

Current Status of Islet Cell Replacement and Regeneration Therapy

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Context: B cell mass and function are decreased to varying degrees in both type 1 and type 2 diabetes. In the future, islet cell replacement or regeneration therapy may thus offer therapeutic benefit to people with diabetes, but there are major challenges to be overcome.

Evidence Acquisition: A review of published peer-reviewed medical literature on β -cell development and regeneration was performed. Only publications considered most relevant were selected for citation, with particular attention to the period 2000–2009 and the inclusion of earlier landmark studies.

Evidence Synthesis: Islet cell regenerative therapy could be achieved by *in situ* regeneration or implantation of cells previously derived *in vitro*. Both approaches are being explored, and their ultimate success will depend on the ability to recapitulate key events in the normal development of the endocrine pancreas to derive fully differentiated islet cells that are functionally normal. There is also debate as to whether β -cells alone will assure adequate metabolic control or whether it will be necessary to regenerate islets with their various cell types and unique integrated function. Any approach must account for the potential dangers of regenerative therapy.

Conclusions: Islet cell regenerative therapy may one day offer an improved treatment of diabetes and potentially a cure. However, the various approaches are at an early stage of preclinical development and should not be offered to patients until shown to be safe as well as more efficacious than existing therapy. (*J Clin Endocrinol Metab* 95: 1034–1043, 2010)

Diabetes is a devastating chronic disease afflicting hundreds of millions of individuals: it is expected to increase inexorably over the coming decades. There is currently no cure for diabetes, and despite great improvements in treatment, the morbidity and increased mortality associated with diabetic complications are still a major concern. Unfortunately, most existing therapies fail to provide adequate control. Thus, there is an urgent need for more effective and durable management of diabetes, and ultimately a cure. This review considers just one such approach: islet cell regeneration therapy, embracing both the

replacement/implantation and *bona fide* regeneration of islet cells in a patient. It is not our purpose to provide a comprehensive review of the literature but rather to describe to the clinician how such therapy may be achieved, where we stand today, and the technical as well as ethical challenges for the future.

Setting the stage: lessons from islet transplantation

The rationale for β -cell replacement therapy is driven by not only the near total deficiency of the cells in type 1

diabetes but also the now accepted finding that β -cell mass is reduced in type 2 diabetes (1, 2). Although many patients benefit from pancreas transplants, the drawbacks remain major surgery with a high complication rate and the need for immunosuppression. With simultaneous pancreas kidney transplants, the major benefit is from the kidney, with increased long-term survival (3); the addition of a pancreas improves quality of life and hypoglycemia unawareness but has only modest effects on diabetic complications (4). Serious work on islet replacement began in the early 1970s with the demonstration that isolated islets could reverse diabetes in rodents (5), but the first convincing clinical demonstration was achieved only in 1989 (6). Although the pioneering centers could only provide short-term insulin independence in about 10% of their transplant recipients, even this seemingly modest success was extremely important for individuals with type 1 diabetes, offering the chance of life without diabetes.

A new era opened in 2000 with the report from Edmonton that seven consecutive subjects with type 1 diabetes were rendered insulin independent with islet transplants (7); more current data suggest that 72% of all recipients became insulin independent (8). Because one of the most common indications for transplantation was disabling hypoglycemia, a major success was the striking elimination of this problem. Another success has been the demonstration that increasing numbers of recipients can become insulin independent with infusion of islets from only one donor (9). Unfortunately, sobering realities accompanied this success. Perhaps the biggest disappointment was lack of durability such that more than 50% of those with insulin independence were back on insulin within 2 yr (10). On the positive side, despite this return to insulin injections, the accompanying continued (low grade) insulin secretion from the remaining graft still smoothed glycemic control in many of these patients and reduced the incidence of hypoglycemia. However, progression to complete graft failure occurred within a few years. This rapid loss of function is in contrast to the better durability of islet autotransplants (11), leading to the conclusion that graft loss is largely due to continuing assault by autoimmunity and allojection as well as toxicity of immunosuppressive drugs. In addition to the problem of graft durability, there are risks from both the transplant procedure and immunosuppression (8).

Whereas we can look forward to better outcomes from continuing improvements in immunosuppression and the quality of islets, because of the limited islet supply, we can expect only hundreds rather than thousands of transplants annually for the next several years (the maximum number of available pancreases in the United States is only about 4000 per year and only a minority of these will provide islets of sufficient quality).

Moving to the postcadaveric islet transplantation era

The continuing work with transplants using cadaver islets prepares us for a new era of better sources of insulin producing cells and protection of these cells from immunological destruction. For the cell source problem, an extraordinary amount of progress is being made with stem cell biology, transdifferentiation, and xenotransplantation, and there are indications that β -cell regeneration within the pancreas may be possible. Although not the major focus of this review, it will obviously be necessary to protect new β -cells, regardless of their origin, from renewed autoimmune attack in individuals with type 1 diabetes and rejection in the example of xeno- or allotransplantation. Suffice it to say that progress is being made with our understanding of immunological tolerance and inflammation, and protective immunological barriers may prove useful.

The possibility of transplanting porcine islet cells continues to receive considerable attention because of their availability and similarities between porcine and human glucose metabolism. The hyperacute rejection of xenotransplants caused by preformed antibodies interacting with the Gal- α -Gal epitope is a major problem with organ transplantation but less so with cellular transplants. Strikingly improved results have been obtained in nonhuman primates using adult porcine islets (12), neonatal pancreatic cell clusters (13), or embryonic pancreatic tissue (14). Although these results are encouraging, there are concerns as to whether the immunosuppression used for these experiments in nonhuman primates will be efficacious in humans and not too toxic. Active research to control xenorejection includes the production of transgenic pigs to make islets more resistant to rejection by various maneuvers including removal of the Gal- α -Gal epitope (13) and the use of immunobarriers. For more than 30 yr, there has indeed been exploration of the potential of semipermeable membranes to protect islets from immune destruction (15). The principle is that glucose, nutrients, and oxygen can reach the encapsulated cells and insulin can be released, but immune cells and perhaps antibodies are excluded. Various configurations have been developed. Those receiving the most attention include microencapsulation in which islets are contained in small (400–1000 μ m) beads of an alginate gel and macroencapsulation in which islets are enclosed within an implantable device consisting of planar sheets.

