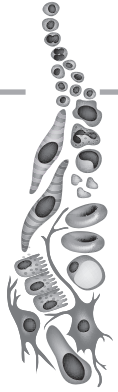

Do stem cell-derived islets represent a commercially viable treatment for Type 1 and 2 diabetes?

"The benefits in terms of overall health, quality of life and cost savings from improved glucose control make a compelling case for cell replacement therapy for the treatment of diabetes."

KEYWORDS: β-cell ■ cell therapy ■ diabetes ■ islet transplant ■ stem cell ■ xenotransplant

Adrianne L Wong

Juvenile Diabetes Research Foundation International, 26 Broadway, 14th floor, NY 10004, USA

Concepcion R Nierras

Author for correspondence:
Juvenile Diabetes Research Foundation International, 26 Broadway, 14th floor, NY 10004, USA
Tel.: +1 212 479 7589
Fax: +1 212 479 7692
cnierras@jdrf.org

The potential of stem cell therapies for the treatment of diabetes has been the cornerstone for successful political action, fundraising and academic research programs. How can these lofty goals be translated? Clinical translation of stem cell therapies for diabetes requires unraveling the underlying biology of pancreatic development and islet function, and then recapitulating development in a defined, scalable and commercially viable manner. The β-cell or surrogate β-cell product must be safe, and must secrete insulin in a physiologically appropriate response to glucose. Xenotransplantation of pig islets would address the shortage of human cadaveric donor islets and would be an alternative to β-cells derived from human pluripotent stem cells. Stem cell-derived β-cells represent a cell replacement therapy for Type 1 diabetes (T1D) and for severe Type 2 diabetes (T2D).

Market & product profile for diabetes cell replacement

Diabetes is the term for a collection of diseases characterized by high levels of blood glucose, as a result of an absolute or relative deficiency of insulin-producing β-cells [101]. T1D is an autoimmune disease in which the insulin-producing β-cells of the pancreatic islets are specifically destroyed by the immune system. People with T1D are unable to produce insulin, and must inject exogenous insulin in order to regulate their blood glucose. By contrast, T2D does not have an autoimmune component: in its early stages, normal insulin secretion is met with insulin resistance, which leads, over time, to a loss of functional β-cell mass. A proportion of people with severe T2D require exogenous insulin injections. In 2007, it was estimated that nearly 24 million Americans had diabetes, and the cost of diabetes exceeded US$174 billion [102]. This estimate included $116 billion in medical expenditures attributed to diabetes, as well as $58 billion in reduced national productivity. Approximately US$1 in every 10 spent on healthcare is attributed to diabetes. People with diagnosed diabetes, on average, have medical expenditures that are approximately 2.3-times higher than the expenditures would be in the absence of diabetes. The loss of glycemic control in diabetes can lead to kidney failure, adult blindness, nontraumatic amputation, nerve damage, stroke and heart attacks. Preventing the development of diabetes complications would improve overall health and realize considerable savings [1].

Cell replacement therapy for diabetes focuses on the replacement of functional β-cell mass. Proof of principle for the efficacy of this therapy is provided by the experience of islet transplantation from cadaveric donors [2]. At present, islet transplantation is an experimental procedure, and is only used to treat a limited number of people with T1D and life-threatening hypoglycemia. Nonetheless, data are emerging that suggest that islet transplantation can prevent the progression of the complications of diabetes [3,4], and that transplant recipients have a much improved quality of life [5]. These improvements have spurred efforts to develop replacement therapies despite the high costs and the requirement for immunosuppression.

The Juvenile Diabetes Research Foundation (JDRF), founded in 1970 to find a cure for T1D and its complications, was an early supporter of stem cell research, recognizing the potential of using stem cell-derived, glucose-responsive, insulin-producing β-cells to treat diabetes [6]. Potential target patient populations for treatment with these β-cells might be patients with T1D, pancreatitis, severe T2D, brittle (i.e., difficult to control) diabetes or more controlled T1D. It is

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the consensus that the juvenile T1D population will probably be the last group to receive cell replacement treatment, owing to safety and ethical considerations. As in the development of islet transplantation [2], patients without autoimmune concerns, such as those with pancreatitis or severe T2D, represent the most straightforward potential patient population. While people with brittle diabetes would have the most to gain from a cell therapy, it has been argued that for initial trials there may be a greater chance of success for the transplanted stem cell-derived β-cells if they were transplanted into a patient with less severe diabetes.

Cell source

Different types of pluripotent stem cells have been proposed as the starting material for deriving β-cells [7]. The advantage of starting with pluripotent stem cells is their ability to differentiate into all cells of the body, including β-cells. However, the risk of teratoma formation from undifferentiated stem cells is well known. In theory, induced pluripotent stem cells are also able to develop into β-cells, with the additional potential for individualized therapy, but questions remain to be answered about the pluripotency, phenotypic stability and ultimate safety of these cells [8].

> "*Although proof of concept for a stem cell-derived pancreatic progenitor cell therapy for diabetes has been achieved in small animal models, a fully functional* β*-cell* in vitro *has not yet been achieved.*"

Alternative approaches to stem cell-based β-cell replacement therapy are under investigation [9]. β-cells can be regenerated in adult mice, an appealing paradigm still to be conclusively demonstrated in humans. Alternatively, other adult pancreatic cell types or tissues sharing a common lineage might be directly reprogrammed into β-cells using transcription factors. Finally, there have been reports identifying a 'pancreatic stem cell' population in the adult pancreas. However, expansion of these populations has proved difficult.

