [25]. Since iPSCs can be taken from the patient/transplant recipient themselves, the issue of transplant rejection and the need for immunosuppression is positively addressed. SCNT also avoids the use of fertilized human embryos by using donated human oocytes with a transplanted nucleus to produce cells similar to hESCs [12]. Finally, intestinal progenitor cells from mature adults can be induced to form IPCs, thereby avoiding embryos as well. Due to the generally more positive public perception surrounding non-embryonic cell lines, it is plausible to argue in favor of iPSC, SCNT, and intestinal cell use over hESCs with regard to regenerative stem cell therapy, especially in the treatment of diabetes.

iPSCs, SCNT, and adult stem cell/progenitor cell lines have also been shown to function comparably to hESCs and even possess certain advantages. When used in tandem with SCNT lines, iPSCs show great promise in the field of full organ regeneration and transplantation and have proven their efficacy in studies of diabetes and the pancreas [12]. IPCs derived from intestinal cells also show remarkable potential for

insulin production, possibly because intestinal cells and pancreatic cells differentiate from a common germ layer. Adipose tissue derived stem cells create islet cell aggregates that can stimulate β cell differentiation at an enhanced rate, compared to alternative methods. Finally, iPSCs have the unique ability to secrete C-peptide, which gives them a distinct therapeutic advantage over other alternatives [17, 26]. Considering the proven efficacy, foreseeable potential, and lesser moral controversy tied to iPSCs, SCNT lines, and adipose/intestinal progenitor cells, we propose the superiority of non-embryonic stem cell therapy in the continuation of diabetes research and treatment [27-29].

Disclosure of conflict of interest

None.

Address correspondence to: Dr. G Ian Gallicano, Department of Biochemistry and Molecular & Cellular Biology, Georgetown University Medical Center, 3900 Reservoir Road NW, Washington DC 20057, USA. Tel: 202-687-0228; Fax: 202-687-9864; E-mail: gig@georgetown.edu

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