Curr Diab Rep (2017) 17: 68
DOI 10.1007/s11892-017-0901-4



# Immunological Issues After Stem Cell-Based β Cell Replacement

**Valeria Sordi**[1] · **Silvia Pellegrini**[1] · **Lorenzo Piemonti**[1]

Published online: 19 July 2017


**Abstract**
*Purpose of Review* Islet and pancreas transplantation prove that β cell replacement can cure the glycemic derangements in type 1 diabetes (T1D). Induced pluripotent stem cells (iPSCs) can differentiate into functional insulin-producing cells, able to restore normoglycemia in diabetic animal models. iPSCs in particular can be derived from the somatic cells of a person with T1D. This review aims to clarify if it is possible to transplant autologous iPSC-derived β cells without immunosuppression or which are the alternative approaches.
*Recent Findings* Several lines of evidence show that autologous iPSC and their derivatives can be immune rejected, and this immunogenicity depends on the reprogramming, the type of cells generated, the transplantation site, and the genetic/epigenetic modifications induced by reprogramming and differentiation. Besides, cell replacement in T1D should keep in consideration also the possibility of autoimmune reaction against autologous stem cell-derived β cells.
*Summary* Autologous iPSC-derived β cells could be immunogenic upon transplantation, eliciting both auto and allogeneic immune response. A strategy to protect cells from immune rejection is still needed. This strategy should be efficacious in protecting the grafted cells, but also avoid toxicity and the risk of tumor formation.

This article is part of the Topical Collection on *Immunology, Transplantation, and Regenerative Medicine*

✉ Lorenzo Piemonti
piemonti.lorenzo@hsr.it

[1] Diabetes Research Institute, IRCCS San Raffaele Scientific Institute, Via Olgettina 60, 20132 Milan, Italy

**Keywords** Type 1 diabetes · Induced pluripotent stem cells · Immunogenicity

## Introduction

Pancreatic islet transplantation, in selected patients with medically unstable type 1 diabetes (T1D), was shown to be able to either restore normoglycemia or be efficacious in improving metabolic control and preventing severe hypoglycemia [1]. Despite improvements in pancreas procurement, islet isolation, and immunosuppressive therapy, major scientific and technical challenges remain to be addressed before pancreatic islet transplantation can be widely adopted for the clinical management of T1D; examples include serious side effects from chronic immunosuppression and the insufficient human islet supply from pancreas donation.

One possible solution is the use of stem cells as an unlimited source of functional new β cells. In the last 20 years, the field of regenerative medicine for T1D has expanded tremendously and shifted the attention from adult stem cells (mainly bone marrow-derived hematopoietic and mesenchymal stem cells) to pluripotent stem cells. Indeed, embryonic stem cells (ESC) were the first stem cells to show a viable path to differentiate into insulin-producing cells, with an in vitro protocol, developed by Novocell (today Viacyte Inc., http://viacyte.com), mimicking the steps of fetal pancreas development [2–5]. Other groups confirmed ESC capacity to differentiate into β cells and to restore normoglycemia in animal models of diabetes [6, 7]. In particular, the successful experience of ViaCyte led to the first clinical trial of cell replacement therapy for diabetes with pancreatic precursor cells derived from ESC (NCT02239354). However, ethical concerns related to the procurement of human embryos and immune rejection are major hurdles to the clinical application of ESC.

In 2006, Yamanaka's group developed the alternative to ESC, the induced pluripotent stem cell (iPSC) [8, 9]. iPSCs, obtained through ectopic expression of four reprogramming factors in terminally differentiated somatic cells, provide an unprecedented opportunity to model human disease, re-understand the basic biology such as development and differentiation, identify new therapeutic targets, and test new therapies. In the field of β cell replacement, iPSCs were reprogrammed from patients with T1D, differentiated into functional β cells, and were able to restore normoglycemia when transplanted in humanized diabetic mice [6, 7, 10•]. Thus, the breakthrough of iPSC technology has raised the possibility that patient-specific iPSC may become a renewable source of autologous cells for cell therapy without the concern of immune rejection led by major histocompatibility complex restriction. However, the immunogenicity of autologous human iPSC-derived cells is not taken for granted and is at the center of an intense scientific debate [11].

## Immunogenicity of Autologous iPSC Is Controversial

### iPSCs Elicit Immune Response

Recently, many publications support the possibility that autologous iPSC and their derivatives elicit an immune response. The first evidence to dampen the hopes on autologous iPSC came from a publication in Nature in 2011: Zhao and colleagues, using a teratoma transplantation model, showed that cells derived from mouse iPSCs were rejected in syngeneic recipients [12]. In this paper, it was reported that some but not all cells derived from mouse iPSCs could be immunogenic and that the immune rejection response was T cell dependent, in fact the immune rejection was totally blocked in Rag knockout recipients. Furthermore, two genes, Hormad1 and Zg16, were abnormally expressed in iPSC-derived teratoma and directly contributed to the immunogenicity of iPSC derivatives, supporting the existence of primed T cells in the mice harboring the iPSC-derived teratomas.

