Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Pharmacology

Pluripotent stem cell replacement approaches to treat type 1 diabetes

Silvia Pellegrini[1], Lorenzo Piemonti[1,2] and Valeria Sordi[1]

Stem cells represent a potential candidate for β cell replacement in type 1 diabetes. Pluripotent stem cells are able to differentiate *in vitro* into functional insulin producing cells, that can restore normoglycemia in diabetic mice. Clinical trials with embryonic stem cell-derived pancreatic progenitors are ongoing. Besides, induced pluripotent stem cells offer the chance of personalized cell therapy. So far, transition to the clinic still needs to face critical issues, such as immunogenicity and safety of stem cell derived β cells. To this purpose, new strategies for immunoprotection, including micro and macro-encapsulation, but also gene editing approaches, are being explored.

Addresses
[1] Diabetes Research Institute – IRCCS San Raffaele Scientific Institute, Italy
[2] Vita-Salute San Raffaele University, Italy

Corresponding author: Sordi, Valeria (sordi.valeria@hsr.it)

Current Opinion in Pharmacology 2018, **43**:20–26

This review comes from a themed issue on **Endocrine & metabolic diseases**

Edited by **SJ Persaud**

https://doi.org/10.1016/j.coph.2018.07.007

Introduction

In type 1 diabetes (T1D), insulin producing β cells of the endocrine pancreas are destroyed by an autoimmune attack [1]. Exogenous insulin administration is necessary to control glycemia, but it cannot replace the highly specialized work done by β cells. As a matter of fact, patients with diabetes face two-sided complications: chronic risk of hyper and hypo-glycemia with correlated risks and long term complications due to the toxic effect of hyperglycemia on brain, nerves, vessels and heart [2]. Restoring the functional β cell mass is a possibility; this is an especially attractive case in cell therapy field because only a single cell type is missing and replacement can occur in non-endogenous sites. This has been extensively demonstrated by whole pancreas or pancreatic islet transplantation procedures as β cell replacement frequently results in euglycemia, but even partial function is able to reduce the risk of severe hypoglycemic events and limit the progression of diabetic complications [3,4]. Despite its effectiveness, this therapy is still limited to a small number of patients with T1D, due to the unavailability of sufficient numbers of organs for transplantation and the difficulty of safely controlling rejection and autoimmunity with immunosuppressant drugs.

In this review we will focus on the possibility to use pluripotent stem cells to overcome the limits of islet transplantation and widen the application of cell replacement therapy to a large number of patients with T1D.

Pluripotent stem cells (ESC and iPSC) and ongoing clinical trials

To overcome the hurdles of islet transplantation, scientific community is currently focusing the attention on human pluripotent stem cells (PSC) as a source of functional β-like cells *in vitro*. As a matter of fact, in the field of *in vitro* differentiation from stem cells the most promising results have been achieved by using human embryonic stem cells (ESC) [5] or human induced pluripotent stem cells (iPSC) [6]. PSC are developmentally immature cells that have self-renewal abilities and hold the potential to become virtually any cell type of the body; indeed, PSC can be induced to undergo specific differentiation stages by exposure to defined combinations of stimuli to activate and/or inhibit signaling pathways to mimic, for instance, human pancreas development [7]. At first indeed the pioneering study of Novocell (now ViaCyte Inc.) focused on the discovery of the stimuli able to effectively specify ESC into cells of the definitive endoderm and subsequently in pancreatic progenitor cells up to insulin-producing cells, with an efficiency of 7% [8]. The group then dedicated to the efficient and large-scale production of proliferative and multipotent pancreatic progenitor cells, identified through co-expression of PDX1 and NKX6-1 [9], following the evidence that, upon *in vivo* transplantation in murine models, a portion of these cells will spontaneously maturate into functional β-like cells [10].

This strategy laid the foundation for the beginning of a prospective, multicenter, open-Label, First-in-Human Phase 1/2 clinical trial (ClinicalTrials.gov identifier: NCT02239354) in 2014 conducted by ViaCyte using a macro-encapsulated allogeneic hESC-derived pancreatic progenitor product (known as VC-01) in subjects with T1D. Next, in 2017, a new clinical trial was started (NCT03163511), testing ViaCyte's PEC-Direct product, a new open device allowing direct vascularization of

pancreatic progenitor (PEC-01) cells but that requires immunosuppressant drugs, highlighting the presence of survival issues of encapsulated cells in human. Both these studies are ongoing and will certainly provide soon a great deal of information regarding a safe and effective use of stem cell-derived β cells in humans.

