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Abbreviations

BMI: Body Mass Index
ESC: Embryonic stem cell
IEQ: Islet Equivalents
IAPP: Islet amyloid polypeptidase
IBMIR: Instant blood-mediated inflammatory reaction
IPITA: International Pancreas and Islet Transplant Association
iPSC: Induced pluripotent stem cells
SRTR: Scientific Registry of Transplant Recipients
T1DM: Type 1 diabetes
PWD: Person/People with diabetes
Tregs: T regulatory cells
Abstract

ß cell replacement with either pancreas or islet transplantation has progressed immensely over the last decades with current 1- and 5-year insulin independence rates of ~85% and ~50%, respectively. Recent advances are largely attributed to improvements in immunosuppressive regimen, donor selection and surgical technique. However, both strategies are compromised by a scarce donor source. Xenotransplantation provides a potential solution by providing a theoretically unlimited supply of islets, but clinical application has been limited by concerns for a potent immune response against xenogeneic tissue. ß cell clusters derived from embryonic or induced pluripotent stem (iPS) cells represent another promising unlimited source of insulin producing cells, but clinical application is pending further advances in the function of the ß cell like clusters. Exciting developments and rapid progress in all areas of ß cell replacement prompted a lively debate by members of the young investigator committee of the International Pancreas and Islet Transplant Association (IPITA) at the 15th IPITA Congress in Melbourne and at the 26th international congress of The Transplant Society (TTS) in Hong Kong. This international group of young investigators debated which modality of ß cell replacement would predominate the landscape in 10 years, and their arguments are summarized here.
Introduction

Within a century, Type 1 Diabetes evolved from fatal diagnosis to a manageable condition with near normal life span, complicated by secondary morbidities\textsuperscript{1-4}. The discovery of insulin in the 1920’s resulted in reduced mortality before the age of 10, plummeting from ~85\% between 1897-1914 and ~ 40\% between 1914-1922 in the preinsulin era, to < 10\% during 1922-1926 and < 1\% in the 1950’s\textsuperscript{1,2}. These remarkable results were seen as a cure at the time\textsuperscript{5}; so that early experimental attempts to replace pancreatic tissue were abandoned\textsuperscript{6}. Decades later it became apparent that exogenous insulin therapy, while averting death from dehydration, could not prevent hitherto unknown secondary complications such as diabetic nephropathy, retinopathy and neuropathy\textsuperscript{7}. This recognition rekindled interest in $\beta$ cell replacement therapy as a truly curative approach, resulting in the first pancreas transplant in 1966\textsuperscript{8} and the development of protocols for islet isolation\textsuperscript{9-11}. While pancreas transplantation was rapidly adapted into clinical practice\textsuperscript{12}, the first clinical islet transplant was performed in 1977\textsuperscript{13,14} but unsatisfactory results prevented widespread use. The International Pancreas and Islet Transplantation Association (IPITA) held its first meeting in 1988 and has since pursued the goal to provide a scientific forum for the transplantation of insulin producing tissue in the treatment and cure of diabetes mellitus\textsuperscript{15}. In addition, the early 1990’s saw the release of 2 milestone trials of large cohorts of people with Type 1 (DCCT) and Type 2 Diabetes (UKPDS) demonstrating that intensified metabolic control is able to reduce or prevent the development and progression of long-term complications\textsuperscript{16-19}. Intensification of insulin therapy however, is limited by the inherent risk of hypoglycaemia\textsuperscript{20-22}. Hence, efforts to broaden the application of Islet and pancreas transplantation increased to resolve this conundrum. In the year 2000 the Edmonton group reported a revised protocol for islet transplantation achieving sustainable islet graft function in a series of nonuremic patients with T1DM\textsuperscript{23,24}, which triggered a worldwide surge in islet transplantation. Incremental improvements since then enabled outcomes like those seen with pancreas transplants\textsuperscript{25-29}. 
Unfortunately, both pancreas and islet transplantation are severely compromised by a limited source of donor tissue and the requirement for immunosuppression. Significant strides in xenotransplantation of porcine islets, address the limited donor pool but are compromised by a rigorous immune response. β cells derived from embryonic or induced pluripotent stem cells (iPSC) are getting closer to clinical translation, but the uncertain functional capacity of these cells and risk of neoplastic transformation will need to be resolved (Table 1). Exciting developments and rapid progress in all areas of β cell replacement prompted a lively debate by members of the Young Investigator Committee of IPITA at the 15th World Congress held in Melbourne, Australia and at the 26th International Congress of The Transplantation Society (TTS) in Hong Kong. This international group and conference attendees pondered which modality of β cell replacement is likely to predominate the landscape in 10 years, and this debate is revisited and explored further in this review.