Progress has been frustrating because impressive success in rodents (16) has not yet been reliably reproduced in large animals or humans. The question of how much permselectivity is needed is very complex. It is possible that exclusion of cells will be the only requirement and that

cytokine penetration will not be an issue. One can wonder about the need to restrict the release of antigens. The bigger issue is probably the biocompatibility of the materials. An inflammatory reaction with neutrophils and macrophages on the outside will result in dead islet cells inside, probably due to suffocation by competition for oxygen rather than by cytokines. If the biomaterials are inert, there should be no attached cells, which raises the question of where cytokines would come from and whether they would ever threaten the islet cells inside. Despite the difficulties, the work continues because safe control of autoimmunity and transplant rejection may take many years to achieve.

Regeneration and transdifferentiation

In human pancreas, it now seems clear that there is a slow rate of β -cell turnover whereby β -cells replicate and new islets are formed, probably from exocrine duct cells through the process of neogenesis (1, 17, 18). The relative rates of these two processes are not known, but the rate of β -cell replication seems to slow with age and neogenesis can be stimulated by injury. After decades of type 1 diabetes, small numbers of β -cells can invariably be found in the pancreas, supporting the concept that β -cells continue to be formed throughout life but are then killed by autoimmunity. Limits to the capacity for regeneration in type 2 diabetes are exemplified by pathological studies that do not find evidence for active regeneration (1).

Approaches to *in situ* regeneration are to stimulate either β -cell replication or neogenesis. *In vitro* expansion is also possible, in a process likely to involve dedifferentiation of β -cells in tissue culture, proliferation, and then redifferentiation (19, 20). Success with such an *in vitro* intervention on islets derived from cadavers could lead to an expanded population of β -cells suitable for transplantation. Finding a molecular intervention that can be safely used *in vivo* seems more challenging but not impossible.

There are ways in which neogenesis might be stimulated to expand β -cell mass. Based on animal experiments, agents that may contribute include exendin-4, an analog of the incretin hormone glucagon-like-peptide-1; gastrin; epidermal growth factor; and islet neogenesis-associated protein (21). Much work is now underway to determine the potential of these agents used alone or in combination.

Transdifferentiation is a process in which a differentiated cell can be reprogrammed to change its identity. A considerable amount of work has been devoted to trying to convert cells in the liver into β -cells (22, 23), and this effort continues (24, 25). An exciting new possibility has emerged with the demonstration that pancreatic acinar cells may possibly be reprogrammed in mice with injections into the pancreas of adenoviruses expressing

just three transcription factors, pancreatic duodenal homeobox-1, musculoaponeurotic fibrosarcoma oncogene homolog A, and neurogenin-3 (26). However, until this study is developed further, it is not possible to distinguish clearly between such reprogramming of acinar cells and β -cell neogenesis from precursor cells.

Recapitulating normal development of the endocrine pancreas

Early attempts to generate β -cells directly from embryonic stem (ES) cells or other cell sources, although apparently successful in producing cells that contained insulin (27–29), failed to produce true β -cells (30, 31). Usually these cells expressed very little insulin, failed to respond adequately to normal secretagogues like glucose, and had non- β -cell characteristics, often looking suspiciously like neurons (32). In fact, during normal development in mammals, certain non- β -cells produce low amounts of insulin, including cells in the extraembryonic membranes and neurons in the central nervous system (33); these early attempts likely generated some of these interesting, but ultimately clinically impractical, cells. One lesson from these efforts was that recapitulation of normal development was the surest route to normal β -cells.

During normal development, the pancreas, and subsequently β -cells, arise from the endoderm germ layer (for basic reviews see Refs. 34–36). Early protocols for generating β -cells from ES cells did not attempt to go through endoderm, and this shortcut may have ultimately doomed those efforts. In the past few years, however, several groups have succeeded in generating endoderm (37, 38). This effort has been rewarded by more efficient derivation from human ES cells of insulin-producing cells containing more insulin and with more β -cell-like characteristics (39). However, these *in vitro*-derived cells still fail to function like normal β -cells [even if maturation *in vivo* after implantation of the β -like cells into mice has been shown to promote their further differentiation toward a more convincing β -cell phenotype (40)], and once again normal development may hold the answers.

From mouse studies we know that there are four phases of β -cell generation. Before embryonic day (e) 13 in the mouse pancreas, the few endocrine cells generated (predominantly glucagon with a few insulin producing cells) differ from normal α - and β -cells (41, 42): the insulin-expressing cells contain low levels of insulin, often coexpress glucagon, and lack the mature β -cell markers Nkx6.1, MafA, and pancreatic duodenal homeobox-1. Then at e13, β -cell neogenesis accelerates dramatically, and these new cells look like mature β -cells (although they may still lack robust glucose stimulated insulin secretion). At the same time, the exocrine acinar cells start to differ-

entiate, and the progenitor cells become restricted to the ducts.

These synchronous differentiation events have been termed the secondary transition (43), and the atypical endocrine cells that differentiate before the secondary transition can be called primary endocrine cells to distinguish them from the mature islet cells that appear later. Although originally discussed 35 yr ago (43), the issue of the difference between primary endocrine cells and mature islet cells remains very relevant today because the insulin-producing cells generated to date from ES cells *in vitro* appear to have the characteristics of primary endocrine cells (39), rather than the normal mature β -cells of the secondary transition.

It is not clear whether the events that occur around the secondary transition are simply time dependent, result from some extracellular signal (44), or a combination of both. Whatever their basis, the pancreatic progenitor cells, from which the endocrine and exocrine cells differentiate, themselves undergo distinct morphological and gene expression changes during the secondary transition (41, 45, 46) and successively acquire the capacity to generate each of the mature islet cell types (45). Therefore, the stage of the progenitor cells may determine the type of endocrine cells they can generate (primary endocrine cells *vs.* mature islet cells). The process of differentiation of progenitor cells down the endocrine lineage is initiated by the transient expression of the transcription factor neurogenin-3, which then activates a cascade of transcription factors that drive differentiation and determine the final characteristics of the resultant endocrine cell (34–36).

The genesis of β -cells from the secondary progenitor cells peaks around e14–15 in the mouse and has largely ceased by e18 (47). The third phase of β -cell formation starts shortly before this termination: existing β -cells start to proliferate, resulting in a marked expansion in β -cells that lasts through the first few weeks of postnatal life (48, 49). A similar expansion occurs in human infants (50).

Unfortunately, we know the least about the final phase of β -cell generation: replacement or expansion of β -cells in the adult. We know it occurs and underlies the ability of β -cell mass to fluctuate in response to metabolic needs (1, 51), but we do not know the signals that drive the process, the source of the new cells, or the pathway by which they are generated. In mice, adult β -cell regeneration depends predominantly on the proliferation of preexisting β -cells rather than neogenesis (52), although in certain forms of pancreatic damage, neogenesis of β -cells through a neurogenin-3-expressing stage does occur (51). Humans, however, may use the two pathways, neogenesis and proliferation, quite differently (1). In any case, the cell of origin for this neogenesis pathway, whether a differentiated

adult cell or a professional stem cell, is not known, although in mice adult duct cells, which share certain characteristics of secondary pancreatic progenitor cells, can contribute to the pathway (18).