In the last 10 years, in parallel to the research on ESC, the study of induced pluripotent stem cells (iPSC) has increasingly taken the field. IPSC, derived from the genetic reprogramming of adult somatic cells [6], have all the phenotypic/functional characteristics of ESC and several protocols of *in vitro* differentiation of iPSC into pancreatic β cells have been reported with encouraging results [11–13]. Human iPSC can be derived from healthy subjects but also from diabetic patients [14,15••], therefore this kind of approach could lead to an autologous β cell replacement therapy. The translation of human iPSC to the clinical practice is already a reality in other fields [16], as the first clinical study using human iPSC-derived retinal pigment epithelial (RPE) cells was initiated in 2014 in Japan for macular degeneration. The treatment proved to be safe and the transplanted tissue demonstrated engraftment and survival at 1 year from the transplant [17••], but the trial was subsequently put on hold due to the identification of two genetic variants in the iPSC of the second prospective patient [18]. The study was then redesigned and the first patient was treated March 28, 2017 [19]; the strategy switched from an autologous to an allogeneic approach, with iPSC from a bank supplied by Kyoto University's Center for iPS Cell Research and Application.

Finally, two other clinical trials with iPSC have recently been approved: (i) allogeneic iPSC-derived mesenchymal stem cells (MSC) for Graft versus Host Disease (GvHD) in UK and Australia (NCT02923375) conducted by the Australian company Cynata Therapeutics and (ii) in Japan an allogeneic iPSC-based study for ischemic heart disease using sheets of cardiac cells to treat severe heart failure patients (Table 1). These latest approved clinical trials highlight that scientific community, despite the feasibility of clinical development of an autologous hiPSC-derived cell replacement product, is focusing on tuning an allogeneic iPSC therapy to minimize costs, time and quality controls of a personalized approach.

Differentiation efficiency, immunogenicity and safety are by now the main challenges that researchers have to face in order to facilitate the transition of PSC-derived β cells to patients with T1D [20•] (Figure 1).

In vitro and *in vivo* differentiation into β cells: efficacy

As reported, active clinical trials by ViaCyte are intended with ESC-derived pancreatic progenitors, as the efficient terminal *in vitro* differentiation into functional β cells comparable to those of adult human islets is still one of the main critical points. The process of β cell differentiation is orchestrated by fine-tuned transcriptional regulation of genes involved in pancreas development that depends on the type, number and timing of differentiation factors administrated and on the stem cell source and culture conditions. Over the last 10 years, multiple variations have been made to ViaCyte first protocol [8], which was able to give rise to insulin-producing cells albeit non-responsive to glucose stimuli. Modified or improved protocols have been established using combinations of cytokines and small molecules, such as many Fibroblast Growth Factors, Sonic hedgehog pathway inhibitors (KAAD-cyclopamine or SANT-1), Retinoic Acid, Nicotinamide, thyroid hormone, BMP inhibitors, protein kinase C activator (Indolactam V, PdBU), γ-secretase inhibitors or TGFβ pathway inhibitors (Alk5 inhibitor, Dorsomorphin or Noggin) [12,21–23]. In two seminal papers [24,25], a novel and efficient approach to generate *in vitro* 20%–50% insulin (C-peptide)-positive cells from PSC was reported; differentiated cells resulted mono-hormonal and glucose responsive. Upon transplantation into immunocompromised mice, the graft (composed of endocrine and ductal cells) restored glycemia within 2 [24] or 6 weeks [25] after transplantation, a tremendous improvement compared to the 2–3 months period required for cell maturation after transplantation of ESC-derived pancreatic progenitor cells [10]. Similar results were obtained also by transplantation of iPSC-derived β cells, with human insulin detected in the sera of mice before and after glucose administration [13,24], even with iPSC derived from patients with T1D [15••].

The necessity to obtain insulin-positive cells with high efficiency has led, in addition to the identification of new molecules able to increase differentiation efficiency, to

Table 1

Ongoing clinical trials with iPSC

Indication	Derivative	Source of iPSC	Phase	Country	Ref
Macular degeneration	Retinal pigment epithelial (RPE) cells	Autologous	I/II	Japan	[17••]
Macular degeneration	RPE cells	Allogeneic	I/II	Japan	Not found
Sever heart failure	Cardiac cell sheet	Allogeneic	I/II	Japan	Not found
Graft versus host disease	Mesenchymal stromal cells (MSC)	Allogeneic	I/II	Australia, UK	NCT02923375