■ R E V I E W

Are stem cells a cure for diabetes?

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A B S T R A C T

With the already heightened demand placed on organ donation, stem cell therapy has become a
tantalizing idea to provide glucose-responsive insulin-producing cells to Type 1 diabetic patients
as an alternative to islet transplantation. Multiple groups have developed varied approaches to
create a population of cells with the appropriate characteristics. Both adult and embryonic stem
cells have received an enormous amount of attention as possible sources of insulin-produc-
ing cells. Although adult stem cells lack the pluripotent nature of their embryonic counterparts,
they appear to avoid the ethical debate that has centred around the latter. This may limit the
eventual application of embryonic stem cells, which have already shown promise in early mouse
models. One must also consider the potential of stem cells to form teratomas, a complication
which would prove devastating in an immunologically compromised transplant recipient. The
present review looks at the progress to date in both the adult and embryonic stem cells fields as
potential treatments for diabetes. We also consider some of the limitations of stem cell therapy
and the potential complications that may develop with their use.

INTRODUCTION

Diabetes mellitus is a devastating disease that, according
to the WHO (World Health Organization), is expected to
affect the lives of 380 million people by the year 2025.
It is also estimated that 5 % of all deaths in the world
are caused by diabetes; a number which will increase
by 50 % in the next 10 years. TIDM (Type 1 diabetes)
is a disease characterized by autoimmune destruction of
pancreatic β-cells, whereas T2DM (Type 2 diabetes) is
due to systemic insulin resistance and inadequate insulin
production by β-cells. In both cases, inadequate glucose
control leads to both micro- and macro-vascular com-
plications that account for the morbidity and mortality
associated with the disease. Clinical trials have already
shown that tight glycaemic control with an intensive
insulin regimen can reduce the risk of developing these
complications [1,2]. However, even with the use of

insulin, most patients with T1DM are unable to maintain
their blood glucose levels in the normal range at all times.
These patients are also prone to dangerous episodes of
hypoglycaemia, a barrier to effective diabetes treatment.
These issues have led physicians and researchers to look
for other means of controlling glucose levels.

In 1966, the first pancreas transplant was carried out
by Kelly and co-workers [3]. Since then, more than 25 000
pancreas transplants have been conducted worldwide,
with 1-year graft survival rates in the range of 77 % for
pancreas transplants alone [4]. However, due to risks
relating to the substantial surgical procedure, pancreas
transplantation is only suitable for a select subgroup,
usually those undergoing simultaneous or previous renal
replacement therapy. Therefore alternative approaches
are actively needed. This led to the introduction of islet
transplantation [5,6]. Now, instead of transplanting the
whole pancreas, the insulin-producing islets themselves

could be transplanted, avoiding the need for a major laparotomy. With further refinements, including the Ricordi method for islet extraction [7], the Edmonton Protocol [8] and more recent refinements, islet transplantation became a viable option for the treatment of T1DM, but requires potent immunosuppression to facilitate graft survival. Initial results were outstanding with 80 % of recipients achieving insulin independence at the 1-year point; however, most patients needed to be maintained on a small dose of insulin by the years 3–5 [9,10]. However, more impressive was that these patients achieved better glucose control with the avoidance of hypoglycaemic events and a substantially improved quality of life [11–13]. These results showed that a cure for diabetes is possible through replenishment of the β-cell mass. That being said, islet transplantation has its limitations, including a limited source of tissue and the need for chronic immunosuppression. The availability of islet cells from brain-dead donors is severely limited; there are only 7000 such donors/year in the U.S.A. [14]. The possibility of using living donors has been explored in Japan, but this does not offer a truly practical approach, as the donor could also be at risk of developing diabetes and has to undergo potentially risky surgery [15,16]. Others have explored the use of alternative animal sources, including pig islet xenografts (neonatal porcine islets) [17]. Pigs are an ideal islet source as they are physiologically similar to humans, breed rapidly and produce large litters. In addition, neonatal islets have the potential benefit of proliferation. However, as with other xenografts, there is an enhanced immune response to them; a situation which may be avoided with co-stimulation blockade [18] or encapsulation [19]. One must also consider the possibility of introducing infections into the host that were derived from the donating animal species: so-called zoonotic diseases. These drawbacks have led researchers to search for other potential sources of glucose-responsive insulin-producing tissue, including the use of stem cells.

In the following sections, we provide a general overview of stem cells, the progress towards their use as a cure for diabetes and some of the limitations encountered thus far.

WHAT ARE STEM CELLS?

Initially identified by Becker, McCulloch and Till in 1963 [20], stem cells are defined by two key features. First, they have the ability to renew themselves through cell division while remaining undifferentiated. Secondly, when given the appropriate signals, stem cells can differentiate into many specialized cell types. Stem cells can be categorized into two subtypes. ESCs (embryonic stem cells) are pluripotent cells derived from the inner cell mass of a 4–5-day old embryo (blastocyst) and have the potential to form derivatives from all three germ cell layers. Initially

derived from mouse embryos [21,22], it was not until 1998 that James Thomson and co-workers were able to isolate and continuously culture these cells from a human blastocyst [23]. It is important to emphasize here that the human ESC is defined as a pluripotent stem cell because it can generate teratomas composed of all lineages when transplanted into immune-compromised mice. In the case of mouse ESCs, they are capable of reconstituting an entire mouse following injection of the blastocyst and germline transmission. At the time that human ESCs were first established in culture, human pluripotent embryonic germ cells were also defined. Since that time, a number of other studies have claimed the generation of pluripotent stem cells derived from the blood [24], amniotic fluid [25] and testis [26]. In contrast, adult stem cells are found in various adult organ compartments and are more restricted in their ability to differentiate. For example, the HSC (haemopoietic stem cell) gives rise to itself and the progenitor cells responsible for the adult blood lineages. Mesenchymal cells are multipotent stem cells capable of generating, for example, fat, bone and cartilage. Adult stem cells with restricted organ-specific differentiation capabilities also reside within the CNS (central nervous system) and skeletal muscle, and may exist in all major organs. In these locations they may be functioning as 'repair' cells to replenish damaged tissue.

Owing to the unique properties of stem cells, a considerable effort has been placed in finding ways to use them to treat various medical conditions, especially those conditions where the patient's endogenous tissues have been damaged. Although as yet there have been no approved treatments using ESCs, adult stem cell use has become fairly common in medical practice. Bone marrow transplantation employs HSCs taken from donor marrow to successfully treat leukaemia and other haematological malignancies. This success has led researchers to explore other uses, including the treatment of stroke [27], blindness [28] and even myocardial infarctions [29].