Islets *vs.* β -cells for regeneration therapy?

β -Cells exist in the highly specialized microenvironment of the islet, with other β - or non- β -cells as their immediate neighbors, in contact with extracellular matrix components deposited by themselves and/or endothelial cells, richly irrigated by the islet microvasculature and innervated. Contacts between islet cells (53) and with the extracellular matrix (54, 55) impact directly on β -cell function, survival, and replication. There is close coordination and cross talk between the different islet endocrine cell types, with profound consequences for normal glycemic control. In particular, postprandial glycemia is contained within physiological limits by the combination of increased insulin and decreased glucagon secretion. Ironically it has been recognized since 1970 that type 2 diabetes is the consequence of dysfunction of the islet as a microorgan, with insufficient insulin and excessive glucagon acting together to drive hyperglycemia (56). Despite new evidence for a direct effect of insulin on glucagon secretion (57), the jury is still out regarding exactly how β -cells speak to α -cells to regulate glucagon secretion (*i.e.* via secretion of insulin, Zn^{2+} or γ -aminobutyric acid); however, there is no doubt that such communication occurs and is important for coordinated islet hormone secretion (58). Finally, neural regulation of both insulin and glucagon secretion was demonstrated more than 30 yr ago (59). Even disregarding the additional complexity of the known cross talk among other islet cell types, it thus seems reasonable to anticipate notable differences in the function of β -cells on their own and those within islets. However, equivalent glycemic control has been demonstrated in diabetic mice transplanted with aggregates of pure β -cells or islets (60), even if no such equivalence has ever been demonstrated clinically.

Quality control and safety

β -Cells derived *in vitro* will have to be characterized completely before any clinical trials. The first step would involve phenotypic characterization, with comprehensive gene profiling and a complete battery of *in vitro* functional tests to monitor the dynamics of insulin secretion in response to glucose and other secretagogues. This would be followed by preclinical testing *in vivo*, first in small animals and next in nonhuman primates. This sounds perfectly straightforward, but in reality there are serious issues preventing clean interpretation of the data. Although we know much about the β -cell, it may hold more secrets.

It will be hard to decide exactly which functional aspects of this highly specialized cell are the most essential (61). What is the gold standard that surrogate β -cells must match to be given the go for clinical testing? Given the known differences between rodent and human β -cells on the one hand and between β -cells within an islet *vs.* pure cells on the other, a preparation of pure primary human β -cells will be the only valid comparator. Whereas it is feasible to obtain a highly enriched population of human β -cells sorted from other islet cell types (62), there are many uncontrollable variables that may impact the quality and performance of these cells, thereby compromising their validity as the comparator *vs.* surrogate β -cells. These would include the age, health, and cause of death of the donor; warm and cold ischemia time of the donor pancreas; and the isolation procedure itself (*i.e.* collagenase digestion and harvesting of islets).

Next, the cells will have to be tested *in vivo*. It is obvious that the first tests will have to be performed in rats or mice, and since 1972 we have certainly gained a lot of experience from studies using transplanted islets (5). However, glucose homeostasis differs from one species to another, and considerable care will have to be taken in extrapolating the data of glucose tolerance tests from rodents to man as discussed previously in greater detail (61).

Any attempt to treat individuals by regeneration therapy must of course first meet the strictest standards of safety. Even though this cannot be the major focus here, it must be recognized that all approaches raise major concerns with regard to safety. Any therapy based on increasing β -cell mass and function must have robust built-in measures to avoid an overshoot, with possible uncontrolled oversecretion of insulin and life-threatening hypoglycemia. Ensuring that newly regenerated or implanted β -cells are close to perfect in terms of differentiated function will be part of this safety measure. If the approach chosen is to make new cells, it is vital that we ensure that self proliferation is in check to avoid excessive insulin production with resultant hypoglycemia. If the chosen approach is to implant β -cells derived from ES or induced pluripotent stem (iPS) cells, there is the inherent risk of teratoma: no remaining pluripotent cells should be present in the implanted cell population. Indeed, the successful *in vivo* differentiation in mice of β -cells derived from human ES cells was accompanied by a high incidence of teratomas (40).

Complex requirements for insulin release by the replacement of β -cells *in vivo*

The development and use of β -cell replacement therapy, no matter what form it may take, will require the ultimate of the surrogate cells: they will have to secrete

insulin in a regulated manner while coping with ever-changing circumstances. These shifting situations will require the cells to not only increase insulin output in response to nutrient ingestion but also to decrease output appropriately as glucose levels decline.

The β -cell's response is larger when nutrients are presented orally than when administered *iv* (63). This difference, known as the incretin effect, results in part from the concomitant release of glucagon-like-peptide-1 and glucose-dependent insulinotropic peptide (64). Thus, the replacement cells will need to incorporate more than a single nutrient response system, and in turn these will need to act in concert to ensure appropriate timing of insulin release.

Whereas many may feel the critical aspect of β -cell function that will need to be reproduced is simply the release of insulin, one cannot overemphasize the vital importance of the timing of insulin exocytosis. It is now very well documented that the insulin response has to occur early if glucose is to be metabolized normally (65, 66). Thus, when this early response is deficient, impaired glucose tolerance or diabetes result (65, 66). Interestingly, under circumstances when glucose intolerance exists, the β -cell will frequently release more insulin late after nutrient presentation, but the timing is inappropriate and release is occurring only because the glucose levels have become excessively elevated. Thus, the replacement cells will have to respond rapidly if they are not simply going to lower glucose but do so efficiently and maintain glycemia well within the normal range. Last but by no means least, this need for a rapid and precise on switch for insulin secretion must be complemented by an equally responsive and dynamic off switch in response to falling glycemia (which would require intact cellular mechanisms responding to inputs such as catecholamines) that will ensure a prompt decrease in β -cell secretion if hypoglycemia is to be avoided.

