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Review

Human Stem Cell Therapy for the Cure of Type 1 Diabetes Mellitus (T1D): A Hurdle Course between Lights and Shadows

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Abstract: Background: T1D is a severe metabolic disorder due to selective autoimmune pancreatic islet β-cell killing, which results in complete abrogation of endogenous insulin secretion. The affected patients, once the disease is clinically overt, must immediately undertake insulin supplementation according to intensive therapy regimens to prevent the onset of acute and chronic complications, some of them potentially lethal. Replacement of the destroyed β-cells with fresh and vital pancreatic endocrine tissue, either of the whole organ or isolated islets transplantation, started a few decades ago with progressively encouraging results, although exogenous insulin withdrawal was obtained in a minor cohort of the treated patients. The restricted availability of donor organs coupled with general immunosuppression treatment of recipients to avoid graft immune rejection may, at least partially, explain the limited success achieved by these procedures. **Results:** The introduction of pluripotent stem cells (either of human embryonic origin or adult cells genetically induced to pluripotency) that can be differentiated toward insulin secretory β-like cells could provide an indefinite resource for insulin-producing cells (IPCs). **Conclusions:** Because the use of human embryos may encounter ethical problems, employment of adult multipotent mesenchymal stem cells (MSCs) extracted from several tissues may represent an alternative option. MSCs are associated with strong immunoregulatory properties that can alter early stages of β-cell-directed autoimmunity in T1D, other than holding the potential to differentiate themselves into β-like cells. Lights and shadows of these new strategies for the potential cure of T1D and their advancement state are reviewed.

Keywords: immunoprotection; regeneration; insulin; blood glucose

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1. Introduction

Insulin, a fundamental hormone secreted by β-cells located in the Islets of Langerhans of the endocrine pancreas, tightly regulates energy metabolism in the body. In fact, insulin is key to glucose entry in target tissues like muscle and fat, in addition to inhibiting glucose output from the liver [1,2].

As a consequence of insulin action, circulating free fatty acids and glucose are reduced, which greatly contributes to the maintenance of glucose homeostasis under physiologic conditions [3].

Obviously, in T1D, where an autoimmune disease process selectively kills β-cells [4], shut-off of insulin production and its finely tuned secretory patterns coincides with an uncontrolled increase in blood glucose levels, often leading to acute (i.e., diabetic ketoacidosis, a medical emergency mainly related to free fatty acids massive release in the absence of endogenous insulin) and over time, chronic complications that severely compromise life expectancy of the affected patients [5,6].

The only therapeutic option, once T1D becomes clinically overt, consists of administering exogenous insulin to the patients, usually by multiple daily injections comprising

long- and short-acting insulin molecules or by means of an insulin infusion pump, based on semi-automatic insulin delivery, according to specific infusion algorithms [7,8].

The aim here is to keep blood glucose levels as controlled as possible by blending together insulin dose, carbohydrate counting, and physical exercise so that the so-called "time in range" of the resulting glycemia and the glycated hemoglobin values are optimal. In this way, the likelihood of developing time-related secondary complications such as diabetic nephropathy, cardiovascular disease, neuropathy, and retinopathy is minimized [9].

Unfortunately, although exogenous insulin is a life-saving medicine, it does not represent a cure, but only a treatment for type 1 diabetes, as per Fredrick Banting's (who discovered insulin in 1921) own statement during his Nobel Prize lecture in 1925. An effective cure for T1D should include either replacement of the diseased/dead β-cells upon elimination of the β-cell directed autoimmune destruction with viable and functional cells or in situ regeneration of β-cells from pancreatic progenitor cells [10]. In other words, we need to think of a possible substitution of exogenous insulin delivery with organ-specific cell and molecular therapy [11–13].

This is not an easy task because of the many and complex (other than still only partially elucidated) underlying mechanisms associated with this specific aim.

2. Cell Therapy for T1D: The Past

Over four decades ago, the first attempts to fulfill the task of physically replacing the destroyed β-cells in T1D consisted of transplanting whole pancreatic organs retrieved from cadaveric donors into T1D patients, according to orthotopic or heterotopic surgical procedures under general recipients' immunosuppression. In front of clinical and substantial success accomplished in many of the over 40,000 whole pancreatic grafts performed since 1966, as shown by attained normoglycemia and suspension of exogenous insulin administration, the grafting procedure, whether conducted alone or in parallel or after a previous kidney transplant intervention (in typical patients with insulin-dependent diabetes and terminal renal insufficiency, who often related to diabetic nephropathy), has always been burdened with severe morbidity and mortality because of its undoubtful invasiveness, in addition to the imminent and adverse effects of the ineludible general pharmacologic immunosuppression administered to recipients [14,15].