As T1DM results from the destruction of pancreatic β-cells, the unique regenerative properties of stem cells could be employed to replenish this deficit. Developing a renewable source of islets would circumvent the current supply/demand issues in islet transplantation and provide patients with a long-term source of insulin-producing β-cells. A key challenge will be to prevent autoimmune-mediated destruction of the new cells. For example, hOKT3-ala-ala, an anti-CD3 monoclonal antibody, and other immunomodulatory therapies have shown great promise in early clinical trials in mitigating this response [30,31]. Chronic immunosuppression, with its attendant side effects of renal toxicity, increased risk of infection and malignancy, will be required until tolerance, immuno-isolation or HLA-identical cell preparations have been developed. New frontiers in immunomodulation, with calcineurin-inhibitor avoidance and use of co-stimulatory blockade, are moving forward with clinical trials of
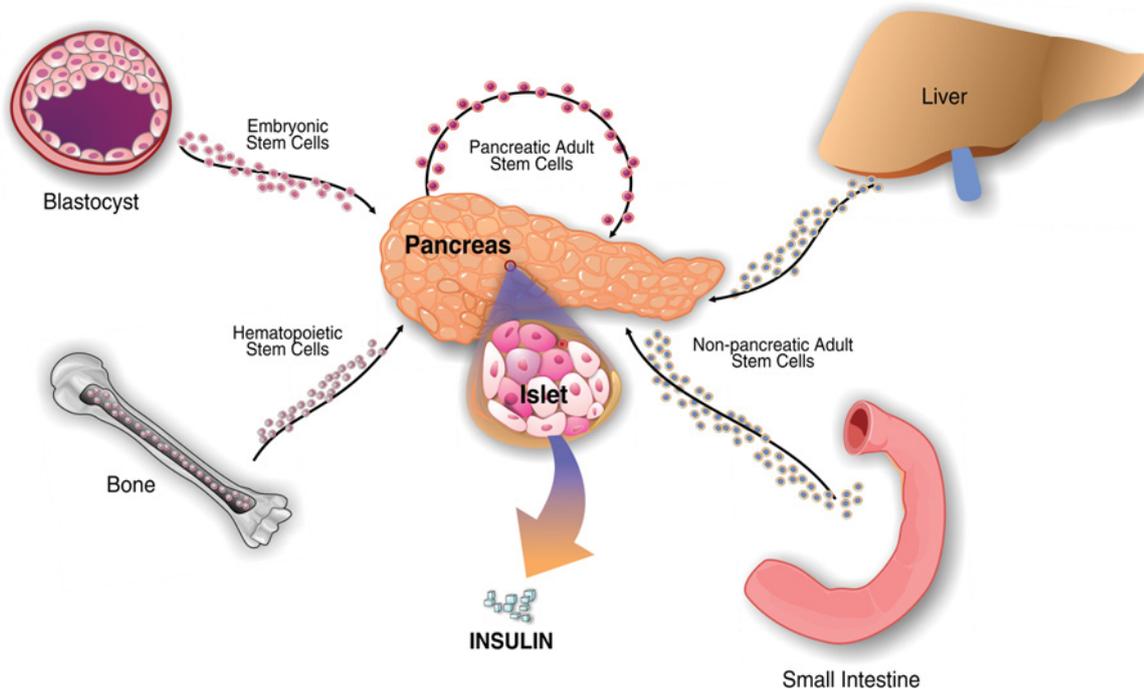
Figure 1   Multiple sources of stem cells that have been developed to produce insulin

belatacept in clinical renal transplantation, and may be one way forward to minimize immunosuppression-related side effects [32,33].

STEM CELLS IN DIABETES

Although crucial research still continues in improving our current islet transplantation protocol, stem cell investigation has erupted in recent years as a tantalizing treatment for diabetes. Studies have focused on a number of approaches, including the use of both pancreatic and non-pancreatic adult stem cells in addition to ESCs (Figure 1). The following sections will provide a review of the progress to date in each of these areas.

Pancreatic adult stem cells

The first place that researchers began looking for potential stem cells was in the pancreas itself. Early studies had shown that the pancreas could replenish its β-cell mass when all but 10 % of a rat pancreas was removed [34]. Although some of this regeneration was due to replication of differentiated β-cells, a significant proportion was thought to be due to cells that were able to dedifferentiate to a more pluripotent form that then produced β-cells. These cells appeared to reside within the pancreatic ducts. Further research using digested human pancreatic tissue proved that this population of ductal cells could, *in vitro*, be cultivated and directed to form islet-like clusters that produced insulin [35,36]. Still further research showed

that these ductal cells re-expressed a key transcription factor, IPF-1/PDX-1 (insulin promoter factor-1/pancreas and duodenal homeobox-1), which is known to play a role in pancreatic development and endocrine cell neogenesis [37]. Finally, in 2004, Seaberg et al. [38] were the first group to develop a clonal population of adult pancreatic precursor cells which were isolated from ductal cells. These cells did express IPF-1 as well as several neural precursor markers causing them to develop into neurons and glial cells, in addition to pancreatic endocrine and exocrine tissue. In addition, the β-like cells produced both C-peptide and insulin, the release of the latter being positively regulated by glucose. At the same time, other groups were exploring the possibility of stem cells residing within the islets themselves. Zulewski and co-workers [39,40] published a series of studies showing that islets, both rodent and human, contain multipotential stem cells which have the ability to differentiate into a number of tissues, including pancreatic endocrine and hepatic phenotypes. These cells were discovered through their common link to neural stem cells, the filament protein nestin. Interestingly enough, nestin-positive cells were also found in pancreatic ducts, providing a potential link between these cells and the previously discovered ductal stem cells [39]. Pancreatic stem cells appeared quite promising.

Even with this evidence, there were some that challenged the existence of a pancreatic adult stem cell. Dor et al. [41] reported, using genetic lineage tracing, that pre-existing β-cells, rather than stem cells, are the

major source for new β-cells during adult life in humans and after injury in mice. This cast doubt on the studies that had preceded it. Xu et al. [41a] have recently reported proof that multipotent progenitor cells do exist in the pancreatic ducts of mice and express both IPF-1 and Ngn3 (neurogenin 3), a transcription factor known to be expressed by endocrine progenitor cells during embryonic development [42,43]. These cells reappear following pancreatic injury (duct ligation) and give rise to new β-cells. Although this conclusively proves that a pancreatic stem cell exists and that β-cells can be formed from non-β-cells, much work still needs to be done to (i) elucidate further the factors that induce certain pancreatic cells to revert to an embryonic mode of development, and (ii) determine whether these stem cells exist in diabetic patients and if they can be somehow activated to promote β-cell formation or isolated and sufficiently expanded *ex vivo* for transplantation. Extensive studies have been performed *in vitro* in which adult rodent acinar cells have been transdifferentiated into β-like cells using combinations of various growth factors and culture methods [44]. So far, these methods have not proven to be applicable to human acinar cells. Most recently, Zhou et al. [45] have demonstrated that fully differentiated exocrine cells from the pancreas can be directly re-programmed into cells that closely resemble adult β-cells, using specific adenoviral-delivered transcription factors for IPF-1, Ngn3 and Mafa (V-maf musculoaponeurotic fibrosarcoma oncogene homologue A). Although this does not argue that stem cells exist, it suggests that adult pancreatic cells can be re-programmed to become β-like cells by supplying key endocrine and β-cell 're-programming' genes.

Non-pancreatic adult stem cells

Haemopoietic progenitor cells
A number of different adult stem cell can be found in the haemopoietic system. These include (i) HSCs which can be isolated from blood, bone marrow or UCB (umbilical cord blood), and (ii) MSCs (mesenchymal stem cells) from bone marrow and UCB. The former are responsible for producing all blood cell types, whereas the latter contribute to the formation of mesenchymal tissues, including bone, muscle and fat.

In recent years, bone-marrow-derived stem cells have been used successfully to treat a number of haematological malignancies due to their ability to reconstitute the haemopoietic system. Research has also shown them to have the ability to transdifferentiate into a number of other cell lineages, including liver, brain, lung and even gastrointestinal tract cells [46–49]. In addition, UCB represents an abundant and easily accessible resource having a heightened ability to differentiate when compared with bone marrow or peripheral blood. For these reasons, a significant amount of work has focused on the possibility of using haemopoietic progenitors to replenish the β-cell population in T1DM.