Adapting to changing β -cell secretory demand: the need for differing β -cell function and mass

The ability of the β -cell to respond to stimulation is also critically modulated by differences in insulin sensitivity (67). Insulin resistance requires increased insulin output both in the basal state and in response to stimulation to maintain normal glucose tolerance, whereas improvements in insulin sensitivity place the β -cell in the position of having to reduce insulin release to avoid hypoglycemia. These changes in insulin sensitivity that require adjustment of insulin output can occur quite rapidly or over longer periods of time. The mechanisms responsible for these changes clearly vary and involve changes in both β -cell function and β -cell mass, although in most instances it appears that functional changes predominate. As dis-

cussed in greater detail previously (61), this flexibility will be an absolute and critical requirement of the replacement β -cells when glucose uptake increases over the short term, as occurs with exercise (68), or decreases rapidly, as an acute illness develops (69). In addition to functional adaptation to such rapid changes in insulin sensitivity, the β -cell must also alter its activity when this critical modulator changes for more prolonged periods. Under such conditions one envisages both β -cell secretory function and β -cell mass playing complementary roles. In pregnancy, during which there is an absolute need for increases in both basal and stimulated insulin release, the rather limited assessment of β -cell mass in humans suggests this to be increased (70), a finding well demonstrated in animals (71). However, function is also increased and typically beyond what the predicted increase in mass could be responsible for (72). Furthermore, the rapid decline in insulin demand that occurs immediately after parturition (73) underscores an additional critical flexibility the replacement β -cells will need to manifest in this instance.

In healthy subjects obesity alters the character of β -cells by requiring them to increase both their function and mass. These functional and morphological changes have been recognized for many years (74, 75), with current estimates being that in obese individuals β -cell function is increased on average about 3-fold (74, 76), whereas mass may be enhanced by only 20–40% (2, 75). Here again the changes are complementary, with the functional aspect being more rapidly regulated as demonstrated by the prompt decline in insulin release after bariatric surgery, well before marked weight loss has occurred (77). What mediates these adaptive changes is uncertain; however, a number of possibilities exist including alterations in plasma levels of glucose, fatty acids, or incretin hormones and changes in neural tone. Whereas the exact mechanism remains unclear, the replacement cells will have to be responsive to these substrates and peptides. If central nervous system control is a critical aspect, then the site of placement of these cells will have to ensure that neural signals reach them and that they are responsive.

Keeping the replacement cell and the recipient out of dangerous territory

The source of replacement cells will also in many ways dictate processes that could handicap cell function and possibly hamper their survival. Cells obtained from sources other than the recipient can be expected to require immunosuppression therapy. Individuals with type 1 diabetes will be confronted with both autoimmunity and allo- or xenorejection, whereas those with type 2 diabetes will be spared from autoimmunity. Immunosuppression is problematic because it can be associated with insulin resistance

(78) and can have an inhibitory effect on insulin release (79, 80), thereby making it even more difficult for the replacement cells to maintain optimal glucose control. Of additional importance is the observation that use of certain of these medications has been associated with an increased risk of malignancy [although proven only after kidney transplantation (81)], raising another specter altogether.

With type 1 diabetes, there may be value in monitoring T cell reactivity and some of the antibodies typically associated with this process such as insulin autoantibodies and glutamic acid decarboxylase, islet cell and IA-2 antibodies (82, 83). Recurrence and/or increasing titers of these antibodies may signal activity of the β -cell destruction process. With the current focus on immunotherapy to prevent the onset of type 1 diabetes in at-risk individuals (84), it is quite possible that advances in this area could also be applied to prevent disease progression in individuals who manifest antibodies after cell replacement therapy. Whether use of encapsulation will provide sufficient protection from immune assault and allow adequate β -cell function remains an important and unanswered question. It has recently been shown that it is possible to derive β -cells using iPS cells from an individual with type 1 diabetes (85). Whereas such cells will surely be useful for studies on the etiology and pathophysiology of type 1 diabetes, until methods have been developed for their protection, it seems likely that they would be targeted for rapid autoimmune destruction if implanted into that same individual.

Should cell replacement therapy prove a viable option for type 2 diabetes and especially if cells can be manufactured through manipulation of stem cells, the approach will surely be evaluated for potential widespread application. The concept of β -cell replacement therapy for type 2 diabetes has been more controversial because a substantial proportion of these patients are not insulin dependent. We know that β -cell replacement in the form of pancreas transplants can normalize glucose levels (86). Thus, transplantation of sufficient numbers of β -cells will also predictably reverse diabetes in these patients. However, if the replacement cells were to be derived from the host, there are two confounding issues. First, the identified genes for type 2 diabetes are predominantly associated with β -cell dysfunction (87, 88). Second, patients with the disease have reduced numbers and function of β -cells (89), the pathogenic basis of which has not yet been clearly delineated but that may be related to alterations in these so-called β -cell genes. Thus, implantation of replacement cells derived from an individual with type 2 diabetes may well not lead to sustained glucose control over time because of a decrease in either or both of these parameters.

This is akin to the situation in type 1 diabetes discussed above, although the autoimmune-based destruction of host-derived β -cells would be predicted to occur more rapidly.

Advances in genetics have clearly identified a number of genes to be associated with reduced β -cell function and/or mass in type 2 diabetes (90), which raises questions about whether such cells might secrete less insulin per cell and be lost to apoptosis at a faster rate. It will be interesting to learn whether the differential effects of medications believed to slow the progression of β -cell disease (91) will also prove beneficial for replacement cells. Another concern is islet amyloid, which occurs in the majority of patients with type 2 diabetes (92) and has recently been shown to occur in transplanted human islets (93) and in those derived from a transgenic mouse model of islet amyloid (94).

Conclusion and Other Considerations

There have been impressive advances in the derivation of β -like cells from human ES cells that provide great hope that such an approach may one day offer a plentiful source of β -cells for transplantation (95, 96). There continues to be remarkable progress in this field, including the identification of small molecules that promote the differentiation of human ES cells into pancreatic progenitor cells (97). There is also great excitement surrounding iPS cells (98) that may provide an alternative to ES cells for *in vitro* derivation of β -cells (99). The field has exploded since the original publication in 2006 (100), and most recently human iPS cells were produced by direct delivery of proteins into somatic cells without the need to introduce the corresponding genes (101). Importantly, iPS cells offer the tantalizing prospect of generating patient-specific β -cells, and they would also circumvent political, religious, or moral objections to use of ES cells that we shall not dwell on further here. *In situ* regeneration of islets may be a distant dream, but progress in the understanding of the development of the endocrine pancreas and the mechanism of adaptive β -cell regeneration (102) has provided a paradigm shift. Transdifferentiation may offer an interesting alternative approach, even if formidable obstacles still lie on the road to safe and controlled islet cell regeneration using either approach.