The variables potentially involved in immunogenicity of iPSC upon transplantation are multiple: the reprogramming technique, the potential genetic and epigenetic abnormalities induced by reprogramming, the expression of neoantigens during differentiation into mature cells, and also the transplantation site.

The contribution of the reprogramming technique to immunogenicity still needs to be understood. One of the first pieces of evidence was that, comparing iPSC derived from the same mouse fibroblasts with two different reprogramming strategies, cells transduced with retroviral vectors were highly immunogenic while those reprogrammed with episomal non-integrating vectors could be immunogenic in syngeneic recipients but their overall immunogenicity was significantly lower [12]. Another study instead reported no difference between lentiviral and episomal methods [13] and lastly another study overturned these findings: lines of iPSC reprogrammed with a non-viral plasmid were associated with a stronger immune rejection upon transplantation in syngeneic recipients, when compared to lentiviral reprogramming [14].

Furthermore, it is widely accepted that reprogramming itself can induce both genetic and epigenetic defects in iPSC [15–20] and these defects could be associated with a more or less immunogenic behavior. For instance, substantial hypermethylation or hypomethylation of cytosine-phosphate-guanine island shores were found in nine human iPSC lines as compared to their parental fibroblasts [15]. In another study, it was reported that hiPSC acquire genetic modifications in addition to epigenetic modifications [19]: 22 iPSC lines reprogrammed using five different methods each contained an average of five protein-coding point mutations in the regions sampled (an estimated six protein-coding point mutations per exome). The majority of these mutations were non-synonymous, non-sense, or splice variants, and were enriched in genes mutated or having causative effects in cancers. At least half of these reprogramming-associated mutations preexisted in fibroblast progenitors at low frequencies, whereas the rest occurred during or after reprogramming. In addition, it was shown that early-passage iPSCs retain a transient epigenetic memory of their somatic cells of origin, which manifests as differential gene expression and altered differentiation capacity for the first passages [18]. Accordingly, low-passage iPSCs derived by reprogramming of adult murine tissues harbor residual DNA methylation signature characteristic of their somatic tissue of origin, which favor their differentiation along lineages related to the donor cell, while restricting alternative cell fates [17]. These data are extremely important for differentiation protocols but also focus the attention on the epigenetic modifications occurring to cultured iPSC, modifications which may render the cells susceptible to immune recognition and rejection. In fact, epigenetic memory of the reprogrammed cell type could result in aberrant surface antigen expression when iPSCs are differentiated into other cell lineages.

Finally, Todorova and colleagues demonstrated that the immune response towards iPSC-derived transplanted cells is dependent also on the immune environment of the transplantation site. In their study, syngeneic iPSC and their differentiated hepatocytes survived under the kidney capsule but were immune rejected when transplanted subcutaneously or intramuscularly. The authors concluded that kidney graft tolerance was due to a lack of functional antigen-presenting cells in the microenvironment, in fact when mature dendritic cells were co-transplanted, iPSC-derived grafts were rejected [21].

### iPSCs Do Not Elicit Immune Response

In recent experiences, however, negligible or limited immunogenicity of transplanted cells differentiated from iPSC was reported. Several research groups differentiated iPSC into different germ layers or cells, transplanted those cells into syngeneic hosts, and evaluated the immunogenicity of iPSC-derived cells. One group examined the immunogenicity of mouse iPSC and ESC derivatives and observed no differences in the rate of success of syngeneic transplantation when skin and bone marrow cells derived from iPSCs or ESCs were compared. Moreover, they reported limited or no immune responses, including T cell infiltration, for tissues derived from both iPSCs or ESCs when transplanted subcutaneously, and no increase in the expression of the immunogenicity-causing genes Hormad1 and Zg16 in teratoma tissues [22].

In another study, murine iPSCs were differentiated into embryoid bodies (EB) or representative cell types of the three embryonic germ layers and their immunogenicity was assessed in vitro and after transplantation into syngeneic recipients. No evidence of increased T cell proliferation in vitro, rejection of syngeneic iPSC-derived differentiated cells after transplantation, or an antigen-specific secondary immune response were observed. Also little evidence of an immune response to undifferentiated syngeneic iPSC was found [13]. Finally, Almeida and colleagues found that autologous iPSC-derived cell grafts evoked self-tolerance mechanisms [14]. In their study, iPSC-derived endothelial cells transplanted subcutaneously in murine models exhibited long-term survival in vivo and prompted a tolerogenic immune response characterized by elevated IL-10 expression. In contrast, undifferentiated iPSCs were rejected in syngeneic hosts, eliciting a very different immune response with high lymphocytic infiltration and elevated IFN-γ, granzyme-B, and perforin intragraft. These data suggest the possibility that the differentiation of iPSCs results in a loss of immunogenicity and leads to the induction of tolerance, despite expected antigen expression differences between iPSC-derived versus original somatic cells. Thus, some data seem to demonstrate that differentiated cells derived from syngeneic iPSCs are not rejected after transplantation and support the idea that iPSC-derived cells could be applied for cell replacement therapy without eliciting immune rejection.