In terms of bone marrow cells, an early mouse study proved promising, showing that these cells had the ability to differentiate *in vivo* into functionally competent β-cells [50]. The group injected bone marrow from male donor mice, whose cells were genetically modified to produce a fluorescent protein if insulin was produced, into irradiated female donor mice. Within pancreatic islets in the donor mice were found fluorescent protein-producing cells that contained a Y chromosome. Thus they were able to show that bone marrow cells were able to populate the pancreatic islet cells. In addition, this treatment was specific, as extra-pancreatic cells did not express the same fluorescent protein. Similar experiments were carried out by other groups using mice with chemically induced diabetes but without similar results [51–53]. They were able to show that bone marrow cells can be targeted to the pancreas and that hyperglycaemia can be reversed [51]; however, they failed to provide evidence of differentiation of bone marrow cells into β-cells. These researchers felt that the marrow cells facilitated endogenous pancreatic regeneration possibly via conversion into endothelial cells or generalized anti-inflammatory activities but they themselves did not become functional β-cells.

At the same time, other groups were looking at blood stem cell populations in the NOD (non-obese diabetic) mouse model [54–57]. Widely used in diabetes research, the NOD mouse is an autoimmune diabetes model. Once again, an initial report looked promising. NOD mice were given a syngeneic islet renal subcapsular transplant to treat their hyperglycaemia. Mice were then given donor splenocytes and complete Freund's adjuvant (previously shown to prevent autoimmune diabetes in NOD mice [58]) and, after 40 days, the islet graft was removed [54]. Normoglycaemia continued. The authors hypothesized that the donor splenocytes differentiated into insulin-producing cells to regenerate the new β-cell mass. Three further studies partially replicated these experiments showing that a proportion of mice could regain endogenous β-cell function, but could not find any evidence that the splenocytes themselves replenished the β-cell mass [55–57].

Similar experiments were performed using UCB cells. In one study, T-cell-depleted mononuclear cells were transplanted into newborn mice [59]. As the mice developed, 0.65% of the insulin-positive cells in the pancreas were shown to be of UCB origin; a process that was both dependent and independent on fusion mechanisms. Another group subjected UCB cells to ESC culture conditions reported previously, leading to production of C-peptide [60,61].

Taking these cells one step further, a cohort of children with T1DM were transplanted with autologous UCB cells [62]. The authors showed lower average daily insulin requirements and lower average HbA$_{1c}$ (glycated

haemoglobin) levels. Unfortunately, it is still unclear whether this was due to differentiation of the transplanted cells or altered immune regulation. Still, even more promising are the recent studies showing improvement in T1DM [63] and T2DM [64] when autologous stem cells were transplanted. Although the patients in the former trial developed a number of complications, these trials provide promising results for the use of autologous HSCs in the treatment of diabetes. In addition, the use of a patient's own cells would probably avoid the ethical dilemma associated with their embryonic counterparts.

Other non-pancreatic adult stem cells

As the pancreas is embryologically derived from endodermal tissue, a logical place to search for pancreatic stem cells is in other endodermal-derived organs. Both the liver and small intestine share a common lineage origin with the pancreas and, as such, both have been extensively studied as potential sources of pancreatic β-cells.

Early work on the liver uncovered multipotent cells able to differentiate into both hepatocytes and bile duct epithelium. These were termed 'hepatic oval cells' [65]. Using this knowledge, researchers began the search for ways to induce the differentiation of these liver cells into insulin-producing cells. Once again, IPF-1/PDX-1 has proved to be a useful tool, with multiple groups successfully transdifferentiating rodent hepatic cells into insulin-producing cells via multiple genetic approaches [66–75]. Most groups used adenovirus-mediated gene transfer techniques to introduce transcription factors (especially PDX-1) into hepatic cells *in vivo* [66,68,72,73,75,76], whereas others found that *in vitro* high glucose concentrations and/or transplantation into a diabetic mouse were necessary to stimulate hepatic cell changes [67,71]. Regardless of the method used, amelioration of hyperglycaemia was achieved in these mouse models, giving hope to researchers searching for extra-pancreatic sources of insulin.

Other potential stem cell sources have also been studied, including the small intestine [76–78], salivary glands [79] and adipose tissue [80]. Varying success has been achieved in this regard and, if a non-pancreatic stem cell source of β-cells is to be utilized in a cure for diabetes, it appears that the liver could be a likely source based on its close embryological proximity and the results obtained to date. Although issues remain, including the expandability and durability of these cell lines, the ability to achieve fully differentiated β-cell phenotypes and the potential necessity of islet structure formation, hepatic production of insulin has the potential to become a viable source for β-cell replacement in the years to come.

Human ESCs

ESCs have received a heightened amount of interest in the last decade owing to their pluripotency and their ability to self-renew. These unique characteristics have led many researchers to explore their use in a number of medical conditions, especially those with a degenerative or destructive aetiology. T1DM fits in this realm of diseases as the initiating insult is an autoimmune destruction. The theory has developed that, if ESCs could be directed to differentiate into pancreatic islet cells and these cells could then be implanted in patients with diabetes, then the β-cell deficit would be overcome. This differentiation task, however, has proven to be complex and difficult.

The first attempts at producing islet cells *in vitro* from mouse ESCs was published in 2000 [81]. The group led by Soria developed insulin-secreting clones from a genetically engineered and drug-selected mouse ESC line. They were able to transplant these cells into diabetic mice and were able to achieve a degree of amelioration of hyperglycaemia that lasted for a few months [81]. Unfortunately, the rate of insulin-positive cell production was quite low, probably because their cell selection process was conducted before full differentiation. As such, some of the cells in their final group were non-islet insulin-producing cells (e.g. neural cells). Over the next few years, a number of other groups have published results with varying degrees of success. These initial studies employed both mouse [60,82–87] and human [88,89] ESCs, and were limited by final cell homogeneity [60], immaturity of the differentiated cells [89], low numbers of insulin-producing cells [90] and a poor insulin-response when the cells were exposed to glucose [86,88]. In general, all of these studies lacked thorough analysis and demonstration of the sequential developmental steps required to ensure formation of foregut pancreatic cells capable of endocrine hormone production. Although these early reports brought some hope, it was certainly proving difficult to create reliable insulin-producing cells with a β-cell phenotype from ESCs.

To add to these difficulties, other groups were beginning to question whether these cells were actually producing insulin at all. Three separate teams were able to show that the majority of the claimed differentiated pancreatic 'insulin-producing' cells did not, in fact, produce any insulin [91–93]. They argued that the insulin found in these cells was due to its uptake from the surrounding medium. In support of this, they showed a lack of C-peptide production [91–93] and a lack of intracellular insulin once the cells were cultured in insulin-free medium [92]. For further progress to be made with ESCs, proof of C-peptide production would have to be shown.

This setback forced researchers to rethink their differentiation strategies. The stages and gene expression involved during normal pancreas development were already known [94–96], including the first major differentiation stage in the development of the endocrine pancreas, the definitive endoderm germ layer [94]. Kubo et al. [97] were able to define the exact culture conditions necessary to convert mouse ESCs into definitive endoderm. This paved the way for D'Amour et al. [98] to refine

this protocol and produce a near 100 % pure definitive endoderm cell population. The same group extended their work on definitive endoderm and developed a five stage *in vitro* differentiation process modelled after *in vivo* development of the pancreas [99]. In that study, they demonstrated the production of pancreatic endocrine-hormone-producing cells that contained both insulin and C-peptide. In fact, the insulin content of these cells was in the range of that for mature human islets. Unfortunately, although C-peptide was released from these cells in response to various stimuli, including KCl and cAMP, neither C-peptide nor insulin was released in response to glucose. Hyperglycaemic responsiveness is a crucial characteristic that is needed for any potential cellular diabetic therapy. In order to overcome this deficit, the group once again looked at earlier research in which fetal human pancreatic anlagen was successfully isolated and grafted into rodents, resulting in functional human islet formation. This time they stopped their *in vitro* differentiation at a point where the cells resembled a 6–9-week-old embryo; they were committed to the pancreatic lineage, but had not yet transitioned to the endocrine progenitor state [100]. A prior report had demonstrated that a 6–9-week human embryonic pancreas (primarily pancreatic epithelium as opposed to endocrine cells) grafted into an immunodeficient diabetic mouse was able to develop and reverse hyperglycaemia further [101]. Using this knowledge, the group transplanted their differentiated cells into the epididymal fat pad of immunodeficient mice. Although C-peptide levels were low 1 month post-transplantation, at the 90 day point, levels were approaching those seen with transplantation of 3000–5000 human islets. Insulin release was also measured and was shown to be released in a glucose-dependent manner. This allowed the cells to both recover mice from STZ (streptozotocin)-induced diabetes as well as prevent it.