β-cell development

Understanding the developmental biology of the β-cell is required for a functional β-cell to be derived, whatever the starting material [10]. Much of the early work on β-cell development was performed by academic groups with government funding using mouse models. Many of these groups were constrained by government restrictions on research using human pluripotent stem cells. This provided an opportunity for commercial entities to undertake the development of β-cells from human embryonic stem cells [11–13]. A current protocol from Viacyte (i.e., Novocell, Cythera) starts with pluripotent cells and differentiates them into definitive endoderm, through a primitive gut, then posterior foregut, to pancreatic endoderm-like cells. These pancreatic progenitor cells are implanted into mice and allowed to mature for 3 months *in situ*, resulting in insulin-expressing cells that exhibit many properties of functional β-cells [13]. This protocol is optimized with Viacyte's own Cythera49 human embryonic stem cell line. Independent verification of these results, especially with different starting cell lines, has not yet been published. It is likely that a commercial entity would either want an exclusive license for the use of a cell line for a particular product or would develop its own verified cGMP line. Although substantial progress has been made, the later stages of development and the functional maturation of the human β-cell remain active areas of study by both academic and commercial groups.

In November 2009, JDRF, the NIH and the US FDA convened a workshop to ask, "how good does a β-cell have to be to receive regulatory approval as a therapeutic?" [103]. The basic requirements for a surrogate β-cell are the ability to process proinsulin into insulin, low basal insulin secretion and physiologically regulated secretion in response to glucose. Of these criteria, physiologically regulated insulin response is likely to be the biggest challenge.

The work leading to the development of a reproducible and efficient β-cell product is far from finished, although many now feel that is a reachable goal [104]. The time required for *in vitro* differentiation of the final β-cell product will have a large impact on scale-up costs. Estimates of the costs for production are unknown. For small-scale experiments, both 2D and 3D cultures have been used; 3D or suspension cultures may be necessary for scale-up, although this has not yet been shown. Characteristics for QC/release criteria should ideally correlate with outcomes in patients, but will have to be agreed with regulatory authorities [14].

Considerations for cell replacement therapy

Clinical transplantation of cadaveric islets, which includes β-cells, has provided the proof of concept for replacement therapy for diabetes

and is the rationale for developing β-cells from pluripotent stem cells [2,15]. More than 20 years of experience in improving cadaveric islet transplantation has provided insights into the number of islet equivalents required, the site of transplantation, delivery and immunosuppression regimens. FDA guidance criteria exist for islet transplantation. This knowledge base will be utilized in the design of clinical trials for stem cell-derived β-cells.

The requirement for immune protection will affect replacement therapy based on stem cell-derived β-cells [8]. In addition to cell source, the platform technology to protect the β-cells from transplant rejection will be essential to the success of any commercialization strategy. If used to treat T1D, it should be kept in mind that there is evidence of recurrent autoimmunity in whole-organ transplants, even in the face of significant immunosuppression [16].

> *"Clinical translation of stem cell therapies for diabetes requires unraveling the underlying biology of pancreatic development and islet function, and then recapitulating development in a defined, scalable and commercially viable manner."*

Encapsulation for β-cells/islets has been investigated for some years, both at the level of microencapsulation coatings of individual islets and macro devices encompassing a larger cell population [17]. These encapsulation strategies are designed to protect the β-cells from the immune system, allowing engraftment, and nutrient and oxygen exchange. The device should ideally be biologically inert and retrievable. In the context of β-cells derived from stem cells, encapsulation may also prevent the escape of cells that are not fully differentiated, which may produce teratomas. However, the presence of a capsule means that regulatory agencies will be considering a combination cell and device product. As both the scale-up of β-cells and the encapsulation device are still being developed, the possible regulatory complexity increases.

Xenotransplants as an alternative model

The use of xenoislets from pigs might be considered as an alternative or even interim model between the use of human cadaveric donor islets and β-cells derived from human pluripotent stem cells. Indeed, a health-economic analysis of porcine islet xenotransplantation found that porcine islet cell xenotransplantation is cost effective when compared with the standard treatment with insulin and the development of complications [18]. In this case, the possible eligible patient profile would be similar (i.e., those with severe hypoglycemia unawareness) [19]. There would be fewer production issues than with β-cells derived from stem cells. Factors unique to xenotransplant trials that need to be considered include the potential for infection by porcine endogenous retroviruses, and the need for life-long monitoring. Since pig-derived cells have to be protected from attack by the human immune system, the use of encapsulated porcine islets may inform the development of capsules that could also be used for stem cell-derived β-cells.

Conclusion

The benefits in terms of overall health, quality of life and cost savings from improved glucose control make a compelling case for cell replacement therapy for the treatment of diabetes. Although proof of concept for a stem cell-derived pancreatic progenitor cell therapy for diabetes has been achieved in small animal models, a fully functional β-cell *in vitro* has not yet been achieved. Given the solid foundation in basic science and continued interest in developing cell therapies for diabetes from academia, biotech and industry, we look forward to the elucidation of a reproducible, efficient and scalable protocol. A stem cell-based replacement product for diabetes will be the first in its class. There will be significant regulatory hurdles to address, including overcoming teratoma risk, the need for immunosuppression, and designing and obtaining approval for a possible combination product. The potential of stem cell-derived β-cells as treatment is competitive with islet transplantation from cadaveric donors and with xenotransplantation using pig islets.

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