Starting from the 1980s, the use of islets of Langerhans isolated and purified from human donor pancreases introduced a much less invasive procedure. In fact, the islets, or cell clusters accounting for restricted graft volume, were infused into the liver via the portal vein system under local anesthesia and colonized the portal triads where they would engraft and initiate insulin production/release. The success of this procedure was limited until 2000 when the Edmonton Group applied the Edmonton protocol for islet transplantation that resulted in the accomplishment of normoglycemia and exogenous insulin withdrawal in 7/7 treated patients with T1D for one year: changes in improved quality of the grafted islets and use of more selective immunosuppressive agents had made this success possible [16]. Nevertheless, in the following years, a series of technical problems emerged, such as activation of the coagulation cascade (instant blood-mediated inflammatory reaction or IBMIR) during the islet infusion process, e.g., reported in the study by Naziruddin et al., who demonstrated that levels of thrombin–anti-thrombin III complex (TAT), and C-peptide increased significantly during islet infusion, indicating activation of coagulation and inflammation [17], with consequential need to refine the peri-transplant pharmacologic therapy. The real issue that has contended the widespread success of islet transplantation as a cure for T1D remains the restricted availability of cadaveric human donor pancreases, also bearing in mind that not always a single pancreas might yield enough isolated islets able to reverse hyperglycemia in one recipient. Hence, this procedure is actually still employed by a few selected centers worldwide, where the islets are grafted into the liver or, more recently, in the omentum of patients that already carry or undergo, in parallel, kidney transplant, or alone in patients with brittle diabetes or uncontrollable hypoglycemia unawareness and elevated glycated hemoglobin levels, despite intensive

insulin therapy regimens, and always under general immunosuppression [18]. A potential alternative to general recipients' immunosuppression came from the use of alginate-based microcapsules that, by enveloping individual islets, constitute a selectively permeable barrier between the islet graft and the host's immune system. In fact, microcapsules, while granting oxygen/nutrient supply to the enveloped cells, interdict access to humoral and cellular mediators of the host's immune system, with no need for the recipient's general immunosuppression [19,20]. Initial human clinical trials of microencapsulated islet grafts showed preliminary interesting results, although they ultimately were unable to provide full and sustained control of T1D [21].

3. Cell Therapy for T1D: The Present Toward Future

3.1. Cell Biology and Molecular Biology Mechanisms Involved in Insulin Gene Expression and Post-Translation Modification

Crucial for the field of cellular therapy for type 1 diabetes mellitus has been the study of the molecular and cellular biology mechanisms underlying insulin synthesis and secretion. As reported in the work of Kaneto et al., PDX-1 is essential for the embryonic development of the pancreas, where it contributes to β-cell differentiation, while in adults, it contributes to the maintenance of mature β-cell function [22]. Another important pancreas β-cell specific transcription factor is MafA, a potent activator of insulin gene transcription. Studies on PDX-1 and MafA allowed us to hypothesize that overexpression of these transcription factors in non-β-cells could induce the expression of β-cell-related genes, including insulin, possibly offering a novel approach for cellular therapy of diabetes [22]. Arcidiacono et al.'s study clarified the cooperation between PDX1 and MafA by introducing the HMGA1 protein into the complex insulin transcription mechanism. This protein, a nuclear architectural factor that organizes chromatin structures and regulates gene expression by forming multi-protein complexes called "enhanceosomes", physically interacts with PDX-1 and MafA, helping regulation of insulin gene expression and beta-cell function in response to glucose concentration [23]. Further insights into insulin secretion have come from understanding post-translational modifications (PTMs), with special regard to how PTMs regulate gene transcription (via phosphorylation, acetylation, ubiquitination, and O-GlcNAcylation) and insulin hormone secretion by multiple signaling pathways, such as SUMOylation and palmitoylation [24].

3.2. Types of Stem Cells (SC) Potentially Eligible for Management of T1D

As mentioned above, a main bottleneck to pancreatic islet graft-based cell therapy and likely a final cure of T1D remains the scarce availability of insulin-producing cells, immune rejection problems aside. A great hint to try overcoming this basic hurdle came from the idea of recapitulating embryogenesis of the pancreas and detecting the original developmental pathways that result in the generation of the endoderm-origin endocrine cells, namely, β-cells, α-cells, δ-cells, γ-cells, and PP cells that are in charge of production of insulin, glucagon, somatostatin, ghrelin, or pancreatic polypeptide [12].