These recent discoveries have paved the way for ESCs to become a strong candidate for cellular replacement therapy in T1DM; however, we are probably still a number of years away from any potential clinical trials with a number of questions still left to be answered, including the long-term stability of stem cell derivatives and their potential side effects.

HOW FAR AWAY IS CELLULAR-REPLACEMENT THERAPY FOR DIABETES?

Although researchers have made enormous strides toward using stem cells as a potential treatment for T1DM, there are a number of issues that need to be addressed. These include concerns of both a scientific and societal nature (Table 1).

First, before any new treatment is used in the human population, it of course needs to undergo rigorous testing

Table 1   Issues of a scientific and societal nature that must be resolved before a clinical application of stem-cell-derived products can be attempted

Issues related to the use of stem cells in the treatment of T1DM
Potential risks, including teratomas
Challenges related to the transplantation of isolated cells lacking vascular and neural support
Need for immunosuppression
Ethical acceptance
Engraftment of a non-marginal mass of insulin-producing cells for human use (exceeding 15 000 IE/kg of body weight)

and screening for potential side effects. This concern for safety is probably even more heightened when it comes to stem cells. One complication that has already arisen in the mouse models is the formation of teratomas with the potential for malignancy. This is especially a concern with ESCs, where groups have already observed teratoma formation when grafts were histologically assessed [85,87,100]. These tumours form due to the implantation of undifferentiated cell populations into an immunodeficient host; such as would be the case if these cells were introduced into a patient on necessary immunosuppressives. It would be difficult to treat patients with a cell-replacement product for diabetes if it could not be demonstrated to be safe with respect to teratoma formation. Future protocols will therefore need some form of purification or screening step in order to eliminate and screen for the presence of unsafe cells respectively. Of course, if the replacement therapy could be administered without immunosuppressive drugs, the possibility of teratoma formation would no doubt be lessened.

The pancreas is a very complex organ with many functions both endocrine and exocrine in nature. Endogenous β-cells develop through a regulated pathway to eventually become the insulin-producing cells which regulate euglycaemia. The mature cells are part of an integrated milieu of cells and cellular signals together with their cellular products. Even the islets transplanted in current clinical islet-transplant programmes contain β-cells along with α- and δ-cells. In addition, endogenous islets are situated in a complex array of vascular and neural supports. How will stem-cell-derived products behave once transplanted? Depending on the transplant site, will they be able to develop these same vascular and neural connections? Even though some groups have shown the production of glucagon and somatostatin in their cell populations, how will these cells interact once transplanted into an unfamiliar environment? Will it be necessary to have a complete islet structure with the appropriate endocrine hormone composition or will it be sufficient to have appropriate numbers of β-cells? Recent studies have demonstrated that purified β-cell

preparations are sufficient to treat the diabetic condition in rodents [102]. In addition, it is likely that β-cells are able to adapt to changes in their glucose environment and adapt to insulin resistance through both neogenesis and cell replication [103]. Will stem-cell-derived β-cells have these abilities or, even worse, will increased insulin resistance cause these β-cells to expand uncontrollably? Further studies will no doubt need to address these issues.

Although a cellular-based replacement therapy for diabetes would overcome one of the major limitations of our current islet transplantation protocol, it is still likely to be subject to the other major limitation. Unless a protocol is developed where stem cells are derived from a patient's own cellular population (and even here the issue of the autoimmune insult which caused the disease needs to be addressed), some form of immunosuppression or an immuno-isolation delivery strategy will be required. Clearly our knowledge of the immune system and therapies targeted at diminishing its effects have made great strides, but patients are faced with unpleasant and, at times, unbearable side effects from immunosuppressive agents. Thus, although stem cells could conceivably circumvent the need to rely on organ donation for a source of insulin-producing tissue, they may do nothing to relieve the toxicity associated with the post-implantation drug therapy, unless additional immunomodulatory regulatory stem cells are co-transplanted or specific tolerization strategies are developed. Any future stem-cell-related therapies will no doubt be facilitated by improvements in the tolerance of our current anti-immune therapies. Underlying this point is the need to continue with the current research into islet transplantation, as any further advances made there will no doubt have a positive impact upon the development of any cellular-based diabetes therapy.

When considering current clinical islet transplantation programmes another interesting issue arises. Current guidelines employ a minimal islet implant mass of 10 000 IE (islet equivalent)/kg of body weight, usually obtained by harvesting two pancreases [9]. Even with this amount of islets, most patients need to return to a small amount of insulin at the 2–3-year mark. It still remains to be seen how stem cells can compare, in terms of insulin production and potency, to this amount of islets. Although Kroon et al. [100] have stated that their stem-cell-derived products are achieving a production rate of C-peptide equivalent to 3000–5000 human islets in their mouse model, this is far short of the levels needed to support an adult human. The scale-up potential of stem cells will, therefore, need to be studied further to provide an excess of transplanted cellular reserve.

The last issue, and certainly by no means the least important, is the intense ethical debate that forms from any discussion of stem cells. Beginning with the cloning of Dolly the sheep in 1997 [104], cultural fires have ignited with any mention of cloning or genetic engineering. These fires have now spread to the field of stem cell research.

Here, it appears the issues revolve mainly around ESCs and their derivation. In short, ESCs are usually derived from unused embryos at *in vitro* fertilization clinics. Full informed consent needs to be given by the donor before these cells can be used. Unfortunately, the embryo, in most cases, needs to be destroyed to harvest the cells it contains. It appears that the majority of the controversy develops from this derivation process and the question of when life actually begins. On the one hand are those that believe that stem cell research violates the sanctity of life. They are of the mind that life is inviolable and begins when a sperm fertilizes an egg. They are in direct contrast with those that take a more utilitarian view on the issue where the potential benefits, in terms of cellular therapies for medical conditions, outweigh the potential costs. Although this debate continues, the full extent of its impact on research using stem cells remains to be seen. Even though adult stem cells will probably avoid much of the negative publicity generated by their embryonic cousins, any potential clinical uses involving stem cells will need to be accompanied by a thorough explanation of their derivation. Although unlikely to end the debate, it will hopefully ease some of the tension that has built up around this topic.

A longer-term solution to the human ESC ethical dilemma will probably be the induced pluripotent stem cell approach [105,106]. In this case, adult cells are reprogrammed to the pluripotent state to be subsequently differentiated to functional β-cells. The further safety concerns associated with *ex vivo* gene therapy with oncogenes which, together with a better understanding of the genetic and epigenetic stability, adds a further safety burden probably to be solved in the future.