The clinical requirements being demanded of the replacement β -cells are challenging. They will need to adapt to ever-changing needs from their host, some of these being more rapid than others. Under some circumstances a change in β -cell mass may be required, and this clearly needs to occur in a controlled manner whether it be increasing or decreasing. Furthermore, the host and replace-

ment cells have to work in concert to reduce the likelihood that β -cells will be lost due to a recurrence of the destructive process that required their replacement in the first place. One could argue that if this were to occur, it may be possible to simply top up the β -cell reservoir. However, whereas this may seem and in reality be easy, we cannot begin to understand the possible long-term implications of doing so.

Of course, in addition to being as safe if not safer than existing therapies, islet cell regeneration therapy should provide a significant improvement in the management of the disease and its complications. Ideally, it should be readily available to all in need, regardless of where they live or their socioeconomic status. Quite understandably, individuals with diabetes and their families are increasingly impatient for better treatment and ultimately a cure. Improved communication of science has increased public awareness of major advances in biomedical research and demand for rapid translation of new discoveries from bench to bedside. Here too diabetes is at the forefront and individuals with this disease are almost always mentioned as likely beneficiaries when a breakthrough in stem cell research is communicated to the general public. This all provides fertile ground for unscrupulous medical practice: stem-cell based therapy is today's snake oil and diabetes is taking center stage in this modern age drama. Direct-to-consumer advertising drives stem cell tourism (103), feeding off patients' suffering and frustration in the absence of controlled investigation. The treatment that is offered has to be set against a background of peer review and clinical trials in accordance with guidelines for transition of stem cell therapy to clinical applications (104, 105), rather than relying on hearsay and unverifiable reports from patients.

In conclusion, there are many reasons to believe that islet cell replacement or regeneration therapy will become a clinical reality, in full respect of good clinical practice and providing true benefit to the patient, so reducing the burden of diabetes on the individual and society.

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References

- Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC 2003 β -cell deficit and increased β -cell apoptosis in humans with type 2 diabetes. *Diabetes* 52:102–110
- Rahier J, Guiot Y, Goebbels RM, Sempoux C, Henquin JC 2008 Pancreatic β -cell mass in European subjects with type 2 diabetes. *Diabetes Obes Metab* 10(Suppl 4):32–42
- Ojo AO, Meier-Kriesche HU, Hanson JA, Leichtman A, Magee JC, Cibrik D, Wolfe RA, Port FK, Agodoa L, Kaufman DB, Kaplan B 2001 The impact of simultaneous pancreas-kidney transplantation on long-term patient survival. *Transplantation* 71:82–90
- Larsen JL 2004 Pancreas transplantation: indications and consequences. *Endocr Rev* 25:919–946
- Ballinger WF, Lacy PE 1972 Transplantation of intact pancreatic islets in rats. *Surgery* 72:175–186
- Scharp DW, Lacy PE, Santiago JV, McCullough CS, Weide LG, Boyle PJ, Falqui L, Marchetti P, Ricordi C, Gingerich RL 1991 Results of our first nine intraportal islet allografts in type 1, insulin dependent diabetic patients. *Transplantation* 51:76–85
- Shapiro AM, Lakey JR, Ryan EA, Korbitt GS, Toth E, Warnock GL, Kneteman NM, Rajotte RV 2000 Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* 343:230–238
- Alejandro R, Barton FB, Hering BJ, Wease S 2008 Update from the Collaborative Islet Transplant Registry. *Transplantation* 86:1783–1788
- Hering BJ, Kandaswamy R, Ansite JD, Eckman PM, Nakano M, Sawada T, Matsumoto I, Ihm SH, Zhang HJ, Parkey J, Hunter DW, Sutherland DE 2005 Single-donor, marginal-dose islet transplantation in patients with type 1 diabetes. *JAMA* 293:830–835
- Ryan EA, Paty BW, Senior PA, Bigam D, Alfadhli E, Kneteman NM, Lakey JR, Shapiro AM 2005 Five-year follow-up after clinical islet transplantation. *Diabetes* 54:2060–2069
- Blondet JJ, Carlson AM, Kobayashi T, Jie T, Bellin M, Hering BJ, Freeman ML, Beilman GJ, Sutherland DE 2007 The role of total pancreatectomy and islet autotransplantation for chronic pancreatitis. *Surg Clin North Am* 87:1477–1501, x