Pancreas transplantation

Since the first pancreas transplant was performed at the University of Minnesota in 1966, more than 50,000 pancreata have been transplanted worldwide. Over this period pancreas transplantation has evolved from an experimental procedure to become a routine transplant in the modern era. Multiple advances in surgical technique, immunosuppression and donor/recipient selection now achieve a high rate of success. The traditional indications for a pancreas transplant include type 1 diabetes (T1DM) with renal failure, and nonuremic T1DM with significant hypoglycemic unawareness. The former is the most common scenario, with 76% of pancreata transplanted as a simultaneous pancreas and kidney (SPK), whereas the remainder are transplanted as a solitary pancreas transplant - either after a kidney (PAK) (11%) or alone (PA) (13%). Five- and 10-year pancreas graft survival rates are 73 and 56% for SPK; 64 and 38% for PAK; and 53 and 36% for PA with outcomes continuing to improve. Given the improving outcomes and the
significant benefits for mortality, quality of life and end-organ complications, indications for pancreas transplantation are expanding to include people with type 2 diabetes (T2DM) and older recipients (age≥60)\textsuperscript{34}.

T2DM comprises the largest of these expanding indications and is increasing, now accounting for 9\% of all SPK recipients with excellent results\textsuperscript{34-36}. This is relevant when considering an expanding elderly population and a projected 552 million people with T2DM by 2030\textsuperscript{37}. Because of the rapidly increasing numbers of uremic T2DM patients, UNOS (The United Network of Organ Sharing) restricts the number of T2DM patients that can receive SPK transplants to those patients with a C peptide < 2 ng/mL and a BMI ≤ 30 kg/m\textsuperscript{2}. This BMI cut-off is likely to increase in the future given that numbers of uremic T2DM patients on the waitlist remain low (UNOS will continue increasing the BMI cut-off every 6 months by 2 kg/m\textsuperscript{2} until 10\% of the waitlist is comprised of people with T2DM). It will be critically important to understand the effect of increasing BMI on outcomes in these T2DM patients. The C peptide cut-off is another source of debate, since it does not fully correlate with diabetes type\textsuperscript{38,39}, and C peptide levels do not predict outcome\textsuperscript{40,41}; prompting some to propose raising the cut-off to 10 ng/ml to include more T2DM patients\textsuperscript{34}.

Risks specific to solid organ pancreas transplantation include those associated with major surgery, increased perioperative cardiovascular risk and high intensity immunosuppression, which can be minimized with appropriate patient selection and surgical technique\textsuperscript{30}. Technical graft loss occurs approximately 5 to 10\% of the time\textsuperscript{42,43}. Overall, pancreas transplantation is currently the most effective method of achieving euglycemia in appropriately selected diabetic patients\textsuperscript{25}. Advantages of solid organ pancreas transplantation have been clearly demonstrated with respect to patient survival and reduction of diabetic complications. Indeed, pancreas transplantation reduces the risk of cardiovascular events, and reverses nephropathy and neuropathy\textsuperscript{44-47}.

Pancreas transplantation in the US has declined despite these excellent results\textsuperscript{36,43}.
The reasons for this decline in the US are not completely understood, but decreased donor organ quality is likely a major factor. For instance, in the US, only approximately 15% of donated pancreata from deceased donors in 2013 were accepted for transplantation\textsuperscript{48}. This is not a surprising trend, given that the donor population is becoming increasingly aged, obese, and diabetic, all factors that adversely affect functional pancreatic graft outcomes\textsuperscript{49}.