CONCLUSIONS

Are stem cells a 'cure for diabetes'? Although great strides have been made towards using stem cells as a cellular replacement therapy for T1DM, many issues still remain. It would appear that ESCs have the advantage thus far owing to their enhanced potential for directed differentiation and replication. Unfortunately, it is ESCs that also are fraught with potential complications, including a tendency to form teratomas and the ethical debate revolving around their derivation. That said, in the last 10 years, we have been able to use the knowledge of normal embryologic development to differentiate stem cells into insulin-producing tissue with the ability to reverse diabetes in an animal model. Who knows what the next 10 years will bring?

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REFERENCES

1 The Diabetes Control and Complications Trial Research Group (1993) The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N. Engl. J. Med. **329**, 977–986

2 UK Prospective Diabetes Study (UKPDS) Group (1998) Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet **352**, 837–853

3 Kelly, W. D., Lillehei, R. C., Merkel, F. K., Idezuki, Y. and Goetz, F. C. (1967) Allotransplantation of the pancreas and duodenum along with the kidney in diabetic nephropathy. Surgery **61**, 827–837

4 Gruessner, A. C. and Sutherland, D. E. (2005) Pancreas transplant outcomes for United States (US) and non-US cases as reported to the United Network for Organ Sharing (UNOS) and the International Pancreas Transplant Registry (IPTR) as of June 2004. Clin. Transplant. **19**, 433–455

5 Ballinger, W. F. and Lacy, P. E. (1972) Transplantation of intact pancreatic islets in rats. Surgery **72**, 175–186

6 Scharp, D. W., Lacy, P. E., Santiago, J. V., McCullough, C. S., Weide, L. G., Falqui, L., Marchetti, P., Gingerich, R. L., Jaffe, A. S., Cryer, P. E. et al. (1990) Insulin independence after islet transplantation into type I diabetic patient. Diabetes **39**, 515–518

7 Ricordi, C., Lacy, P. E. and Scharp, D. W. (1989) Automated islet isolation from human pancreas. Diabetes **38** (Suppl. 1), 140–142

8 Shapiro, A. M. J., Lakey, J. R., Ryan, E. A., Korbutt, G. S., Toth, E., Warnock, G. L., Kneteman, N. M. and Rajotte, R. V. (2000) Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N. Engl. J. Med. **343**, 230–238

9 Shapiro, A. M. J., Ricordi, C., Hering, B. J., Auchincloss, H., Lindblad, R., Robertson, R. P., Secchi, A., Brendel, M. D., Berney, T., Brennan, D. C. et al. (2006) International trial of the Edmonton protocol for islet transplantation. N. Engl. J. Med. **355**, 1318–1330

10 Ryan, E. A., Paty, B. W., Senior, P. A., Bigam, D., Alfadhli, E., Kneteman, N. M., Lakey, J. R. and Shapiro, A. M. J. (2005) Five-year follow-up after clinical islet transplantation. Diabetes **54**, 2060–2069

11 Ryan, E. A., Lakey, J. R., Paty, B. W., Imes, S., Korbutt, G. S., Kneteman, N. M., Bigam, D., Rajotte, R. V. and Shapiro, A. M. J. (2002) Successful islet transplantation: continued insulin reserve provides long-term glycemic control. Diabetes **51**, 2148–2157

12 Ryan, E. A., Shandro, T., Green, K., Paty, B. W., Senior, P. A., Bigam, D., Shapiro, A. M. J. and Vantyghem, M. C. (2004) Assessment of the severity of hypoglycemia and glycemic lability in type 1 diabetic subjects undergoing islet transplantation. Diabetes **53**, 955–962

13 Toso, C., Shapiro, A. M. J., Bowker, S., Dinyari, P., Paty, B., Ryan, E. A., Senior, P. and Johnson, J. A. (2007) Quality of life after islet transplant: impact of the number of islet infusions and metabolic outcome. Transplantation **84**, 664–666

14 Sheehy, E., Conrad, S. L., Brigham, L. E., Luskin, R., Weber, P., Eakin, M., Schkade, L. and Hunsicker, L. (2003) Estimating the number of potential organ donors in the United States. N. Engl. J. Med. **349**, 667–674

15 Matsumoto, S., Okitsu, T., Iwanaga, Y., Noguchi, H., Nagata, H., Yonekawa, Y., Liu, X., Kamiya, H., Ueda, M., Hatanaka, N. et al. (2006) Follow-up study of the first successful living donor islet transplantation. Transplantation **82**, 1629–1633

16 Matsumoto, S., Okitsu, T., Iwanaga, Y., Noguchi, H., Nagata, H., Yonekawa, Y., Yamada, Y., Fukuda, K., Tsukiyama, K., Suzuki, H. et al. (2005) Insulin independence after living-donor distal pancreatectomy and islet allotransplantation. Lancet **365**, 1642–1644

17 Kin, T., Korbutt, G. S., Kobayashi, T., Dufour, J. M. and Rajotte, R. V. (2005) Reversal of diabetes in pancreatectomized pigs after transplantation of neonatal porcine islets. Diabetes **54**, 1032–1039

18 Cardona, K., Korbutt, G. S., Milas, Z., Lyon, J., Cano, J., Jiang, W., Bello-Laborn, H., Hacquoil, B., Strobert, E., Gangappa, S. et al. (2006) Long-term survival of neonatal porcine islets in nonhuman primates by targeting costimulation pathways. Nat. Med. **12**, 304–306

19 Kobayashi, T., Arefanian, H., Harb, G., Tredget, E. B., Rajotte, R. V., Korbutt, G. S. and Rayat, G. R. (2008) Prolonged survival of microencapsulated neonatal porcine islet xenografts in immune-competent mice without antirejection therapy. Cell Transplant. **17**, 1243–1256

20 Becker, A. J., McCulloch, E. and Till, J. E. (1963) Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. Nature **197**, 452–454

21 Evans, M. J. and Kaufman, M. H. (1981) Establishment in culture of pluripotential cells from mouse embryos. Nature **292**, 154–156

22 Martin, G. R. (1981) Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. Proc. Natl. Acad. Sci. U.S.A. **78**, 7634–7638

23 Thomson, J. A., Itskovitz-Eldor, J., Shapiro, S. S., Waknitz, M. A., Swiergiel, J. J., Marshall, V. S. and Jones, J. M. (1998) Embryonic stem cell lines derived from human blastocysts. Science **282**, 1145–1147

24 Jaing, T. H., Sun, C. F., Lee, W. I., Wen, Y. C., Yang, C. P. and Hung, I. J. (2008) Successful unmanipulated peripheral blood progenitor cell transplantation from an HLA haploidentical 2-locus-mismatched mother in a thalassemic patient with primary graft failure after transplantation of bone marrow and cord blood from unrelated donors. Pediatr. Transplant. **12**, 232–234

25 De Coppi, P., Bartsch, Jr, G., Siddiqui, M. M., Xu, T., Santos, C. C., Perin, L., Mostoslavsky, G., Serre, A. C., Snyder, E. Y., Yoo, J. J. et al. (2007) Isolation of amniotic stem cell lines with potential for therapy. Nat. Biotechnol. **25**, 100–106

26 Conrad, S., Renninger, M., Hennenlotter, J., Wiesner, T., Just, L., Bonin, M., Aicher, W., Buhring, H. J., Mattheus, U., Mack, A. et al. (2008) Generation of pluripotent stem cells from adult human testis. Nature **456**, 344–349