- Hering BJ, Wijkstrom M, Graham ML, Hårdstedt M, Aasheim TC, Jie T, Ansite JD, Nakano M, Cheng J, Li W, Moran K, Christians U, Finnegan C, Mills CD, Sutherland DE, Bansal-Pakala P, Murtaugh MP, Kirchoff N, Schuurman HJ 2006 Prolonged diabetes reversal after intraportal xenotransplantation of wild-type porcine islets in immunosuppressed nonhuman primates. *Nat Med* 12:301–303
- Cardona K, Korbitt GS, Milas Z, Lyon J, Cano J, Jiang W, Bello-Laborin H, Hacquoil B, Strobert E, Gangappa S, Weber CJ, Pearson TC, Rajotte RV, Larsen CP 2006 Long-term survival of neonatal porcine islets in nonhuman primates by targeting costimulation pathways. *Nat Med* 12:304–306
- Hecht G, Eventov-Friedman S, Rosen C, Shezen E, Tchorsh D, Aronovich A, Freud E, Golan H, El-Hasid R, Katchman H, Hering BJ, Zung A, Kra-Oz Z, Shaked-Mishan P, Yusim A, Shtabsky A, Idelevitch P, Tobar A, Harmelin A, Bachar-Lustig E, Reisner Y 2009 Embryonic pig pancreatic tissue for the treatment of diabetes in a nonhuman primate model. *Proc Natl Acad Sci USA* 106:8659–8664
- Colton CK 1995 Implantable biohybrid artificial organs. *Cell Transplant* 4:415–436
- Duvivier-Kali VF, Omer A, Parent RJ, O'Neil JJ, Weir GC 2001 Complete protection of islets against allojection and autoimmunity by a simple barium-alginate membrane. *Diabetes* 50:1698–1705
- Bonner-Weir S 2000 Life and death of the pancreatic beta cells. *Trends Endocrinol Metab* 11:375–378
- Inada A, Nienaber C, Katsuta H, Fujitani Y, Levine J, Morita R, Sharma A, Bonner-Weir S 2008 Carbonic anhydrase II-positive pancreatic cells are progenitors for both endocrine and exocrine pancreas after birth. *Proc Natl Acad Sci USA* 105:19915–19919
- Ouziel-Yahalom L, Zalzman M, Anker-Kitai L, Knoller S, Bar Y, Glandt M, Herold K, Efrat S 2006 Expansion and redifferentiation of adult human pancreatic islet cells. *Biochem Biophys Res Commun* 341:291–298
- Bar Y, Russ HA, Knoller S, Ouziel-Yahalom L, Efrat S 2008 HES-1 is involved in adaptation of adult human β -cells to proliferation *in vitro*. *Diabetes* 57:2413–2420
- Bonner-Weir S, Weir GC 2005 New sources of pancreatic β -cells. *Nat Biotechnol* 23:857–861
- Ferber S, Halkin A, Cohen H, Ber I, Einav Y, Goldberg I, Barshack I, Seiffers J, Kopolovic J, Kaiser N, 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
- Meivar-Levy I, Ferber S 2003 New organs from our own tissues: liver-to-pancreas transdifferentiation. *Trends Endocrinol Metab* 14:460–466
- Yeohor V, Liu V, Espiritu C, Paul A, Oka K, Kojima H, Chan L 2009 Neurogenin3 is sufficient for transdetermination of hepatic progenitor cells into neo-islets *in vivo* but not transdifferentiation of hepatocytes. *Dev Cell* 16:358–373
- Nagaya M, Katsuta H, Kaneto H, Bonner-Weir S, Weir GC 2009 Adult mouse intrahepatic biliary epithelial cells induced *in vitro* to become insulin-producing cells. *J Endocrinol* 201:37–47
- Zhou Q, Brown J, Kanarek A, Rajagopal J, Melton DA 2008 *In vivo* reprogramming of adult pancreatic exocrine cells to β -cells. *Nature* 455:627–632
- Hori Y, Rulifson IC, Tsai BC, Heit JJ, Cahoy JD, Kim SK 2002 Growth inhibitors promote differentiation of insulin-producing tissue from embryonic stem cells. *Proc Natl Acad Sci USA* 99:16105–16110
- Lumelsky N, Blondel O, Laeng P, Velasco I, Ravin R, McKay R 2001 Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. *Science* 292:1389–1394
- Soria B, Roche E, Berná G, León-Quinto T, Reig JA, Martín F 2000 Insulin-secreting cells derived from embryonic stem cells normalize glycemia in streptozotocin-induced diabetic mice. *Diabetes* 49:157–162
- Hansson M, Tonning A, Frandsen U, Petri A, Rajagopal J, Englund MC, Heller RS, Håkansson J, Fleckner J, Sköld HN, Melton D, Semb H, Serup P 2004 Artificial insulin release from differentiated embryonic stem cells. *Diabetes* 53:2603–2609
- Rajagopal J, Anderson WJ, Kume S, Martinez OI, Melton DA 2003 Insulin staining of ES cell progeny from insulin uptake. *Science* 299:363
- Sipione S, Eshpeter A, Lyon JG, Korbitt GS, Bleackley RC 2004 Insulin expressing cells from differentiated embryonic stem cells are not β cells. *Diabetologia* 47:499–508
- Devaskar SU, Giddings SJ, Rajakumar PA, Carnaghi LR, Menon RK, Zahm DS 1994 Insulin gene expression and insulin synthesis in mammalian neuronal cells. *J Biol Chem* 269:8445–8454
- Jørgensen MC, Ahnfelt-Rønne J, Hald J, Madsen OD, Serup P, Hecksher-Sørensen J 2007 An illustrated review of early pancreas development in the mouse. *Endocr Rev* 28:685–705
- Murtaugh LC 2007 Pancreas and β -cell development: from the actual to the possible. *Development* 134:427–438
- Wilson ME, Scheel D, German MS 2003 Gene expression cascades in pancreatic development. *Mech Dev* 120:65–80
- Kubo A, Shinozaki K, Shannon JM, Kouskoff V, Kennedy M, Woo