Of course, β-cells represent the main focus here. In general, stem cells can be classified as follows (Figure 1). Each stem cell generates an identical cell and a cell that undertakes a chain of differentiation steps toward finite somatic or germinal cells. Since stem cells generate the embryonic leaflets from where all 200 types of human cells derive, stem cell therapy gained progressive attention as a treatment option for several diseases. For our purpose, the availability of insulin-producing cells derived from stem cells, upon application of precise differentiation protocols, could permit access to a possibly indefinite number of cells for treating T1D. The main stem cell types possibly suitable for treating T1D are the following:

Pluripotent Stem Cells

1. Embryonic stem cells (ESCs): because these cells form the inner mass of the blastocyst, they are pluripotent and originate all possible differentiated cell types, including pancreatic endocrine β-cells. Many protocols have been reported in the literature,

detailing passages required to obtain a stable somatic cell type. A major problem with this research line lies in ethical issues that, in many countries, prevent the use of human embryos [25];

2. Induced pluripotent stem cells (iPSCs): this approach started from the initial discovery from Yamanaka. He showed that starting from differentiated somatic cells (i.e., fibroblasts, blood cells, etc.), upon their transaction with basic stem genes (*Sox2, c-Myc, OCT4, Klf4*), it was possible to revert them into an undifferentiated, pluripotent state, thereby exposing the obtained pluripotent cell elements to differentiation protocols toward finite somatic cells. Apparently easy, in theory, this approach is very difficult and expensive, depending upon techniques used for maturation [26–29].

Multipotent Stem Cells

1. Mesenchymal stem cells (or stromal cells): these mesoderm-derived cells are adult; hence, they are usable in countries where employment of ESCs is prohibited by law, are of mesodermal origin, retrieved from many tissue sources like bone marrow, adipose tissue, post-natal umbilical cord Wharton Jelly (WJ), placenta, etc. Their vocational orientation is to generate mesodermal tissues/organs with special regard to bone, cartilage, and heart cells, although transdifferentiation pathways toward the production of other tissue types are possible [30–32].