27 Haas, S., Weidner, N. and Winkler, J. (2005) Adult stem cell therapy in stroke. Curr. Opin. Neurol. **18**, 59–64

28 Adler, R. (2008) Curing blindness with stem cells: hope, reality, and challenges. Adv. Exp. Med. Biol. **613**, 3–20

29 Meyer, G. P., Wollert, K. C. and Drexler, H. (2007) The role of stem cells in the post-MI patient. Curr. Heart Failure Rep. **4**, 198–203

30 Herold, K. C., Gitelman, S. E., Masharani, U., Hagopian, W., Bisikirska, B., Donaldson, D., Rother, K., Diamond, B., Harlan, D. M. and Bluestone, J. A. (2005) A single course of anti-CD3 monoclonal antibody hOKT3γ1(Ala-Ala) results in improvement in C-peptide responses and clinical parameters for at least 2 years after onset of type 1 diabetes. Diabetes **54**, 1763–1769

31 Herold, K. C., Hagopian, W., Auger, J. A., Poumian-Ruiz, E., Taylor, L., Donaldson, D., Gitelman, S. E., Harlan, D. M., Xu, D., Zivin, R. A. and Bluestone, J. A. (2002) Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. N. Engl. J. Med. **346**, 1692–1698

32 Larsen, C. P., Pearson, T. C., Adams, A. B., Tso, P., Shirasugi, N., Strobertm, E., Anderson, D., Cowan, S., Price, K., Naemura, J. et al. (2005) Rational development of LEA29Y (belatacept), a high-affinity variant of CTLA4-Ig with potent immunosuppressive properties. Am. J. Transplant. **5**, 443–453

33 Vincenti, F., Larsen, C., Durrbach, A., Wekerle, T., Nashan, B., Blancho, G., Lang, P., Grinyo, J., Halloran, P. F., Solez, K. et al. (2005) Costimulation blockade with belatacept in renal transplantation. N. Engl. J. Med. **353**, 770–781

34 Bonner-Weir, S., Baxter, L. A., Schuppin, G. T. and Smith, F. E. (1993) A second pathway for regeneration of adult exocrine and endocrine pancreas. A possible recapitulation of embryonic development. Diabetes **42**, 1715–1720

35 Bonner-Weir, S., Taneja, M., Weir, G. C., Tatarkiewicz, K., Song, K. H., Sharma, A. and O'Neil, J. J. (2000) *In vitro* cultivation of human islets from expanded ductal tissue. Proc. Natl. Acad. Sci. U.S.A. **97**, 7999–8004

36 Gao, R., Ustinov, J., Pulkkinen, M. A., Lundin, K., Korsgren, O. and Otonkoski, T. (2003) Characterization of endocrine progenitor cells and critical factors for their differentiation in human adult pancreatic cell culture. Diabetes **52**, 2007–2015

37 Gmyr, V., Kerr-Conte, J., Belaich, S., Vandewalle, B., Leteurtre, E., Vantyghem, M. C., Lecomte-Houcke, M., Proye, C., Lefebvre, J. and Pattou, F. (2000) Adult human cytokeratin 19-positive cells reexpress insulin promoter factor 1 *in vitro*: further evidence for pluripotent pancreatic stem cells in humans. Diabetes **49**, 1671–1680

38 Seaberg, R. M., Smukler, S. R., Kieffer, T. J., Enikolopov, G., Asghar, Z., Wheeler, M. B., Korbutt, G. and van der Kooy, D. (2004) Clonal identification of multipotent precursors from adult mouse pancreas that generate neural and pancreatic lineages. Nat. Biotechnol. **22**, 1115–1124

39 Zulewski, H., Abraham, E. J., Gerlach, M. J., Daniel, P. B., Moritz, W., Muller, B., Vallejo, M., Thomas, M. K. and Habener, J. F. (2001) Multipotential nestin-positive stem cells isolated from adult pancreatic islets differentiate *ex vivo* into pancreatic endocrine, exocrine, and hepatic phenotypes. Diabetes **50**, 521–533

40 Eberhardt, M., Salmon, P., von Mach, M. A., Hengstler, J. G., Brulport, M., Linscheid, P., Seboek, D., Oberholzer, J., Barbero, A., Martin, I. et al. (2006) Multipotential nestin and Isl-1 positive mesenchymal stem cells isolated from human pancreatic islets. Biochem. Biophys. Res. Commun. **345**, 1167–1176

41 Dor, Y., Brown, J., Martinez, O. I. and Melton, D. A. (2004) Adult pancreatic β-cells are formed by self-duplication rather than stem-cell differentiation. Nature **429**, 41–46

41a Xu, X., D'Hoker, J., Stangé, G., Bonné, S., De Leu, N., Xiao, X., Van de Casteele, M., Mellitzer, G., Ling, Z., Pipeleers, D. et al. (2008) β Cells can be generated from endogenous progenitors in injured adult mouse pancreas. Cell **132**, 197–207

42 Apelqvist, A., Li, H., Sommer, L., Beatus, P., Anderson, D. J., Honjo, T., Hrabe de Angelis, M., Lendahl, U. and Edlund, H. (1999) Notch signalling controls pancreatic cell differentiation. Nature **400**, 877–881

43 Gu, G., Dubauskaite, J. and Melton, D. A. (2002) Direct evidence for the pancreatic lineage: NGN3+ cells are islet progenitors and are distinct from duct progenitors. Development **129**, 2447–2457

44 Baeyens, L., De Breuck, S., Lardon, J., Mfopou, J. K., Rooman, I. and Bouwens, L. (2005) *In vitro* generation of insulin-producing β cells from adult exocrine pancreatic cells. Diabetologia **48**, 49–57

45 Zhou, Q., Brown, J., Kanarek, A., Rajagopal, J. and Melton, D. A. (2008) In vivo reprogramming of adult pancreatic exocrine cells to β-cells. Nature **455**, 627–632

46 Krause, D. S., Theise, N. D., Collector, M. I., Henegariu, O., Hwang, S., Gardner, R., Neutzel, S. and Sharkis, S. J. (2001) Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. Cell **105**, 369–377

47 Brazelton, T. R., Rossi, F. M., Keshet, G. I. and Blau, H. M. (2000) From marrow to brain: expression of neuronal phenotypes in adult mice. Science **290**, 1775–1779

48 Jiang, Y., Jahagirdar, B. N., Reinhardt, R. L., Schwartz, R. E., Keene, C. D., Ortiz-Gonzalez, X. R., Reyes, M., Lenvik, T., Lund, T., Blackstad, M. et al. (2002) Pluripotency of mesenchymal stem cells derived from adult marrow. Nature **418**, 41–49

49 Lagasse, E., Connors, H., Al-Dhalimy, M., Reitsma, M., Dohse, M., Osborne, L., Wang, X., Finegold, M., Weissman, I. L. and Grompe, M. (2000) Purified hematopoietic stem cells can differentiate into hepatocytes *in vivo*. Nat. Med. **6**, 1229–1234

50 Ianus, A., Holz, G. G., Theise, N. D. and Hussain, M. A. (2003) *In vivo* derivation of glucose-competent pancreatic endocrine cells from bone marrow without evidence of cell fusion. J. Clin. Invest. **111**, 843–850

51 Hess, D., Li, L., Martin, M., Sakano, S., Hill, D., Strutt, B., Thyssen, S., Gray, D. A. and Bhatia, M. (2003) Bone marrow-derived stem cells initiate pancreatic regeneration. Nat. Biotechnol. **21**, 763–770

52 Choi, J. B., Uchino, H., Azuma, K., Iwashita, N., Tanaka, Y., Mochizuki, H., Migita, M., Shimada, T., Kawamori, R. and Watada, H. (2003) Little evidence of transdifferentiation of bone marrow-derived cells into pancreatic β cells. Diabetologia **46**, 1366–1374