A study on human cells indicated that also the type of cells generated from iPSCs may influence the susceptibility to the immune response: using a humanized mouse model reconstituted with a functional human immune system, it was reported that autologous human iPSC-derived smooth muscle cells appeared to be highly immunogenic, while iPSC-derived retinal pigment epithelial cells (RPE) were immune tolerated even in non-ocular locations. This differential immunogenicity was likely due to an abnormal expression of immunogenic antigens in the muscle but not RPE cells. These preclinical findings supported the feasibility of developing hiPSC-derived RPE for treating macular degeneration [23]. In a clinical study in Japan, the first to use iPSC-derived cells in humans, RPE cells differentiated from autologous iPSC were transplanted in a patient with age-related macular degeneration: at 1 year after surgery, the transplanted sheet remained intact, without signs of immune rejection; however, the experimental procedure was not performed on further individuals when serious spontaneous mutations were identified in the next patient's iPSC [24••].

To sum up, it is possible that reprogramming, differentiation, and implant site may render iPSC and their derivatives more or less susceptible to immune rejection. Genetic and epigenetic defects seem to be directly or indirectly associated to the immunogenicity of iPSC derivatives. This immunogenicity, although weaker compared to the allograft, can elicit serious rejection responses, leading to the complete rejection of the transplanted tissue. All these studies suggest that (1) extensive genetic/epigenetic screening should become a standard procedure to ensure iPSC safety before clinical use and (2) immunogenicity of cells derived from patient-specific iPSC should be evaluated before any clinic application of these autologous cells into patients.

## Immunogenic or Not, Autoimmunity Is Still There

Even if autologous iPSC proved not immunogenic, it should be considered that β cell replacement in a patient with T1D meets another hurdle, the autoimmunity against islet antigens, an additional threat to transplanted β cells. It has been demonstrated in fact that autoimmune reaction is per se sufficient to destroy new β cells, as demonstrated by (i) T1D transfer between siblings as a consequence of bone marrow transplantation [25, 26]; (ii) T1D development in a case of islet autotransplantation following total pancreatectomy, within the first year after transplantation, resulting in complete loss of β cell function, where the patient had no evidence of presurgical β cell autoimmunity [27]; (iii) equal contribution of auto and alloimmune reactions to β cell survival after islet transplantation [28]. Accordingly, it is possible that the exposure to autologous islet antigen will trigger the immune system to attack transplanted iPSC-derived β cells. In our experience, insulin-producing cells generated from iPSC express autoantigens like GAD65 (glutamic acid decarboxylase), ZnT8 (zinc transporter 8), Tspan7 (tetraspannin 7), and IA-2 (insulinoma antigen 2) (unpublished observations).

In the natural history of T1D, the timing between the initial trigger and clinical onset, marking the destruction of a significant proportion of β cells, is not known. The duration is thought to be highly variable, ranging from months to decades, as T1D can manifest at a wide age

range [29] and most patients are seropositive for one or more autoantibodies very early in life [30]. Accordingly, the recurrence of diabetes after pancreas transplantation shows similarities to chronic rejection and does not appear as a rapid loss of graft function [31]. Thus, if autoimmunity recurrence causes a slow and gradual death of iPSC-derived β cells, this may allow the cell graft to function for several years and the possibility to repeat the procedure. However, the recurrent exposure to β cell antigens may lead to sensitization and an accelerated immune response to subsequent grafts.

In evaluating the use of autologous patient-derived iPSC for cell therapy of T1D, the risk of an allogeneic rejection and the induction of an autoimmune response, both capable of destroying the graft, should therefore be considered. This risk, together with the time and the cost required for personalized iPSC generation and differentiation into β cells, leads to consider the use of allogeneic iPSC, combined to a strategy to overcome immune rejection in a transplant recipient with T1D.

## Approaches to Shield Transplanted Cells from the Immune System

If allogeneic pluripotent stem cells are planned to be used, efficacious approaches to protect the new β cells will be required. Escape from the immune system can be obtained acting on different targets (Fig. 1).

### Act on the Recipient: Immunosuppression

Theoretically, the immunogenicity of transplanted cells could be addressed by conventional immunosuppressive drugs. The common immunosuppressive protocols used in clinical islet transplantation include induction therapy with either ATG or IL-2 receptor monoclonal antibody, followed by maintenance treatment including tacrolimus and sirolimus [32]. However, due to their toxicity and the associated risk of malignancy, they are not a desirable option for autologous iPSC-derived cell therapies [33].

Reducing such toxic immunosuppressive regimens is the most imposing clinical objective in transplantation field in general, which will ultimately have implications to the utility of iPSC-derived tissues in regenerative medicine. Possible solutions to these problems include the development of mild immunosuppressive regimens (e.g., monoclonal antibodies targeting NK cells and/or T cell subsets) sufficient to induce tolerance to autologous iPSC-derived cells or a highly specific immunosuppressive therapy for iPSC-derived differentiated cells. However, any level of immunosuppression would likely increase the risk that rare pluripotent cells in an iPSC-derived population could form teratomas.