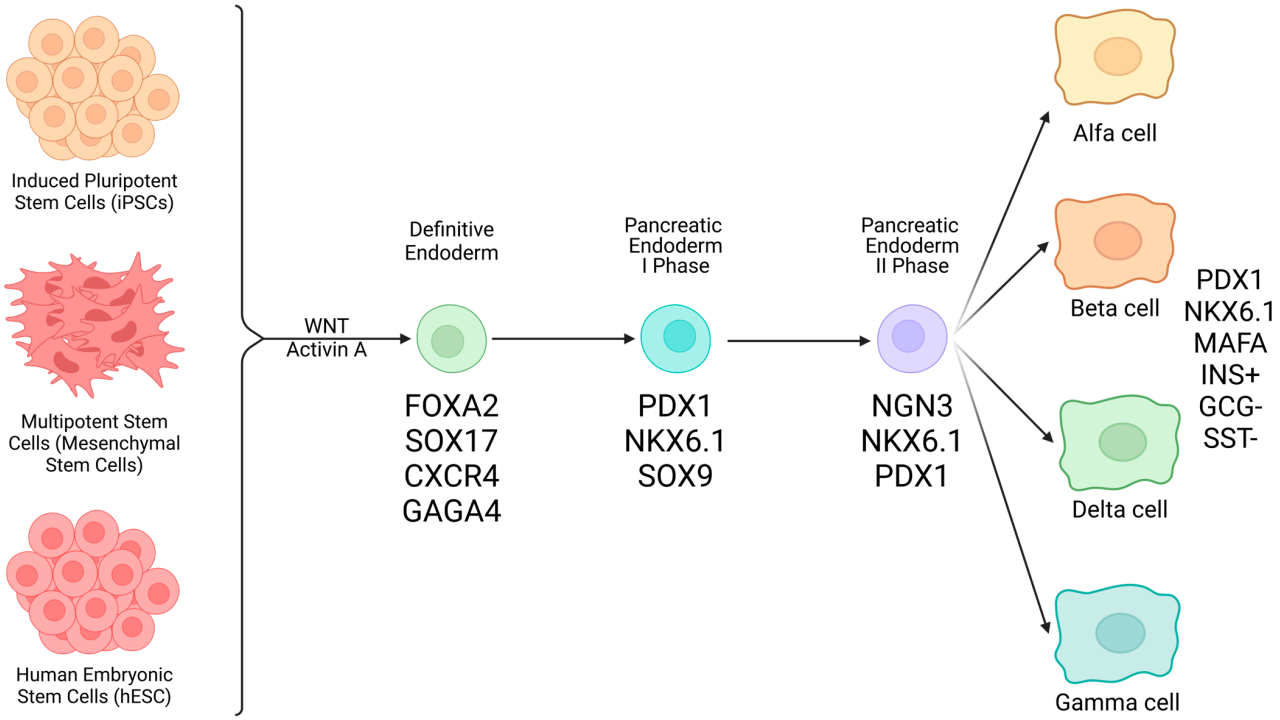

Figure 1. Schematic differentiation of SCs to insulin−producing β−cells. The molecules of key developmental stages of pancreatic islet β−cells are illustrated. Created with BioRender.com.

3.3. Generation of β-like Cells Forms Human SC

1. hESCs: These cells are part of the inner cell mass of the blastocyst and are then associated with pluripotency or the possibility of giving origin to all cells and tissues of the human body. Keller, in 1995, first described the in vitro differentiation of human ESCs into β-like cells [33]. Since then, many authors challenged protocols to produce β-like cells, especially in rodents, with partial results. D'Amour first described the first detailed method to create ESC-derived progenitor cells that contained the PDX-1 master gene [34]. Nevertheless, results from studies of different authors showed evident variability with regard to yield in true β-like cells versus other cell types,

including teratoma cells with all associated risks, clinical application-wise. One of the latest seven-step differentiation protocols [13,35] led to the generation of cells that exhibited MAFA, a marker of mature β-cells, and showed glucose-coupled insulin secretory responsiveness. Clusters of these cells implanted in mice were associated with controlling hyperglycemia [35,36];

2. iPSCs: These cells are pluripotent, similar to ESCs, although they originally derive from adult somatic cells. Theoretically, once functionally viable, iPSCs could be used within an autologous graft system where no immune consequences would occur. Of course, meticulous differentiation of iPSCs into β-cell-like elements requires sequential steps consisting of cell exposure to different signaling stimuli in an attempt to recapitulate embryogenesis of the endocrine pancreas [13,35–37]. From definitive endoderm, through intermediate steps (pancreatic endocrine progenitors, etc.), final β-cell-like cells expressing β-cell markers like NKX6.1, PDX1, and NEUROD1 are obtained [37,38]. This process is not easy or straightforward, with the possible contamination from non-endocrine and possibly tumorigenic cells, as mentioned above. Several protocols have been developed to contrast the presence of contaminating cells in the final preparation with variable results, including the employment of small molecules interfering with wrong developmental pathways [35–39]. Future results will confirm the viability and efficacy of these approaches;
3. MSCs: These cells, as a substantial difference from the former two described cell types, are adult, multipotent stem cells that would not incur any ethical restrictions. These, as said, are associated with a ban that many countries apply to the use of human embryonic material. MSCs are usually derived from extra-embryonic tissue sources, like placenta, amniotic fluid, umbilical cord WJ, bone marrow (stroma), adipose tissue, dental pulp, liver, and bone. With special regard to WJ-derived MSCs, they do not express hematopoietic markers like CD34 and CD45 [30], and because of their specific anatomical situation at the maternal-fetal interface, they possess powerful immunoregulatory properties associated with the production of a number of cytokines and molecular factors. These, overall, inhibit activation of Natural Killer, T cells (Tc), B cells [40], Macrophages, and dendritic cells, as well as hypoxia-induced apoptosis. These favorable properties help contrast autoimmune-directed β-cell destruction in T1D and are coupled with the absence of induced teratogenesis. MSCs are also known to release exosomes containing active molecules that could be exploited for cell therapy. MSCs do not express MHC Class II antigens, another property that reinforces their intrinsic immune privilege. In terms of direct differentiation of MSCs into β-like cells by use of molecules like activin A, EGF, Nicotinamide, and others, no univocal results have been so far obtained. Hence, mechanistically, the beneficial pathways orchestrated by MSCs in T1D consist of the following:
    (a) potential differentiation into β-like/insulin-producing cells (IPCs);
    (b) induction of native β-cells regeneration;
    (c) immunoregulatory and anti-apoptotic effects.

As for (a), especially Wharton Jelly-derived MSCs, because of their higher expression of PDX-1 and C-peptide mRNA, have been deemed to possibly support transdifferentiation into β-like cells [31].