53 Mathews, V., Hanson, P. T., Ford, E., Fujita, J., Polonsky, K. S. and Graubert, T. A. (2004) Recruitment of bone marrow-derived endothelial cells to sites of pancreatic β-cell injury. Diabetes **53**, 91–98

54 Kodama, S., Kuhtreiber, W., Fujimura, S., Dale, E. A. and Faustman, D. L. (2003) Islet regeneration during the reversal of autoimmune diabetes in NOD mice. Science **302**, 1223–1227

55 Nishio, J., Gaglia, J. L., Turvey, S. E., Campbell, C., Benoist, C. and Mathis, D. (2006) Islet recovery and reversal of murine type 1 diabetes in the absence of any infused spleen cell contribution. Science **311**, 1775–1778

56 Chong, A. S., Shen, J., Tao, J., Yin, D., Kuznetsov, A., Hara, M. and Philipson, L. H. (2006) Reversal of diabetes in non-obese diabetic mice without spleen cell-derived β cell regeneration. Science **311**, 1774–1775

57 Suri, A., Calderon, B., Esparza, T. J., Frederick, K., Bittner, P. and Unanue, E. R. (2006) Immunological reversal of autoimmune diabetes without hematopoietic replacement of β cells. Science **311**, 1778–1780

58 Sadelain, M. W., Qin, H. Y., Lauzon, J. and Singh, B. (1990) Prevention of type I diabetes in NOD mice by adjuvant immunotherapy. Diabetes **39**, 583–589

59 Yoshida, S., Ishikawa, F., Kawano, N., Shimoda, K., Nagafuchi, S., Shimoda, S., Yasukawa, M., Kanemaru, T., Ishibashi, H., Shultz, L. D. and Harada, M. (2005) Human cord blood–derived cells generate insulin-producing cells *in vivo*. Stem Cells **23**, 1409–1416

60 Lumelsky, N., Blondel, O., Laeng, P., Velasco, I., Ravin, R. and McKay, R. (2001) Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. Science **292**, 1389–1394

61 Denner, L., Bodenburg, Y., Zhao, J. G., Howe, M., Cappo, J., Tilton, R. G., Copland, J. A., Forraz, N., McGuckin, C. and Urban, R. (2007) Directed engineering of umbilical cord blood stem cells to produce C-peptide and insulin. Cell Prolif. **40**, 367–380

62 Haller, M. J., Viener, H. L., Wasserfall, C., Brusko, T., Atkinson, M. A. and Schatz, D. A. (2008) Autologous umbilical cord blood infusion for type 1 diabetes. Exp. Hematol. **36**, 710–715

63 Couri, C. E., Oliveira, M. C., Stracieri, A. B., Moraes, D. A., Pieroni, F., Barros, G. M., Madeira, M. I., Malmegrim, K. C., Foss-Freitas, M. C., Simoes, B. P. et al. (2009) C-peptide levels and insulin independence following autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus. JAMA, J. Am. Med. Assoc. **301**, 1573–1579

64 Estrada, E. J., Valacchi, F., Nicora, E., Brieva, S., Esteve, C., Echevarria, L., Froud, T., Bernetti, K., Cayetano, S. M., Velazquez, O. et al. (2008) Combined treatment of intrapancreatic autologous bone marrow stem cells and hyperbaric oxygen in type 2 diabetes mellitus. Cell Transplant. **17**, 1295–1304

65 Petersen, B. E., Goff, J. P., Greenberger, J. S. and Michalopoulos, G. K. (1998) Hepatic oval cells express the hematopoietic stem cell marker Thy-1 in the rat. Hepatology **27**, 433–445

66 Ferber, S., Halkin, A., Cohen, H., Ber, I., Einav, Y., Goldberg, I., Barshack, I., Seijffers, R., Kopolovic, J., Kaiser, N. and Karasik, A. (2000) Pancreatic and duodenal homeobox gene 1 induces expression of insulin genes in liver and ameliorates streptozotocin-induced hyperglycemia. Nat. Med. **6**, 568–572

67 Yang, L., Li, S., Hatch, H., Ahrens, K., Cornelius, J. G., Petersen, B. E. and Peck, A. B. (2002) *In vitro* trans-differentiation of adult hepatic stem cells into pancreatic endocrine hormone-producing cells. Proc. Natl. Acad. Sci. U.S.A. **99**, 8078–8083

68 Ber, I., Shternhall, K., Perl, S., Ohanuna, Z., Goldberg, I., Barshack, I., Benvenisti-Zarum, L., Meivar-Levy, I. and Ferber, S. (2003) Functional, persistent, and extended liver to pancreas transdifferentiation. J. Biol. Chem. **278**, 31950–31957

69 Kojima, H., Fujimiya, M., Matsumura, K., Younan, P., Imaeda, H., Maeda, M. and Chan, L. (2003) NeuroD-βcellulin gene therapy induces islet neogenesis in the liver and reverses diabetes in mice. Nat. Med. **9**, 596–603

70 Zalzman, M., Gupta, S., Giri, R. K., Berkovich, I., Sappal, B. S., Karnieli, O., Zern, M. A., Fleischer, N. and Efrat, S. (2003) Reversal of hyperglycemia in mice by using human expandable insulin-producing cells differentiated from fetal liver progenitor cells. Proc. Natl. Acad. Sci. U.S.A. **100**, 7253–7258

71 Cao, L. Z., Tang, D. Q., Horb, M. E., Li, S. W. and Yang, L. J. (2004) High glucose is necessary for complete maturation of Pdx1-VP16-expressing hepatic cells into functional insulin-producing cells. Diabetes **53**, 3168–3178

72 Kaneto, H., Nakatani, Y., Miyatsuka, T., Matsuoka, T. A., Matsuhisa, M., Hori, M. and Yamasaki, Y. (2005) PDX-1/VP16 fusion protein, together with NeuroD or Ngn3, markedly induces insulin gene transcription and ameliorates glucose tolerance. Diabetes **54**, 1009–1022

73 Kaneto, H., Matsuoka, T. A., Nakatani, Y., Miyatsuka, T., Matsuhisa, M., Hori, M. and Yamasaki, Y. (2005) A crucial role of MafA as a novel therapeutic target for diabetes. J. Biol. Chem. **280**, 15047–15052

74 Kim, S., Shin, J. S., Kim, H. J., Fisher, R. C., Lee, M. J. and Kim, C. W. (2007) Streptozotocin-induced diabetes can be reversed by hepatic oval cell activation through hepatic transdifferentiation and pancreatic islet regeneration. Lab. Invest. **87**, 702–712

75 Sapir, T., Shternhall, K., Meivar-Levy, I., Blumenfeld, T., Cohen, H., Skutelsky, E., Eventov-Friedman, S., Barshack, I., Goldberg, I., Pri-Chen, S. et al. (2005) Cell-replacement therapy for diabetes: Generating functional insulin-producing tissue from adult human liver cells. Proc. Natl. Acad. Sci. U.S.A. **102**, 7964–7969

76 Yoshida, S., Kajimoto, Y., Yasuda, T., Watada, H., Fujitani, Y., Kosaka, H., Gotow, T., Miyatsuka, T., Umayahara, Y., Yamasaki, Y. and Hori, M. (2002) PDX-1 induces differentiation of intestinal epithelioid IEC-6 into insulin-producing cells. Diabetes **51**, 2505–2513

77 Kojima, H., Nakamura, T., Fujita, Y., Kishi, A., Fujimiya, M., Yamada, S., Kudo, M., Nishio, Y., Maegawa, H., Haneda, M. et al. (2002) Combined expression of pancreatic duodenal homeobox 1 and islet factor 1 induces immature enterocytes to produce insulin. Diabetes **51**, 1398–1408