### Act on the iPSC: Haplobank

The opportunity to preselect donors for the generation of iPSC lines opens up an opportunity not possible with human ESC lines, which is to create a bank of cell lines specifically chosen to match the widest possible number of recipients worldwide

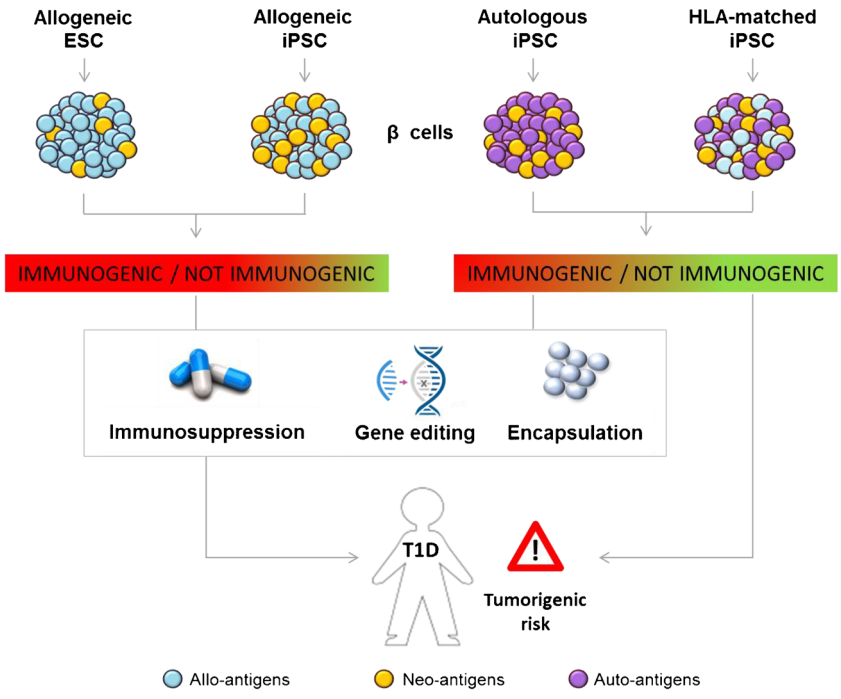


**Fig. 1** Schematic representation of the immunological issues raised by the use of β cells derived from pluripotent stem cells. These new β cells can elicit an immune response towards alloantigens if HLA mismatched, towards islet-specific autoantigens if autologous or in any case towards neoantigens, induced by cell reprogramming and differentiation. Immunosuppression, gene editing, and micro/macroencapsulation are strategies to avoid immune rejection. Finally, the risk of tumorigenicity must be addressed. *ESC* embryonic stem cells, *iPSC* induced pluripotent stem cells

[34]. The creation of a bank of allogeneic clinical good manufacturing practices (GMP) cell lines raises the issues of how such a bank, or network of banks, could be established and, in particular, how immune incompatibility can best be managed. HLA incompatibility between donor cells, tissues, or organs and the recipient gives rise to rejection. One would therefore wish to match as many HLA class I and II loci as is practical in order to minimize this risk and the degree of immunosuppression required. Over 9000 alleles have been identified within the HLA system, which makes this difficult to achieve, but linkage disequilibrium within the HLA region raises the possibility that individuals exist who are homozygous for common HLA haplotypes. Such individuals will produce stem cell lines that are HLA compatible with a high proportion of the population. Creation of a "haplobank" of iPSC lines homozygous for a range of HLAs, representative of different geographical populations and ethnic groups could simplify HLA matching, providing matches for a reasonable percentage of a target population. According to one estimate, an iPSC bank from 150 selected homozygous HLA-typed volunteers could match 93% of the UK population with a minimal requirement for immunosuppression [34]. Similarly, due to their limited diversity, as few as 50 such lines could potentially match 90% of the Japanese population [35]. However, more diverse populations will require more lines [36]. Surely, the diversity of HLA types means that it is highly unlikely that any single regional or national bank could contain sufficient cell lines to cover all people within their population base, and therefore, international collaboration between cell banks will be the key to equity of access. This is very similar to the situation of hematopoietic stem cell registries and cord blood banks, whereby international collaboration enables access to a much larger pool of HLA-typed potential donors than that provided by individual countries [37, 38]. Many researchers involved in the development of GMP-grade iPSC lines have joined in a Global Alliance for iPSC Therapies [38, 39], with the aim to establish a global GMP iPSC haplobank, with shared standards of donor selection and screening, iPSC manufacture, and regulatory compliance.

## Act on the iPSC: Gene Editing

Recently, much effort has been drawn to the generation of universally compatible pluripotent stem cells (mainly ESC) by silencing or deleting HLA or genes essential for HLA expression or function and by expressing genes encoding immunosuppressive molecules [40]. In 2011, the first attempt to create a universally compatible hESC line knocking down class I HLA using HLA I RNA interference and intrabody technology was published [41, 42]. Transplantation of engineered stem cells resulted in decreased T cell activation, antibody production, and graft-infiltrating immune cells, while graft survival was prolonged.