- S, Fehling HJ, Keller G 2004 Development of definitive endoderm from embryonic stem cells in culture. *Development* 131:1651–1662
38. D'Amour KA, Agulnick AD, Eliazar S, Kelly OG, Kroon E, Baetge EE 2005 Efficient differentiation of human embryonic stem cells to definitive endoderm. *Nat Biotechnol* 23:1534–1541
 39. D'Amour KA, Bang AG, Eliazar S, Kelly OG, Agulnick AD, Smart NG, Moorman MA, Kroon E, Carpenter MK, Baetge EE 2006 Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. *Nat Biotechnol* 24:1392–1401
 40. Kroon E, Martinson LA, Kadoya K, Bang AG, Kelly OG, Eliazar S, Young H, Richardson M, Smart NG, Cunningham J, Agulnick AD, D'Amour KA, Carpenter MK, Baetge EE 2008 Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells *in vivo*. *Nat Biotechnol* 26:443–452
 41. Kim SK, MacDonald RJ 2002 Signaling and transcriptional control of pancreatic organogenesis. *Curr Opin Genet Dev* 12:540–547
 42. Wilson ME, Kalamaras JA, German MS 2002 Expression pattern of IAPP and prohormone convertase 1/3 reveals a distinctive set of endocrine cells in the embryonic pancreas. *Mech Dev* 115:171–176
 43. Pictet R, Rutter WJ 1972 Development of the embryonic endocrine pancreas. In: Society AP, Steiner DF, Frenkel N, eds. *Handbook of physiology*. Washington, DC: Williams and Wilkins; 25–66
 44. Levine S, Pictet R, Rutter WJ 1973 Control of cell proliferation and cytodifferentiation by a factor reacting with the cell surface. *Nat New Biol* 246:49–52
 45. Johansson KA, Dursun U, Jordan N, Gu G, Beermann F, Gradwohl G, Grapin-Botton A 2007 Temporal control of neurogenin3 activity in pancreas progenitors reveals competence windows for the generation of different endocrine cell types. *Dev Cell* 12:457–465
 46. Lynn FC, Smith SB, Wilson ME, Yang KY, Nekrep N, German MS 2007 Sox9 coordinates a transcriptional network in pancreatic progenitor cells. *Proc Natl Acad Sci USA* 104:10500–10505
 47. Schwitzgebel VM, Scheel DW, Conners JR, Kalamaras J, Lee JE, Anderson DJ, Sussel L, Johnson JD, German MS 2000 Expression of neurogenin3 reveals an islet cell precursor population in the pancreas. *Development* 127:3533–3542
 48. Finegood DT, Scaglia L, Bonner-Weir S 1995 Dynamics of β -cell mass in the growing rat pancreas. Estimation with a simple mathematical model. *Diabetes* 44:249–256
 49. Sander M, Sussel L, Conners J, Scheel D, Kalamaras J, Dela Cruz F, Schwitzgebel V, Hayes-Jordan A, German M 2000 Homeobox gene Nkx6.1 lies downstream of Nkx2.2 in the major pathway of β -cell formation in the pancreas. *Development* 127:5533–5540
 50. Meier JJ, Butler AE, Saisho Y, Monchamp T, Galasso R, Bhushan A, Rizza RA, Butler PC 2008 β -cell replication is the primary mechanism subserving the postnatal expansion of β -cell mass in humans. *Diabetes* 57:1584–1594
 51. Xu X, D'Hoker J, Stangé G, Bonnè S, De Leu N, Xiao X, Van de Castele M, Mellitzer G, Ling Z, Pipeleers D, Bouwens L, Scharfmann R, Gradwohl G, Heimberg H 2008 β Cells can be generated from endogenous progenitors in injured adult mouse pancreas. *Cell* 132:197–207
 52. Dor Y, Brown J, Martinez OI, Melton DA 2004 Adult pancreatic β -cells are formed by self-duplication rather than stem-cell differentiation. *Nature* 429:41–46
 53. Jaques F, Jousset H, Tomas A, Prost AL, Wollheim CB, Irminger JC, Demaurex N, Halban PA 2008 Dual effect of cell-cell contact disruption on cytosolic calcium and insulin secretion. *Endocrinology* 149:2494–2505
 54. Hammar E, Parnaud G, Bosco D, Perriraz N, Maedler K, Donath M, Rouiller DG, Halban PA 2004 Extracellular matrix protects pancreatic β -cells against apoptosis: role of short- and long-term signaling pathways. *Diabetes* 53:2034–2041
 55. Parnaud G, Hammar E, Ribaux P, Donath MY, Berney T, Halban PA 2009 Signaling pathways implicated in the stimulation of β -cell proliferation by extracellular matrix. *Mol Endocrinol* 23:1264–1271
 56. Müller WA, Faloona GR, Aguilar-Parada E, Unger RH 1970 Abnormal α -cell function in diabetes. Response to carbohydrate and protein ingestion. *N Engl J Med* 283:109–115
 57. Kawamori D, Kurpad AJ, Hu J, Liew CW, Shih JL, Ford EL, Herrera PL, Polonsky KS, McGuinness OP, Kulkarni RN 2009 Insulin signaling in α cells modulates glucagon secretion *in vivo*. *Cell Metab* 9:350–361
 58. Gromada J, Franklin I, Wollheim CB 2007 α -Cells of the endocrine pancreas: 35 years of research but the enigma remains. *Endocr Rev* 28:84–116
 59. Marliss EB, Girardier L, Seydoux J, Wollheim CB, Kanazawa Y, Orci L, Renold AE, Porte Jr D 1973 Glucagon release induced by pancreatic nerve stimulation in the dog. *J Clin Invest* 52:1246–1259
 60. King AJ, Fernandes JR, Hollister-Lock J, Nienaber CE, Bonner-Weir S, Weir GC 2007 Normal relationship of β - and non- β -cells not needed for successful islet transplantation. *Diabetes* 56:2312–2318
 61. Halban PA, Kahn SE, Lernmark A, Rhodes CJ 2001 Gene and cell-replacement therapy in the treatment of type 1 diabetes: how high must the standards be set? *Diabetes* 50:2181–2191
 62. Parnaud G, Bosco D, Berney T, Pattou F, Kerr-Conte J, Donath MY, Bruun C, Mandrup-Poulsen T, Billestrup N, Halban PA 2008 Proliferation of sorted human and rat β cells. *Diabetologia* 51:91–100
 63. Creutzfeldt W 1979 The incretin concept today. *Diabetologia* 16:75–85
 64. Drucker DJ 2006 The biology of incretin hormones. *Cell Metab* 3:153–165
 65. Jensen CC, Cnop M, Hull RL, Fujimoto WY, Kahn SE, American Diabetes Association GENNID Study Group 2002 β -Cell function is the major determinant of oral glucose tolerance in four ethnic groups in the United States. *Diabetes* 51:2170–2178
 66. Mitrakou A, Kelley D, Mokan M, Veneman T, Pangburn T, Reilly J, Gerich J 1992 Role of reduced suppression of glucose production and diminished early insulin release in impaired glucose tolerance. *N Engl J Med* 326:22–29
 67. Kahn SE, Prigeon RL, McCulloch DK, Boyko EJ, Bergman RN, Schwartz MW, Neifing JL, Ward WK, Beard JC, Palmer JP, Porte Jr D 1993 Quantification of the relationship between insulin sensitivity and B-cell function in human subjects. Evidence for a hyperbolic function. *Diabetes* 42:1663–1672
 68. Goodyear LJ, Kahn BB 1998 Exercise, glucose transport, and insulin sensitivity. *Annu Rev Med* 49:235–261
 69. Zauner A, Nimmerichter P, Anderwald C, Bischof M, Schiefermeier M, Ratheiser K, Schneeweiss B, Zauner C 2007 Severity of insulin resistance in critically ill medical patients. *Metabolism* 56:1–5
 70. Van Assche FA, Aerts L, De Prins F 1978 A morphological study of the endocrine pancreas in human pregnancy. *Br J Obstet Gynaecol* 85:818–820
 71. Sorenson RL, Brelje TC 1997 Adaptation of islets of Langerhans to pregnancy: β -cell growth, enhanced insulin secretion and the role of lactogenic hormones. *Horm Metab Res* 29:301–307
 72. Buchanan TA, Metzger BE, Freinkel N, Bergman RN 1990 Insulin sensitivity and B-cell responsiveness to glucose during late pregnancy in lean and moderately obese women with normal glucose tolerance or mild gestational diabetes. *Am J Obstet Gynecol* 162:1008–1014
 73. Jovanovic L, Peterson CM 1982 Optimal insulin delivery for the pregnant diabetic patient. *Diabetes Care* 5(Suppl 1):24–37
 74. Bagdade JD, Bierman EL, Porte Jr D 1967 The significance of basal insulin levels in the evaluation of the insulin response to glucose in diabetic and nondiabetic subjects. *J Clin Invest* 46:1549–1557
 75. Klöppel G, Löhr M, Habich K, Oberholzer M, Heitz PU 1985 Islet pathology and the pathogenesis of type 1 and type 2 diabetes mellitus revisited. *Surv Synth Pathol Res* 4:110–125