However, experimental evidence has not confirmed so far that the detected insulin activity associated with MSC graft in animal models clearly depends on MSC differentiation. As for (b), a restricted number of experiments in rodents showed that infusion of human MSCs resulted in the appearance of neo-generated β-cells, but this observation needs to be confirmed. As for (c), these MSC properties have been demonstrated by several laboratories and are based on the ability of MSCs to inhibit Tc responses to mitogens, inhibit dendritic cell differentiation, and inhibit B cell proliferation. In particular, WJ-derived MSCs specifically are associated with increased production of anti-inflammatory cytokines (i.e., TNFα, TFGβ), the soluble HLA-G5, and upregulation of Bcl2 with potentiation of anti-apoptotic effects.

Some trials have been conducted in patients with T1D who have been receiving an e.v. infusion of MSCs, either intra-portally or systemically, and using different MSC sources. Major functional criteria to prove/disprove MSC therapy function were HbA1c levels, plasma glues, C-peptide response to stimulation, and eventual reduction in daily exogenous insulin dosage [41,42].

In front of anecdotal and no statistically proven results, the majority of the conducted trials did not show evidence of clinical relevance, while further pre-clinical trials are likely warranted [43].

3.4. Gene Editing

Among the latest research trends for the treatment of diabetes mellitus type 1 is CRISPR-Cas9 technology. CRISPR-Cas9 can correct genetic variants that cause diabetes and, at the same time, improve the differentiation of autologous stem cells, thereby allowing for the differentiation of pancreatic β-cells protected from the immune system. Protection of the cells by the immune system is achieved by editing HLA genes, while gene editing with CRISPR-Cas9 can improve the efficiency of stem cell differentiation into functional β-cells [44,45]. Despite the great potential of this technique and the attention paid by the scientific community to its use, the genetic editing carried out by CRISPR/Cas9 is not riskless, and, in particular:

- Incorrectly engineered cell products could behave like a 'Trojan horse' that will induce, after transplantation, possible tumorigenicity of modified β-cells, cell suicides, altered function of the surrounding pre-existing genes;
- DNA double-strand breaks caused by CRISPR/Cas9 may stimulate DNA repair processes that could lead to unwanted insertions or deletions;

In light of these issues, this technique deserves an in-depth critical revision to minimize the risks associated with genome editing and ensure the safety of cellular products [46].

4. Hurdles to Clinical Application of Stem Cells to the Cure of T1D

Immunity. Obviously, stem cell-derived β-like cell/IPC grafts cannot elude the host's immune-surveillance system. Hence, anti-immune rejection measures must be taken to avoid cell graft-directed immune destruction (Figure 2). Moreover, T1D is an autoimmune disorder with selective β-cell killing. In general, several strategies may be pursued to prevent immunity:

(a) general recipients' immunosuppression;
(b) cell graft immune-isolation;
(c) gene editing and molecular engineering.

As for (a), this approach is usually employed for classic pancreatic and islet cell transplantation. Though efficient, especially according to the latest pharmacologic protocols, general immunosuppression is not devoid of severe side effects that may compromise the function of different organs and systems in the body [47]. As for (b), several products based on microencapsulation or macroencapsulation of cells/cell clusters have been devised over the years by a number of centers worldwide, including our own [48]. Microencapsulation, based on a wide array of biomaterials—usually hydrogels associated with favorable compatibility properties, such as alginates, agarose, cellulose, collagen, and others— has been associated with a high acceptance rate upon transplant due to their similarity to natural ECM [48,49]. However, difficulties in long-term oxygen/nutrient supply to the embodied cells were often due to the inadequacy of the selected graft site and difficulty in graft retrieval, which made the macrodevices the preferred method for immunoprotection for stem cell grafts in pilot clinical trials.