More recently, breakthroughs in targeted genome editing by artificial endonucleases have made it possible to precisely modify and engineer PSC genome; in particular, some groups reported the knockout of HLA [27] and genes essential for HLA expression, including β-2-microglobulin (B2M) for class I HLA [42–45] and class II MHC transactivator (CIITA) for class II HLA [46] expression. Moreover, hypoimmunogenic hESC have been obtained also by ectopically expressing a modified form of HLA-G [47] or through the disruption of T cell costimulatory pathways with cytotoxic T lymphocyte antigen 4 fused with immunoglobulin (CTLA4-Ig) and simultaneous activation of the T cell inhibitory pathway with programmed death ligand-1 (PD-L1) [48].

Therefore, the main strategies to prevent immune reaction by gene editing are the following:

1) Inhibiting the expression of HLA-I on the surface of iPSC by silencing or deleting its heavy chain or the light-chain B2M. iPSC without HLA-I on their surface become invisible to the T cells of the host, thus not eliciting an immune response.
2) Inhibiting expression of HLA-II by silencing or deleting CIITA, a transcriptional regulator of HLA-II. In this way, iPSC will not express HLA-II avoiding HLA-II-dependent immune rejection. In addition, CIITA can also regulate the transcription of other genes, including HLA-I.
3) Expressing immunosuppressive molecules HLA-G or -E. Ectopic expression of non-classical HLAs on iPSC-derived cells can inhibit activation of NK cells by interacting with the NK inhibitory receptors ILT2 and KIRs, repress the proliferation of activated T cells by interacting with ILT2 or TCR, and induce the apoptosis of activated or effector CD8+ T cell by interacting with CD8.
4) Expressing immunosuppressive molecules like CTLA4-Ig and PD-L1. Ectopic CTLA4-Ig can competitively bind to CD80/86 expressed on dendritic cells to block the activation of T cells, which then leads to T cell anergy or tolerance. In addition, ectopic PD-L1 can interact with PD-1 expressed on effector T cells to reduce their activity.

In either case, the main concern regarding universally compatible stem cells is tumorigenicity; in fact, gene-edited iPSCs gain the ability to escape immune surveillance, and strategies to address this concern are needed. An approach that addresses simultaneously the two issue, immunogenicity and tumorigenicity, is the use of cell encapsulation.

## Separating the Graft: Micro and Macroencapsulation

One way of bypassing allo- and autoimmunity, in fact, is to physically isolate the cells within semi-permeable solid

membranes or scaffolds allowing diffusion of glucose, nutrients, and insulin but not of larger molecules, cells, or antibodies [49, 50]. This system has the theoretical advantage of precluding the need for immunosuppression and allowing the use of various cell types including β cells derived from stem cell sources; moreover, it offers the possibility to co-transplant different cell types and/or co-localize cells and drugs. It also sequesters the cells, thus avoiding dissemination of potentially tumorigenic derivatives of pluripotent cells.

In the islet transplantation field, two main approaches have been tested in the last decades [51]: micro and macroencapsulation. The fundamental distinction is a matter of scale: the microencapsulation approach uses many microscale capsules (400 to 800 μm in diameter) with each one containing one or a few islets, improving surface-to-volume ratios and exchange of nutrients and molecules [52]. However, as islets are individually encapsulated, thousands of microcapsules are required for each transplant. Macrocapsules instead may house a large number of cells or islets and have been developed in different shapes like tubes [53] or sheets [54]. These larger devices allow for greater control over membrane parameters compared to microcapsules [55] and one of the main advantages is the ease of implantation and removal, if needed. On the other hand, the permeability of the macrocapsule is reduced because of the thicker membrane and the chemistry and mechanical properties of materials that are typically associated with these devices can lead to a foreign body response and subsequent device failure from fibrotic encapsulation [56].

The material used for encapsulation has to be biocompatible and promote the survival of the cells, inert to avoid triggering a host tissue response and need to permit bi-directional diffusion of nutrients. The most used biomaterial for islet encapsulation is alginate, but also others like polysulfon, polyethylene glycol, or polycaprolactone have been explored [57]. The first assessment of alginate encapsulated islet functionality was performed in 1980 with the omental transplantation of microencapsulated islets that succeeded in achieving normoglycemia in diabetic rats for 2 weeks [58]. The results of the first clinical trial of microencapsulated islet transplantation in 6 patients with T1D without immunosuppression were published in 2006 and 2011 by Calafiore's group in Italy. In this trial, after intraperitoneal transplantation of alginate encapsulated islets in T1D patients, blood glucose levels and exogenous insulin requirements decreases, but they slightly but progressively increased at 24 months after transplant. Although the metabolic outcomes highlighted loss of function, immunological studies did not reveal an antibody-mediated immune response against islets [59, 60]. Due to the shortage of organ donors, many clinical and preclinical encapsulation studies are now focused on the use of xenogenic (mostly porcine) or stem cell-derived β cells. For ESC or iPSC-derived insulin-producing cells in particular, the scientific community is focusing on the development of a macrodevice to simultaneously protect the graft from the immune attack, prevent the escape of residual undifferentiated cell, and allow removal. In 1990s, Baxter Healthcare developed one of the first modern prototype of macrodevice consisting of two membranes sealed at all sides with a loading port [61], that was then continuously modified to produce a clinically relevant format, called TheraCyte®. Its development was supported by ViaCyte. In 2014, preclinical data supported the potential of human ESC-derived pancreatic progenitor cells of ViaCyte to differentiate into β cells when loaded into TheraCyte device [62, 63]. This device was further tested in different preclinical transplant settings to assess its immunoisolation potential [64–66]. Finally, in 2014, ViaCyte started a phase 1/2 clinical trial (NCT02239354) to test the safety, tolerability, and efficacy of TheraCyte Device (final version called "Encaptra® drug delivery system") combined with ESC-derived pancreatic progenitor cells (PEC-01) implanted under the skin of patients with T1D. Viacyte is actively recruiting patients in the USA and Canada and results are expected to be released soon. This clinical study is actually at the forefront in the field of diabetes as it represents the first-in-man study both for the use of ESC-derived insulin-producing cells as alternative to pancreatic islets and for the use of biocompatible immunoisolating device.