To increase the availability of pancreas organs for transplant, some centers have successfully transplanted pancreata from marginal donors, including donation after cardiac death (DCD)\textsuperscript{50-54}. Cardiac death donors have yielded excellent outcomes but are currently limited to only 3% of pancreas donors with current acceptance criteria limited to Maastricht Class III and IV (controlled) donors with age<50, BMI<30, and warm ischemia time <30 min\textsuperscript{53-57}. Furthermore, machine perfusion is being tested for hypo and normothermic preservation as well as for organ reconditioning to improve the quality of marginal organs\textsuperscript{58}. The use of living pancreas donors is a new field of study; currently 160 transplants using pancreas tissue from living donors have been performed in highly-specialized centers with excellent success rates for the recipient and minimal adverse postsurgical events for the donors\textsuperscript{59,60}. However, up to a quarter of living pancreas donors developed diabetes in the long run\textsuperscript{61}. Overall, foreseeable efforts to increase the donor pool are unlikely to meet the demand of all patients with T1DM but may help to serve the population of patients with end stage renal disease or severe hypoglycemic episodes. Surgical technique is constantly evolving, as exemplified by the introduction of duodenoduodenostomy that facilitates graft monitoring and timely antirejection therapy\textsuperscript{62,63} and the use of robotic assistance to perform vascular and enteric anastomosis which could further decrease surgical morbidity\textsuperscript{64}.

In conclusion, pancreas transplantation is the current gold standard for \(\beta\) cell replacement therapy in the properly selected diabetic patient and will likely continue to perform well in the next 10 years. In this timeframe, room for improvements lies in expanding donor utilization and quality, expanding recipient selection, a more systematic use
of pancreatic biopsies and improving immunosuppression strategies. In the next few decades, it is likely that pancreas transplantation will continue to develop and be considered as the standard that newer β cell replacement therapies will be measured against.

**Islet transplantation**

Clinical islet transplantation demonstrates that long-term insulin independence is achievable, with registry data reporting 47% insulin independence at 3 years. Since the introduction of the Edmonton protocol, immunosuppressive regimens have continued to be modified and improved. One significant advance being the introduction of potent induction therapy in addition to anticytokine management pretransplant, leading to 5-year insulin independence rates of 50% and exceeding 50% in the 2014 Collaborative Islet Transplant Registry (CITR) report. These improved outcomes place islet transplantation on par with pancreas transplantation. Importantly, the Islet transplant procedure has proven to be safe and able to effectively correct hypoglycaemia unawareness, reduce severe hypoglycaemic episodes, and substantially improve quality of life even when complete exogenous insulin independence is not achieved. Therefore, Islet transplantation has made the transition from an experimental, rarely successful therapy to a routine clinical procedure with predictable efficacy. Thus, islet transplantation has gained the status as standard of care for a selected population with T1DM in many countries. In the US, this status shift is pending the implementation of a recently completed multicentre phase 3 trial into FDA approval of Biological Licensure.

Up to now, the widespread use of Islet transplantation has been limited by the low efficiency in harvesting enough islet equivalents from a single donor to achieve insulin independence or optimal primary graft function. The less invasive nature of the Islet transplant procedure, compared to solid organ pancreas transplantation favours recipients at cardiovascular risk with the potential for reversal of secondary complications. In autoislet
transplantation the reintroduction of a patient’s own isolated islets has successfully been used to prevent surgically induced diabetes in patients undergoing pancreatic surgery for nonmalignant and malignant diseases.\textsuperscript{79-84} Due to steady improvements in all aspects of the islet isolation process, such as donor selection, pancreas preservation, tissue digestion and purification, and islet culture conditions, higher yields of good quality islets are now routinely achieved in specialized centers worldwide.\textsuperscript{26-28,73,85-88} The US Consortium for Islet Transplantation has developed a unified protocol to standardize the manufacturing process.\textsuperscript{89} Exciting new technologies to further automate the isolation process into a closed circuit, with automated renewal of culture media are currently under investigation, which could reduce cost and processing time and increase the quality of isolated islets for autologous or allogeneic Islet transplantation.\textsuperscript{90} Research into alternative transplant sites is crucial as significant islet graft loss (mainly due to activation of the instant blood-mediated inflammatory reaction; IBMIR) and suboptimal engraftment remain as limitations to both auto and allosislet transplantation with intrahepatic infusion.\textsuperscript{91-93} In addition, islet infusion into the liver restricts the transplantable tissue volume since increased tissue volume is associated with portal hypertension and acute thrombosis.\textsuperscript{94,95} Outcomes from completed and ongoing clinical trials using the omentum (ClinicalTrials.gov id NCT02213003, NCT02803905), subcutaneous tissue (NCT01652911), gastric submucosa (NCT02402439), intramuscular (NCT02872571, NCT01967186), bone marrow,\textsuperscript{96} anterior chamber of the eye (NCT02916680, NCT02846571) or encapsulation devices (NCT02064309) may yield new insights for defining extrahepatic sites for Islet transplantation. Therapeutic intervention for intrahepatic grafts or extrahepatic sites may avoid the early graft loss due to IBMIR and improve engraftment, potentially decreasing the donor to recipient ratio and increasing graft longevity.\textsuperscript{93,97} But more (pre) clinical studies are needed to establish an alternative site for clinical transplantation applicable to not just allogeneic and xenogeneic donor derived islets but also alternative \(\beta\) cell sources such as PSC derived \(\beta\) cells or bioengineered cells.
The donor pool for both pancreas and Islet transplantation is steadily changing worldwide, becoming older and more commonly impacted by metabolic disease\(^9\), both of which will jeopardize the functional success achieved from a single islet infusion. Understanding the molecular ultrastructure of the pancreas and the impact of such donor variables upon it, may enable the development of tailored isolation strategies for each different donor type, resulting in improved isolation outcomes from the full range of donors available\(^{99-101}\).