76. Beard JC, Ward WK, Halter JB, Wallum BJ, Porte Jr D 1987 Relationship of islet function to insulin action in human obesity. *J Clin Endocrinol Metab* 65:59–64
77. Mari A, Manco M, Guidone C, Nanni G, Castagneto M, Mingrone G, Ferrannini E 2006 Restoration of normal glucose tolerance in severely obese patients after bilio-pancreatic diversion: role of insulin sensitivity and β cell function. *Diabetologia* 49:2136–2143
78. Beard JC, Halter JB, Best JD, Pfeifer MA, Porte Jr D 1984 Dexamethasone-induced insulin resistance enhances B-cell responsiveness to glucose level in normal men. *Am J Physiol* 247:E592–E596
79. Kalhan SC, Adam PAJ 1975 Inhibitory effect of prednisone on insulin secretion in man: model for duplication of blood glucose concentration. *J Clin Endocrinol Metab* 41:600–610
80. Matsumoto K, Yamasaki H, Akazawa S, Sakamaki H, Ishibashi M, Abiru N, Uotani S, Matsuo H, Yamaguchi Y, Tokuyama K, Nagataki S 1996 High-dose but not low-dose dexamethasone impairs glucose tolerance by inducing compensatory failure of pancreatic β -cells in normal men. *J Clin Endocrinol Metab* 81:2621–2626
81. Vajdic CM, van Leeuwen MT 2009 Cancer incidence and risk factors after solid organ transplantation. *Int J Cancer* 125:1747–1754
82. Laughlin E, Burke G, Pugliese A, Falk B, Nepom G 2008 Recurrence of autoreactive antigen-specific CD4+ T cells in autoimmune diabetes after pancreas transplantation. *Clin Immunol* 128:23–30
83. Huurman VA, Hilbrands R, Pinkse GG, Gillard P, Duinkerken G, van de Linde P, van der Meer-Prins PM, Versteeg-van der Voort Maarschalk MF, Verbeeck K, Alizadeh BZ, Mathieu C, Gorus FK, Roelen DL, Claas FH, Keymeulen B, Pipeleers DG, Roep BO 2008 Cellular islet autoimmunity associates with clinical outcome of islet cell transplantation. *PLoS One* 3:e2435
84. Brusko TM, Putnam AL, Bluestone JA 2008 Human regulatory T cells: role in autoimmune disease and therapeutic opportunities. *Immunol Rev* 223:371–390
85. Mahr R, Chen S, Snitow M, Ludwig T, Yagasaki L, Goland R, Leibel RL, Melton DA 2009 Generation of pluripotent stem cells from patients with type 1 diabetes. *Proc Natl Acad Sci USA* 106:15768–15773
86. Nath DS, Gruessner AC, Kandaswamy R, Gruessner RW, Sutherland DE, Humar A 2005 Outcomes of pancreas transplants for patients with type 2 diabetes mellitus. *Clin Transplant* 19:792–797
87. Staiger H, Machicao F, Fritsche A, Häring HU 2009 Pathomechanisms of type 2 diabetes genes. *Endocr Rev* 30:557–585
88. Groop L, Lyssenko V 2009 Genetic basis of β -cell dysfunction in man. *Diabetes Obes Metab* 11(Suppl 4):149–158
89. Kahn SE, Zraika S, Utzschneider KM, Hull RL 2009 The β cell lesion in type 2 diabetes: there has to be a primary functional abnormality. *Diabetologia* 52:1003–1012
90. Florez JC 2008 Newly identified loci highlight β cell dysfunction as a key cause of type 2 diabetes: where are the insulin resistance genes? *Diabetologia* 51:1100–1110
91. Kahn SE, Haffner SM, Heise MA, Herman WH, Holman RR, Jones NP, Kravitz BG, Lachin JM, O'Neill MC, Zinman B, Viberti G 2006 Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. *N Engl J Med* 355:2427–2443
92. Hull RL, Westermark GT, Westermark P, Kahn SE 2004 Islet amyloid: a critical entity in the pathogenesis of type 2 diabetes. *J Clin Endocrinol Metab* 89:3629–3643
93. Westermark GT, Westermark P, Berne C, Korsgren O 2008 Widespread amyloid deposition in transplanted human pancreatic islets. *N Engl J Med* 359:977–979
94. Udayasankar J, Kodama K, Hull RL, Zraika S, Aston-Mourney K, Subramanian SL, Tong J, Faulenbach MV, Vidal J, Kahn SE 2009 Amyloid formation results in recurrence of hyperglycaemia following transplantation of human IAPP transgenic mouse islets. *Diabetologia* 52:145–153
95. Evans-Molina C, Westermark GL, Mirmira RG 2009 Development of insulin-producing cells from primitive biologic precursors. *Curr Opin Organ Transplant* 14:56–63
96. Guo T, Hebrok M 2009 Stem cells to pancreatic β -cells: new sources for diabetes cell therapy. *Endocr Rev* 30:214–227
97. Chen S, Borowiak M, Fox JL, Mahr R, Osafune K, Davidow L, Lam K, Peng LF, Schreiber SL, Rubin LL, Melton D 2009 A small molecule that directs differentiation of human ESCs into the pancreatic lineage. *Nat Chem Biol* 5:258–265
98. Takahashi K, Yamanaka S 2006 Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126:663–676
99. Tateishi K, He J, Taranova O, Liang G, D'Alessio AC, Zhang Y 2008 Generation of insulin-secreting islet-like clusters from human skin fibroblasts. *J Biol Chem* 283:31601–31607
100. Baker M 2009 Stem cells: fast and furious. *Nature* 458:962–965
101. Kim D, Kim CH, Moon JI, Chung YG, Chang MY, Han BS, Ko S, Yang E, Cha KY, Lanza R, Kim KS 2009 Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins. *Cell Stem Cell* 4:472–476
102. Sachdeva MM, Stoffers DA 2009 Minireview: meeting the demand for insulin: molecular mechanisms of adaptive postnatal β -cell mass expansion. *Mol Endocrinol* 23:747–758
103. Lau D, Ogbogu U, Taylor B, Stafinski T, Menon D, Caulfield T 2008 Stem cell clinics online: the direct-to-consumer portrayal of stem cell medicine. *Cell Stem Cell* 3:591–594
104. Lindvall O, Hyun I 2009 Medical innovation versus stem cell tourism. *Science* 324:1664–1665
105. Hyun I, Lindvall O, Ahrlund-Richter L, Cattaneo E, Cavazzana-Calvo M, Cossu G, De Luca M, Fox IJ, Gerstle C, Goldstein RA, Hermerén G, High KA, Kim HO, Lee HP, Levy-Lahad E, Li L, Lo B, Marshak DR, McNab A, Munsie M, Nakauchi H, Rao M, Rooke HM, Valles CS, Srivastava A, Sugarman J, Taylor PL, Veiga A, Wong AL, Zoloth L, Daley GQ 2008 New ISSCR guidelines underscore major principles for responsible translational stem cell research. *Cell Stem Cell* 3:607–609