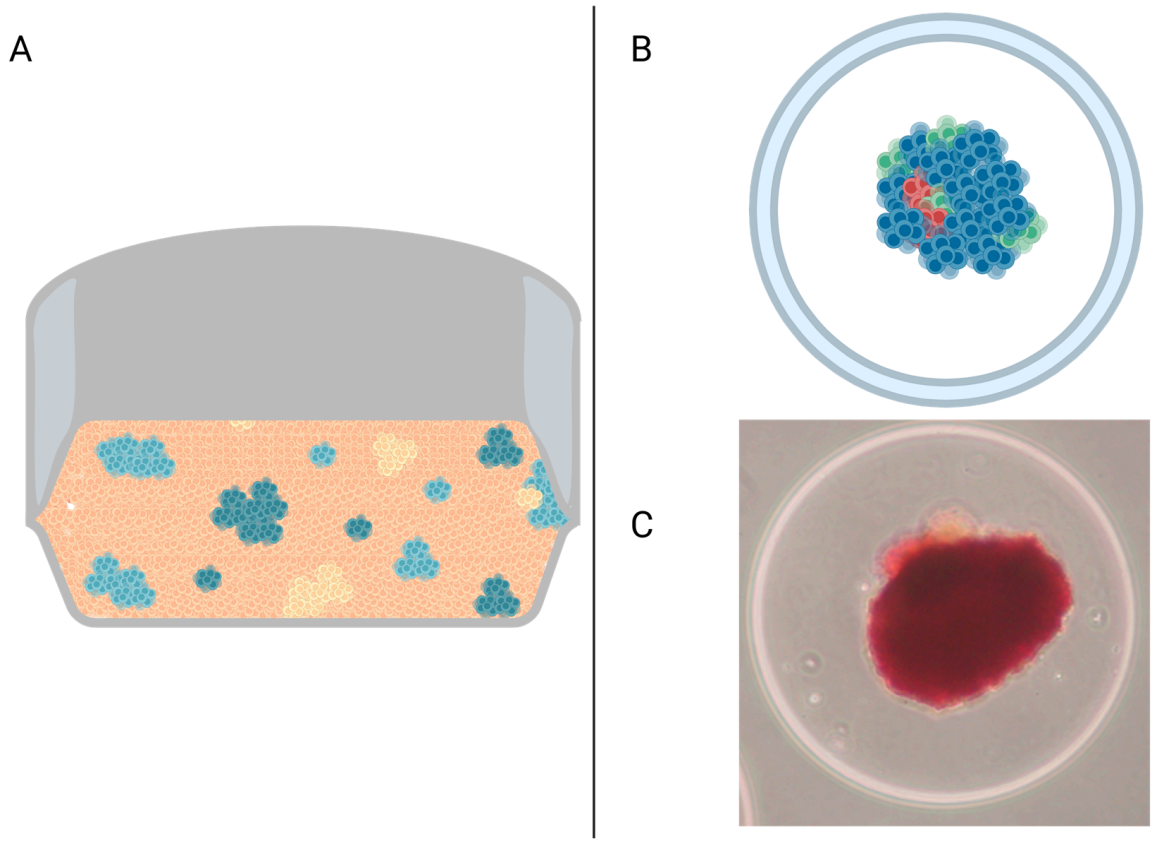

Figure 2. Schematic (**A**,**B**) representation of macrodevices/microcapsules containing human pluripotent stem cells and actual micrograph (**C**) of microcapsules containing human islet cells. Created with BioRender.com.

Macrodevices possibly offer less selective permeability compared to microcapsules and are more prone to elicit fibrotic tissue overgrowth. This is why work is in progress to use refined materials, like nanofibers, that couple immune-isolating properties with stimulation of a capillary network around the device to enhance blood/nutrient supply to the embodied cells and access to graft sites like the subcutaneous tissue that is notoriously associated with low-oxygen tension [50]. Finally, the immune-isolation approach may benefit from the co-encapsulation of cell strains or the addition of molecules that provide additional immunoprotection to the main cell product or the stem cells/IPCs. As for (c), although micro-/macrocapsules may effectively counteract if fabricated properly, they still may not prevent reduced nutrient supply and fibrosis [50]. Another option, although not always easy to apply, could consist of deleting antigen-presenting proteins, like HLA, from pluripotent stem cells or trying to magnify the activity of immune checkpoint inhibitors (i.e., PDL1, etc.). Engineering human pluripotent stem cells could also result in the production of anti-inflammatory and tolerogenic molecules that facilitate cell engraftment [51,52].

Graft site. With regard to naked cells, and particularly pancreatic islets for the treatment of T1D, grafting procedures have been performed, in the majority of cases, in the liver. In fact, by cannulation of the portal vein system, the islets reach the portal triads, where they can easily engraft and start insulin production. Aside from the ineludible need for the treated patients to undertake general immunosuppression, the portal infusion system may be adversely affected by technical flaws, like bleeding, local inflammatory response, and local activation of the coagulation system. As briefly touched above, subcutaneous tissue, in front of important advantages, like easy accessibility for grafting and eventual graft retrieval procedures, hence great risk reduction as compared to the portal vein delivery system, is adversely affected by the lack of vascularization that is typical of this site, with

consequential lack of oxygen/nutrient supply. A possible approach to counteracting this anatomic limit has been studied for many years in pre-clinical trials. Typically, a hollow fiber with a rod is implanted in the subcutaneous tissue to create a foreign body tissue reaction with a lot of surrounding capillary growth. Thereafter, the rod was removed, leaving a pre-vascularized bed where the islets were lodged. Lights and shadows were associated with this strategy. Today, the use of very selected biomaterials has revived attention and interest in this graft site for human pluripotent stem cells. Another possible graft site that is actively being pursued in several schools worldwide, initially for islets but possibly extendable to stem cells, is the omentum. The clear advantage associated with this site is a dense vasculature that provides sufficient oxygen and nutrient supply. Another advantage is that the omentum can be reached by less invasive and usually safe laparoscopy, where an omental pouch is either created artificially or the cell suspension is injected in the virtual omental cavity. Some groups performing islet transplantation in T1D diabetic recipients are engaged in grafting in this site, either in North America or Europe [53,54].