The need for life-long treatment with immunosuppression, in combination with a limited source of available donor organs, are today the most significant obstacles preventing the wider application of allogeneic Islet transplantation, including the treatment of children newly diagnosed with T1DM\(^{102}\). However, autologous Islet transplant recipients exhibit a slow loss of insulin independence despite absence of allogeneic and autoimmunity\(^{103-105}\) revealing a significant contribution of nonimmune factors in long-term graft dysfunction that must be identified and controlled. To combine other cellular therapies with Islet transplantation, such as the use of autologous or allogeneic mesenchymal stem cells (MSCs) or Tregs, are innovative strategies that could support islet health, revascularization, and induce immune tolerance\(^{106,107}\). Close collaborations with the sharing of findings and increased participation in registries are warranted to improve standard clinical practice and obtain proper reimbursement from health authorities worldwide.

In summary, the experience with pancreas and islet transplantation is an important proof of principle that β cell replacement is capable of restoring normal glucose metabolism, preventing cardiovascular disease, preserving kidney function and reversing neuropathy\(^{31,44,46,47}\). Importantly, islet transplantation has been shown to provide superior glycaemic control with less hypoglycaemia compared to continuous insulin infusion or multiple daily injections of insulin\(^{108}\). Therefore, β cell replacement offers better medical and quality of life outcomes for the individual with comparable or reduced health care utilization.
and cost, compared to exogenous insulin therapy\textsuperscript{27,109-111}. However, the cost and risk of the required immunosuppression maintain conventional insulin therapy as favourable for most patients. Finally, the demand for β cell replacement therapy by 422 million people with Diabetes worldwide (approximately 40 million people with T1DM)\textsuperscript{112-115}, a number that is expected to increase in upcoming decades\textsuperscript{115-117}, emphasizes the urgent need for an alternative β cell source. Currently, xenogeneic, porcine islets and PSC derived β cells are racing to fill the gap and other innovative approaches such as bioengineered glucose responsive cells and organs grown in chimeric animals have recently emerged.

\textbf{Xenoislet transplantation}

Porcine islet transplantation has similarity to allogeneic islet transplantation and therefore may benefit from the decades of experience accumulated in the allogeneic islet transplantation field. Porcine islets were first shown to produce C peptide and survive in humans under standard immunosuppression in 1994\textsuperscript{118}; and in 2006 to have the capacity to restore euglycemia in nonhuman primates (NHPs) when used with experimental, potent immunosuppression\textsuperscript{119,120}. Current studies in porcine-islet xenotransplantation are focused on improving islet functionality and survival in NHPs with the use of less potent immunosuppressive regimens through the utilisation of new technologies including genetic engineering, cellular therapy and encapsulating devices. These approaches have been applied in preclinical NHP models of porcine islet transplantation (cell therapy\textsuperscript{121}; genetic engineering\textsuperscript{122-124}; encapsulation devices\textsuperscript{125-129}) with islet survival rates approaching or surpassing 1 year.