## Conclusions

Replacing the endocrine function of the pancreas with a cell therapy is possible, as demonstrated by islet transplantation in T1D. iPSCs can be derived from patients with T1D and in vitro differentiated into patient-specific β cells. However, these new β cells (i) are at risk of rejection, both allogeneic and autoimmune, upon transplantation; (ii) require a huge investment in terms of time and money for their derivation; and (iii) may have tumorigenic potential. Despite these obstacles, we believe that the potential usefulness of these cells is enormous and we that substantial efforts should be made to bring these cells to the clinic. These efforts include new advances in immunosuppressive drugs, cell engineering, and micro/macroencapsulation strategies.

**Acknowledgments** Research was supported by grant from the European Commission H2020 681070.

**Compliance with Ethical Standards**

**Conflict of Interest** Valeria Sordi, Silvia Pellegrini, and Lorenzo Piemonti declare that they have no conflict of interest.

**Human and Animal Rights and Informed Consent** This article does not contain any studies with human or animal subjects performed by any of the authors.

## References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance


1. Hering BJ, Clarke WR, Bridges ND, et al. (2016) Phase 3 trial of transplantation of human islets in type 1 diabetes complicated by severe hypoglycemia. Diabetes Care dc151988.
2. D'Amour KA, Agulnick AD, Eliazer S, Kelly OG, Kroon E, Baetge EE. Efficient differentiation of human embryonic stem cells to definitive endoderm. Nat Biotechnol. 2005;23:1534–41.
3. D'Amour KA, Bang AG, Eliazer S, Kelly OG, Agulnick AD, Smart NG, et al. Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. Nat Biotechnol. 2006;24:1392–401.
4. Kroon E, Martinson LA, Kadoya K, et al. Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. Nat Biotechnol. 2008;26:443–52.
5. Schulz TC, Young HY, Agulnick AD, et al. A scalable system for production of functional pancreatic progenitors from human embryonic stem cells. PLoS One. 2012;7:e37004.
6. Rezania A, Bruin J, Xu J, et al. Reversal of diabetes with insulin-producing cells derived in vitro from human pluripotent stem cells. Nat Biotechnol. 2014; doi:10.1038/nbt.3033.
7. Pagliuca FW, Millman JR, Gürtler M, Segel M, Van Dervort A, Ryu JH, et al. Generation of functional human pancreatic β cells in vitro. Cell. 2014;159:428–39.
8. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006;126:663–76.
9. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007;131:861–72.
10. • Millman JR, Xie C, Van Dervort A, Gürtler M, Pagliuca FW, Melton DA. Generation of stem cell-derived b-cells from patients with type 1 diabetes. Nat Commun. 2016; doi:10.1038/ncomms11463. **This paper shows the possibility to obtain β cells from iPSC derived from patients with T1D.**
11. Cao J, Li X, Lu X, Zhang C, Yu H, Zhao T. Cells derived from iPSC can be immunogenic—yes or no? Protein Cell. 2014;5:1–3.
12. Zhao T, Zhang Z-N, Rong Z, Xu Y. Immunogenicity of induced pluripotent stem cells. Nature. 2011;474:212–5.
13. Guha P, Morgan JW, Mostoslavsky G, Rodrigues NP, Boyd AS. Lack of immune response to differentiated cells derived from syngeneic induced pluripotent stem cells. Cell Stem Cell. 2013;12:407–12.
14. De Almeida P, Meyer EH, Kooreman NG, et al. Transplanted terminally differentiated induced pluripotent stem cells are accepted by immune mechanisms similar to self-tolerance. Nat Commun. 2014;5:3903.
15. Doi A, Park I-H, Wen B, et al. Differential methylation of tissue- and cancer-specific CpG island shores distinguishes human induced pluripotent stem cells, embryonic stem cells and fibroblasts. Nat Genet. 2009;41:1350–3.