Teratoma formation risk. If viral vectors (i.e., retroviral or lentiviral) are used (as it commonly happens) to deliver gene constructs in the human pluripotent stem cells to guide their differentiation toward definitive endoderm and following stages, insertional mutagenesis may occur, with adverse consequences on the cells transcriptome and potential development of malignant cells. Moreover, properly differentiated pluripotent stem cells can still be contaminated by impure cells that are undifferentiated and can evolve to teratoma formation [55,56].

This outcome has been demonstrated in mice grafted with impure pluripotent stem cells only partially differentiated into definitive endoderm. Potential ways out from this stringent problem could consist of either (a) identifying cell surface molecules that may help select pancreatic progenitors from unwanted cells (i.e., by transcriptomic, proteomic analysis, or single cell mRNA sequencing) or (b) eliminating impure cells by targeting stem cells' specific antigens by use of ad hoc antibodies that result in deletion of undifferentiated, possibly dangerous cells. Refined but still preliminary approaches pursue the idea of eliminating dangerous cells by insertion of suicide genes by gene editing technologies [57–59].

5. Platforms for the Potential Clinical Application of Human Pluripotent Stem Cells

Continuous progress in pluripotent stem cell differentiation into endocrine progenitor cells has permitted the initiation of early pilot clinical trials in diabetic recipients (Table 1). Everything started in 2014 when Viacyte Inc. of San Diego, CA, USA, inaugurated a clinical trial (NCT02239354) to assess the safety and efficacy of a device (VC-01) incorporating human embryonic origin pancreatic endoderm cells. The encapsulation device, once grafted, would protect the cells from the host's immune reaction. Unfortunately, VC-01, upon subcutaneous graft, did not translate into success due to fibrotic overgrowth of the device, possibly related to insufficient oxygen/nutrient supply. Subsequently, ViaCyte developed VC-02, where the device-seeded cells were in direct contact with the vasculature. However, since the device was not immunoprotective, the recipients undertook general immunosuppression. In the subsequent years, ViaCyte started additional clinical trials with VC-02 in patients with T1D (NCT03163511) [60]. The preliminary outcome of this multi-center (phase I/II) clinical trial indicated that the product was safe in spite of general recipients' immunosuppression, with no malignant cell development. While the grafted devices were associated with cell survival throughout 26 weeks of transplant, and the embodied cells differentiated enough through insulin production, as proven by C-peptide secretion in response to stimulation, no patients ever reached insulin independence. Possibly, the insufficient functionally mature β-cell fraction embodied in the device was responsible for this outcome. In collaboration with CRISPR Therapeutics, ViaCyte has initiated another clinical trial using hypoimmune stem cell-derived endocrine progenitors by the above-mentioned gene-editing technologies. An important point to make is the following: although the initial cells progressively differentiated in the device upon in vivo

transplant, it took a great deal of time to obtain at least partially functionally competent cells. Moreover, β-like cells constituted only a minor fraction of the endocrine cell mass: whether this preliminary outcome may depend on the higher oxygen demand of β-cells compared to other endocrine cells in a system where vascular supply is anyway limited is the subject of actual investigation. Whether the solution to this problem would be to use fully differentiated β-cells is yet to be proven. For this purpose, Vertex Pharmaceuticals initiated a phase I/II clinical trial in 2021 (NCT04786262) using human embryonic-derived fully differentiated β-cells (VX-880), grafted intraportally in patients with T1D and brittle blood glucose control. At the IPITA/IXA/CTRMS joint Congress held in San Diego, CA, in October 2023, Vertex communicated that 2/16 grafted patients had gradually lowered their exogenous insulin daily dose until insulin withdrawal after 270 days of transplant. Two remitters died subsequently, but the FDA declared in Spring 2024 that their deaths were unrelated to treatment despite the fact that the patients were generally immunosuppressed. To try to surmount this hurdle, Vertex announced another trial approved by the FDA (NCT05791201), where the cells were encased within an immunoprotective macrodevice and implanted subcutaneously, with no recipients' immunosuppression.