But why use porcine islets? The short gestation and rapid growth rate of pigs would facilitate a supply of islets adjusted to demand under controlled conditions\textsuperscript{130,131}. The use of pigs as a source of tissue would raise little ethical concerns as pigs are already used for human consumption and medicinal products such as insulin, heparin, heart valves and
With a single amino acid difference, porcine insulin binds human receptors and is secreted in a biphasic manner in response to blood glucose, maintaining a fasting blood glucose level similar to humans. Despite these similarities, relevant interspecies differences do exist. For example, manufactured porcine, human and macaque islets exhibit distinct metabolic profiles exemplified by varied glucose stimulation indices of 1.5, 3.8 and 7.7, respectively. This may indicate that more porcine islet equivalents may be required to reach euglycemia in humans or NHPs, relative to allogeneic islet transplantation. These differences also provide advantages; for example, the reduced metabolic demand of porcine islets compared to human islets may mean that pig islets could better tolerate encapsulation strategies that suffer from limitations of hypoxia. This is a significant consideration as the protection of porcine islets requires aggressive immunosuppression, islet encapsulation provides an avenue to drastically reduce or obviate the need for immunosuppression. Encapsulation strategies have been shown to extend the survival of porcine islet grafts from between ~1-4 weeks to greater than 6 months, and up to 803 days without immunosuppression in NHPs and up to 16 weeks in autoimmune nonobese diabetic (NOD) mice. However, the metabolic function of encapsulated porcine islets was insufficient in NHP models and in a clinical trial.

Approaches such as encapsulation are critical for the future success of porcine islet transplantation, as they are subject to vigorous cellular and humoral rejection. For instance, the IBMIR response is a formidable barrier in the xenogeneic setting due to the high level of endogenous preformed circulating antibodies that recognize carbohydrate antigen on porcine cells, which are absent in humans. A prominent example, anti-galactose-α1,3-galactose (α-Gal) antibodies, represent up to 1% of the immunoglobulin repertoire in humans, triggering an immediate, potent activation of complement and thrombosis upon exposure to α-Gal.
Nevertheless, the genetic modification of porcine islets successfully mitigates IBMIR in NHPs. In a significant advance, the α1,3-galactosyltransferase gene was successfully targeted (GalTKO) to remove the α-Gal xenopeitope\textsuperscript{144,155}, which improved islet engraftment and function\textsuperscript{156}. Genetic overexpression of human (h) complement-inhibitory factor CD46, was found to prolong the survival of porcine islets in immunosuppressed diabetic NHPs from 46 days, to up to 1 year posttransplantation. This protective effect was mediated by the mitigation of humoral rejection with no detectable reduction in IBMIR-mediated early islet destruction\textsuperscript{122}. In contrast, IBMIR and early thrombosis were dramatically suppressed when GalTKO porcine islets transgenic for the complement regulatory factors CD55\textsuperscript{+}, CD59\textsuperscript{+} were transplanted intraportally into diabetic, immunosuppressed baboons\textsuperscript{124}.

Studies are now underway to generate additional multitransgenic and knock out (KO) pigs to provide further protective benefits to islets when transplanted\textsuperscript{123,157-159}. For example, it is recognized that other nongalactose xenoreactive human natural antibodies react with transplanted porcine islets\textsuperscript{160-163}. The generation of extensive multi-KO and transgenic pigs to remove all identified detrimental antigens from the xenoislet repertoire and express protective genes represents 1 option. Yet, creating a multi-KO pig for all possible xenoantigens that exists\textsuperscript{160} represents a dauntingly long and expensive process with the use of conventional technology\textsuperscript{164}. The emergence of endonuclease-gene editing technology (particularly the CRISPR/Cas9 system\textsuperscript{164}) has greatly facilitated high throughput generation of multi-KO pig lines\textsuperscript{164-167}. Indeed, Zinc Finger endonuclease was used for the rapid generation (7 months) of a pig line with 2 xenoepitopes knocked out, α-Gal and N-Glycolylneuraminic acid (NeufGc)\textsuperscript{168}. Cells from these double KO pigs have been shown to exhibit reduced human antibody binding and cytotoxicity\textsuperscript{169,170}. Moreover, the power of cas9 genetic modification with somatic cell nuclear transfer has been demonstrated by developing pigs lacking GGTA1/CMAH/β4GalNT2, GGTA1/CMAH, GGTA1/SLA Class I or GGTA1 genes. Of which, GGTA1/CMAH/β4GalNT2 KO porcine cells exhibited significantly reduced human
antibody binding to near background levels\textsuperscript{171}. These studies illustrate the potential of leveraging gene editing to develop a clinically acceptable IBMIR and xenoreactivity evading donor pig for xenoislet transplantation.