16. Bar-Nur O, Russ HA, Efrat S, Benvenisty N. Epigenetic memory and preferential lineage-specific differentiation in induced pluripotent stem cells derived from human pancreatic islet beta cells. Cell Stem Cell. 2011;9:17–23.
17. Kim K, Doi A, Wen B, et al. Epigenetic memory in induced pluripotent stem cells. Nature. 2010;467:285–90.
18. Polo JM, Liu S, Figueroa ME, et al. Cell type of origin influences the molecular and functional properties of mouse induced pluripotent stem cells. Nat Biotechnol. 2010;28:848–55.
19. Gore A, Li Z, Fung H-L, et al. Somatic coding mutations in human induced pluripotent stem cells. Nature. 2011;471:63–7.
20. Lister R, Pelizzola M, Kida YS, et al. Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells. Nature. 2011;471:68–73.
21. Todorova D, Kim J, Hamzeinejad S, He J, Xu Y. Brief report: immune microenvironment determines the immunogenicity of induced pluripotent stem cell derivatives. Stem Cells. 2016;34:510–5.
22. Araki R, Uda M, Hoki Y, et al. Negligible immunogenicity of terminally differentiated cells derived from induced pluripotent or embryonic stem cells. Nature. 2013;494:100–4.
23. Zhao T, Zhang Z, Westenskow PD, et al. Humanized mice reveal differential immunogenicity of cells derived from autologous induced pluripotent stem cells. Cell Stem Cell. 2015;17:353–9.
24. •• Mandai M, Watanabe A, Kurimoto Y, et al. Autologous induced stem-cell–derived retinal cells for macular degeneration. N Engl J Med. 2017;376:1038–46. **The first in human clinical trial with iPSC-derived cells.**
25. Lampeter EF, Homberg M, Quabeck K, Schaefer UW, Wernet P, Bertrams J, et al. Transfer of insulin-dependent diabetes between HLA-identical siblings by bone marrow transplantation. Lancet. 1993;341:1243–4.
26. Sutherland DE, Goetz FC, Sibley RK. Recurrence of disease in pancreas transplants. Diabetes. 1989;38(Suppl 1):85–7.
27. Bellin MD, Moran A, Wilhelm JJ, O'Brien TD, Gottlieb PA, Yu L, et al. Development of autoimmune-mediated β cell failure after total pancreatectomy with autologous islet transplantation. Am J Transplant. 2015;15:1991–4.
28. Piemonti L, Everly MJ, Maffi P, et al. Alloantibody and autoantibody monitoring predicts islet transplantation outcome in human type 1 diabetes. Diabetes. 2013;62:1656–64.
29. Van Belle TL, Coppieters KT, von Herrath MG. Type 1 diabetes: etiology, immunology, and therapeutic strategies. Physiol Rev. 2011;91:79–118.
30. Ziegler AG, Rewers M, Simell O, et al. Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. JAMA. 2013;309:2473.
31. Burke GW, Vendrame F, Pileggi A, Ciancio G, Reijonen H, Pugliese A, et al. Recurrence of autoimmunity following pancreas transplantation. Curr Diab Rep. 2011;11:413–9.
32. Barton FB, Rickels MR, Alejandro R, et al. Improvement in outcomes of clinical islet transplantation: 1999-2010. Diabetes Care. 2012;35:1436–45.
33. Johnson JD, Ao Z, Ao P, et al. Different effects of FK506, rapamycin, and mycophenolate mofetil on glucose-stimulated insulin release and apoptosis in human islets. Cell Transplant. 2009;18:833–45.
34. Taylor CJ, Peacock S, Chaudhry AN, Bradley JA, Bolton EM. Generating an iPSC bank for HLA-matched tissue transplantation based on known donor and recipient HLA types. Cell Stem Cell. 2012;11:147–52.
35. Nakatsuji N, Nakajima F, Tokunaga K. HLA-haplotype banking and iPS cells. Nat Biotechnol. 2008;26:739–40.
36. Gourraud P-A, Gilson L, Girard M, Peschanski M. The role of human leukocyte antigen matching in the development of multiethnic "haplobank" of induced pluripotent stem cell lines. Stem Cells. 2012;30:180–6.
37. Barry J, Hyllner J, Stacey G, Taylor CJ, Turner M. Setting up a haplobank: issues and solutions. Curr stem cell reports. 2015;1:110–7.
38. Turner M, Leslie S, Martin NG, Peschanski M, Rao M, Taylor CJ, et al. Toward the development of a global induced pluripotent stem cell library. Cell Stem Cell. 2013;13:382–4.