Table 1. Main clinical trials of human pluripotent stem cells in patients with T1D.

Company	Code of Trial	Type of Device	Population	Results
Viacyte Inc. (San Diego)	NCT02239354 (Phase I/II clinical trial)	Macrodevice (VC-01) incorporating human embryonic origin pancreatic endoderm cells.	Two cohorts to evaluate safety, tolerability, and efficacy in patients with Type 1 Diabetes Mellitus.	Fibrotic overgrowth of the device, possibly related to insufficient oxygen/nutrient supply.
Viacyte Inc. (San Diego)	NCT03163511 (Phase I/II clinical trial)	Device-seeded cells were in direct contact with the vasculature; the device was not immune-protective, so the subjects needed immune suppression.	Subjects with Type 1 Diabetes and hypoglycemia unawareness; cells viable throughout two years.	The product was safe; no malignant cells developed; cells survived throughout 26 weeks of transplant. Embodied cells differentiated enough and were able to produce insulin, as measured by C-peptide secretion in response to stimulation. No patients ever achieved insulin independence [60].
CRISPR Therapeutics	NCT05210530 (Phase I clinical trial)	"Low immunity" stem cell-derived endocrine progenitors (editing technologies).	Safety and Tolerability of VCTX210A combination product in subjects with Type 1 Diabetes Mellitus.	Cells took a great deal of time to become at least partially functional, competent cells; β-like cells constituted only a minor fraction of total endocrine cell mass.
Vertex Pharmaceuticals	NCT04786262 (Phase I/II clinical trial)	Embryonic-derived fully differentiated β-cells (VX-880), grafted intraportally.	Safety, tolerability, and efficacy of VX-880 infusion in the liver in patients with Type 1 Diabetes mellitus (T1D), impaired awareness of hypoglycemia (IAH), and severe hypoglycemia.	2/16 grafted patients had gradually lowered their exogenous insulin daily dose until insulin withdrawal was obtained after 270 days of transplant. These two remitters died later, but FDA declared that their death was unrelated to the treatment despite the fact that the patients were on pharmacologic immunosuppression.
Vertex Pharmaceuticals	NCT05791201 (Phase I/II clinical trial)	Cells encased within an immune-protective macro-device, implanted subcutaneously.	Safety, tolerability, and efficacy of VX-264 in participants with type 1 diabetes (T1D).	In progress.

6. Critique and Outlook

The restricted availability of human donor pancreases has considerably hampered the progress of islet transplantation into clinical trials in patients with T1D. Hence, the availability of potentially unlimited sources of IPCs could help solve at least part of the problem, bearing in mind that islet graft-directed immune destruction required other

strategies than general recipients' immunosuppression. The introduction of human stem cells, such as IPCs or β-like cells, has changed the future perspectives for cell and molecular therapy of T1D. Whether hESC, hiPSC, or MSCs are possibly used to fulfill the task of replacing the destroyed β-cells and restoring insulin production and secretion, a new era has started, looking at the ongoing, still pilot, clinical trials. In spite of this possible "Copernican revolution" in the strives to cure T1D, some limits with stem cell therapy are evident and require special attention, should the success of this approach be desirable:

1. hPSC/hiPSC differentiation process is lengthy since it may require months in vivo and is not always associated with final pure β-like cells: this is a double-faceted problem in terms of insufficient functional β-cell mass and risk for the development of teratomas. Consequently, complex and time-consuming procedures should be applied to accomplish a purer β-cell fraction out of the total differentiated cells;
2. MSCs are still difficult to differentiate into β-like cells in reasonable yield. Because of strong MSC regulatory properties, their use should be addressed to interrupt the T1D disease process at an early stage of the β-cell-directed immune attack when the residual β-cell mass is still sufficient to avoid exogenous insulin supplementation;
3. General immunosuppression of the recipients to grant the survival of grafted hPSC/hiPSC should be avoided. Immunoprotection devices, in terms of either macrodevices or microcapsules, are the easiest way to go;
4. More complex immune-engineering technologies, such as CRISP/Cas9, could be used to alter the immunogeneicity and functionalities of the grafted cells. However, further studies and clinical trials are needed to minimize the risks related to gene-editing technologies.

In conclusion, stem cell therapy represents a new era for the cell and molecular therapy of T1D, although more pre-clinical and pilot clinical trials are necessary before we can safely apply this new strategy to humans suffering from this devastating disease.

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