78 Suzuki, A., Nakauchi, H. and Taniguchi, H. (2003) Glucagon-like peptide 1 (1–37) converts intestinal epithelial cells into insulin-producing cells. Proc. Natl. Acad. Sci. U.S.A. **100**, 5034–5039

79 Okumura, K., Nakamura, K., Hisatomi, Y., Nagano, K., Tanaka, Y., Terada, K., Sugiyama, T., Umeyama, K., Matsumoto, K., Yamamoto, T. and Endo, F. (2003) Salivary gland progenitor cells induced by duct ligation differentiate into hepatic and pancreatic lineages. Hepatology **38**, 104–113

80 Timper, K., Seboek, D., Eberhardt, M., Linscheid, P., Christ-Crain, M., Keller, U., Muller, B. and Zulewski, H. (2006) Human adipose tissue-derived mesenchymal stem cells differentiate into insulin, somatostatin, and glucagon expressing cells. Biochem. Biophys. Res. Commun. **341**, 1135–1140

81 Soria, B., Roche, E., Berna, G., Leon-Quinto, T., Reig, J. A. and Martin, F. (2000) Insulin-secreting cells derived from embryonic stem cells normalize glycemia in streptozotocin-induced diabetic mice. Diabetes **49**, 157–162

82 Hori, Y., Rulifson, I. C., Tsai, B. C., Heit, J. J., Cahoy, J. D. and Kim, S. K. (2002) Growth inhibitors promote differentiation of insulin-producing tissue from embryonic stem cells. Proc. Natl. Acad. Sci. U.S.A. **99**, 16105–16110

83 Kahan, B. W., Jacobson, L. M., Hullett, D. A., Ochoada, J. M., Oberley, T. D., Lang, K. M. and Odorico, J. S. (2003) Pancreatic precursors and differentiated islet cell types from murine embryonic stem cells: an *in vitro* model to study islet differentiation. Diabetes **52**, 2016–2024

84 Blyszczuk, P., Asbrand, C., Rozzo, A., Kania, G., St-Onge, L., Rupnik, M. and Wobus, A. M. (2004) Embryonic stem cells differentiate into insulin-producing cells without selection of nestin-expressing cells. Int. J. Dev. Biol. **48**, 1095–1104

85 Blyszczuk, P., Czyz, J., Kania, G., Wagner, M., Roll, U., St-Onge, L. and Wobus, A. M. (2003) Expression of Pax4 in embryonic stem cells promotes differentiation of nestin-positive progenitor and insulin-producing cells. Proc. Natl. Acad. Sci. U.S.A. **100**, 998–1003

86 Miyazaki, S., Yamato, E. and Miyazaki, J. (2004) Regulated expression of pdx-1 promotes *in vitro* differentiation of insulin-producing cells from embryonic stem cells. Diabetes **53**, 1030–1037

87 Leon-Quinto, T., Jones, J., Skoudy, A., Burcin, M. and Soria, B. (2004) *In vitro* directed differentiation of mouse embryonic stem cells into insulin-producing cells. Diabetologia **47**, 1442–1451

88 Assady, S., Maor, G., Amit, M., Itskovitz-Eldor, J., Skorecki, K. L. and Tzukerman, M. (2001) Insulin production by human embryonic stem cells. Diabetes **50**, 1691–1697

89 Segev, H., Fishman, B., Ziskind, A., Shulman, M. and Itskovitz-Eldor, J. (2004) Differentiation of human embryonic stem cells into insulin-producing clusters. Stem Cells **22**, 265–274

90 Hori, Y., Gu, X., Xie, X. and Kim, S. K. (2005) Differentiation of insulin-producing cells from human neural progenitor cells. PLoS Med. **2**, e103

91 Hansson, M., Tonning, A., Frandsen, U., Petri, A., Rajagopal, J., Englund, M. C., Heller, R. S., Hakansson, J., Fleckner, J., Skold, H. N. et al. (2004) Artifactual insulin release from differentiated embryonic stem cells. Diabetes **53**, 2603–2609

92 Rajagopal, J., Anderson, W. J., Kume, S., Martinez, O. I. and Melton, D. A. (2003) Insulin staining of ES cell progeny from insulin uptake. Science **299**, 363

93 Sipione, S., Eshpeter, A., Lyon, J. G., Korbutt, G. S. and Bleackley, R. C. (2004) Insulin expressing cells from differentiated embryonic stem cells are not β cells. Diabetologia **47**, 499–508

94 Tam, P. P., Williams, E. A. and Chan, W. Y. (1993) Gastrulation in the mouse embryo: ultrastructural and molecular aspects of germ layer morphogenesis. Microsc. Res. Tech. **26**, 301–328

95 Wells, J. M. and Melton, D. A. (1999) Vertebrate endoderm development. Annu. Rev. Cell Dev. Biol. **15**, 393–410

96 Wilson, M. E., Scheel, D. and German, M. S. (2003) Gene expression cascades in pancreatic development. Mech. Dev. **120**, 65–80

97 Kubo, A., Shinozaki, K., Shannon, J. M., Kouskoff, V., Kennedy, M., Woo, S., Fehling, H. J. and Keller, G. (2004) Development of definitive endoderm from embryonic stem cells in culture. Development **131**, 1651–1662

98 D'Amour, K. A., Agulnick, A. D., Eliazer, S., Kelly, O. G., Kroon, E. and Baetge, E. E. (2005) Efficient differentiation of human embryonic stem cells to definitive endoderm. Nat. Biotechnol. **23**, 1534–1541

99 D'Amour, K. A., Bang, A. G., Eliazer, S., Kelly, O. G., Agulnick, A. D., Smart, N. G., Moorman, M. A., Kroon, E., Carpenter, M. K. and Baetge, E. E. (2006) Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. Nat. Biotechnol. **24**, 1392–1401

100 Kroon, E., Martinson, L. A., Kadoya, K., Bang, A. G., Kelly, O. G., Eliazer, S., Young, H., Richardson, M., Smart, N. G., Cunningham, J. et al. (2008) Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells *in vivo*. Nat. Biotechnol. **26**, 443–452

101 Castaing, M., Peault, B., Basmaciogullari, A., Casal, I., Czernichow, P. and Scharfmann, R. (2001) Blood glucose normalization upon transplantation of human embryonic pancreas into β-cell-deficient SCID mice. Diabetologia **44**, 2066–2076

102 King, A. J., Fernandes, J. R., Hollister-Lock, J., Nienaber, C. E., Bonner-Weir, S. and Weir, G. C. (2007) Normal relationship of β- and non-β-cells not needed for successful islet transplantation. Diabetes **56**, 2312–2318

103 Bonner-Weir, S. (2000) Islet growth and development in the adult. J. Mol. Endocrinol. **24**, 297–302

104 Wilmut, I., Schnieke, A. E., McWhir, J., Kind, A. J. and Campbell, K. H. (1997) Viable offspring derived from fetal and adult mammalian cells. Nature **385**, 810–813

105 Yu, J., Vodyanik, M. A., Smuga-Otto, K., Antosiewicz-Bourget, J., Frane, J. L., Tian, S., Nie, J., Jonsdottir, G. A., Ruotti, V., Stewart, R. et al. (2007) Induced pluripotent stem cell lines derived from human somatic cells. Science **318**, 1917–1920

106 Holm, S. (2008) Time to reconsider stem cell ethics: the importance of induced pluripotent cells. J. Med. Ethics **34**, 63–64