39. Wilmut I, Leslie S, Martin NG, Peschanski M, Rao M, Trounson A, et al. Development of a global network of induced pluripotent stem cell haplobanks. Regen Med. 2015;10:235–8.
40. Zheng D, Wang X, Xu R-H. Concise review: one stone for multiple birds: generating universally compatible human embryonic stem cells. Stem Cells. 2016;34:2269–75.
41. Deuse T, Seifert M, Phillips N, et al. Immunobiology of naïve and genetically modified HLA-class-I-knockdown human embryonic stem cells. J Cell Sci. 2011;124:3029–37.
42. Deuse T, Seifert M, Phillips N, et al. Human leukocyte antigen I knockdown human embryonic stem cells induce host ignorance and achieve prolonged xenogeneic survival. Circulation. 2011;124:S3–9.
43. Wang D, Quan Y, Yan Q, Morales JE, Wetsel RA. Targeted disruption of the $\beta_2$-microglobulin gene minimizes the immunogenicity of human embryonic stem cells. Stem Cells Transl Med. 2015;4:1234–45.
44. Feng Q, Shabrani N, Thon JN, et al. Scalable generation of universal platelets from human induced pluripotent stem cells. Stem Cell Reports. 2014;3:817–31.
45. Karabekian Z, Ding H, Stybayeva G, et al. HLA class I depleted hESC as a source of hypoimmunogenic cells for tissue engineering applications. Tissue Eng Part A. 2015;21:2559–71.
46. Chen H, Li Y, Lin X, Cui D, Cui C, Li H, et al. Functional disruption of human leukocyte antigen II in human embryonic stem cell. Biol Res. 2015;48:59.
47. Zhao L, Teklemariam T, Hantash BM. Heterelogous expression of mutated HLA-G decreases immunogenicity of human embryonic stem cells and their epidermal derivatives. Stem Cell Res. 2014;13:342–54.
48. Rong Z, Wang M, Hu Z, et al. An effective approach to prevent immune rejection of human ESC-derived allografts. Cell Stem Cell. 2014;14:121–30.
49. De Vos P, Spasojevic M, Faas MM. Treatment of diabetes with encapsulated islets. Adv Exp Med Biol. 2010;670:38–53.
50. Vaithilingam V, Tuch BE. Islet transplantation and encapsulation: an update on recent developments. Rev Diabet Stud. 2011;8:51–67.
51. Scharp DW, Marchetti P. Encapsulated islets for diabetes therapy: history, current progress, and critical issues requiring solution. Adv Drug Deliv Rev. 2014;67-68:35–73.
52. Desai TA, West T, Cohen M, Boiarski T, Rampersaud A. Nanoporous microsystems for islet cell replacement. Adv Drug Deliv Rev. 2004;56:1661–73.
53. Hayashi H. Application of a novel B cell line MIN6 to a mesh-reinforced polyvinyl alcohol hydrogel tube and three-layer agarose microcapsules: an in vitro study. Cell Transplant. 1996;5:S65–9.
54. Lee JI, Nishimura R, Sakai H, Sasaki N, Kenmochi T. A newly developed immunoisolated bioartificial pancreas with cell sheet engineering. Cell Transplant. 2008;17:51–9.
55. Desai T, Shea LD. Advances in islet encapsulation technologies. Nat Rev Drug Discov. 2016; doi:10.1038/nrd.2016.232.
56. Ward WK. A review of the foreign-body response to subcutaneously-implanted devices: the role of macrophages and cytokines in biofouling and fibrosis. J Diabetes Sci Technol. 2008;2:768–77.
57. Johannesson B, Sui L, Freytes DO, Creusot RJ, Egli D. Toward beta cell replacement for diabetes. EMBO J. 2015;34:841–55.
58. Lim F, Sun AM. Microencapsulated islets as bioartificial endocrine pancreas. Science. 1980;210:908–10.
59. Calafiore R, Basta G, Luca G, Lemmi A, Montanucci MP, Calabrese G, et al. Microencapsulated pancreatic islet allografts into nonimmunosuppressed patients with type 1 diabetes: first two cases. Diabetes Care. 2006;29:137–8.
60. Basta G, Montanucci P, Luca G, Boselli C, Noya G, Barbaro B, et al. Long-term metabolic and immunological follow-up of nonimmunosuppressed patients with type 1 diabetes treated with microencapsulated islet allografts: four cases. Diabetes Care. 2011;34:2406–9.
61. Brauker JH, Carr-Brendel VE, Martinson LA, Crudele J, Johnston WD, Johnson RC. Neovascularization of synthetic membranes directed by membrane microarchitecture. J Biomed Mater Res. 1995;29:1517–24.
62. Motté E, Szepessy E, Suenens K, Stangé G, Bomans M, Jacobs-Tulleneers-Thevissen D, et al. Composition and function of macroencapsulated human embryonic stem cell-derived implants: comparison with clinical human islet cell grafts. Am J Physiol Endocrinol Metab. 2014;307:E838–46.
63. Bruin JE, Rezania A, Xu J, Narayan K, Fox JK, O'Neil JJ, et al. Maturation and function of human embryonic stem cell-derived pancreatic progenitors in macroencapsulation devices following transplant into mice. Diabetologia. 2013;56:1987–98.
64. Yang Z, Chen M, Fialkow LB, Ellett JD, Wu R, Nadler JL. Survival of pancreatic islet xenografts in NOD mice with the theracyte device. Transplant Proc. 2002;34:3349–50.
65. Kumagai-Braesch M, Jacobson S, Mori H, Jia X, Takahashi T, Wernerson A, et al. The TheraCyte™ device protects against islet allograft rejection in immunized hosts. Cell Transplant. 2013;22:1137–46.
66. Boettler T, Schneider D, Cheng Y, Kadoya K, Brandon EP, Martinson L, et al. Pancreatic tissue transplanted in TheraCyte™ encapsulation devices is protected and prevents hyperglycemia in a mouse model of immune-mediated diabetes. Cell Transplant. 2016;25:609–14.