Nonetheless current genetically modified or encapsulated islets are eventually rejected\textsuperscript{122-129}, highlighting that porcine \( \beta \) cell replacement therapy will require alternate strategies to combat cellular rejection and promote graft tolerance. Interestingly, CD4\(^+\) T cells are necessary and sufficient for islet xenograft rejection\textsuperscript{147,172-174}, underscored by the success of anti-CD154 that significantly extends xenogeneic-islet survival in NHPs when added to a conventional immunosuppressive protocol\textsuperscript{119,175}. However, the use of anti-CD154 results in severe thrombotic complications when used in humans\textsuperscript{176}. Strategies that aim to induce xenogeneic-graft tolerance and control the CD4 response include 1) the reengineering of anti-CD154 antibodies, or development of new drugs targeting this pathway\textsuperscript{177,178} while avoiding thrombotic complications but maintaining their capacity to decrease cytotoxic T cell activation and induce antigen specific Tregs\textsuperscript{179-181}, 2) the induction of central tolerance by thymic xenografting or mixed chimerism, or 3) the establishment of peripheral tolerance via the in vivo or ex vivo expansion of antigen-specific Tregs. Seminal studies have found the use of xenoislets is compatible with tolerizing modalities\textsuperscript{182}, such as mixed chimerism\textsuperscript{183-186} or the in vivo or ex vivo expansion and delivery of antigen-specific Tregs, as human Tregs operate in the xenogenic setting\textsuperscript{186-188}.

The transmission of porcine endogenous retroviruses (PERV) has been a major safety concern, although thus far no transmission has been observed in human or NHP recipients\textsuperscript{189-192}. Nonetheless the potential risk is taken extremely seriously with several pre and posttransplant screening processes designed to eliminate endogenous retrovirus\textsuperscript{191,193-196}, as well as microbial infectious risk\textsuperscript{196,197}. Prescreening of donor herds and postscreening of recipients are 2 obligate processes for all future xenotransplantation trials as outlined by the International Xenotransplantation Association (IXA)\textsuperscript{198-200}. The generation of transgenic pigs
expressing endogenous retrovirus small interfering RNAs has been a strategy to remove the potential PERV risk\textsuperscript{201,202}. However, advances in genome editing have now made it possible to inactivate all 62 copies of PERV in a porcine cell line\textsuperscript{159}. Lastly, subcutaneous macroencapsulation will allow the ‘packaging’ of xenogeneic-islets into devices that can be easily and safely retrieved if necessary. The provision of a replenishable O\textsubscript{2} supply may facilitate this approach by addressing the issues of hypoxia and nutrient starvation\textsuperscript{141,203}.

Together, these data highlight the utility of porcine xenogeneic islet transplantation for the treatment of T1DM. Indeed, we believe that a future successful xenogeneic-islet regimen will include: 1) an encapsulation strategy with local O\textsubscript{2} delivery to avoid direct immune-contact of porcine islets, prevent hypoxia and allow their safe retrieval if necessary; 2) the generation of a multi-KO and multi-KI source pig, accelerated by endonuclease technology. Molecules chosen for KO or KI will be those that address islet loss mediated by IBMIR, humoral and cellular rejection, and 3) a tolerizing therapy aimed at generating antigen-specific Tregs, which may be combined with a central tolerance inducing regimen to provide long-lasting durable tolerance\textsuperscript{204}. Lastly, the use of reengineered anti-CD154 monoclonal antibodies represents a promising adjunct therapy for the induction of xenoislet tolerance\textsuperscript{119,120,175,180}.

Additional questions to be answered during the next 10 years relate to practicalities of translating xenoislet transplantation, which will be critical for its success. These include decisions to be made by the xeno community, such as the optimal donor line to use and its genetic background, donor age and standardised preparation of pig islets\textsuperscript{205-207}. These issues have recently been critically reviewed\textsuperscript{208}. The consensus statement made by IXA provides a framework for conducting clinical trials\textsuperscript{206}. Together these advances in technology and policy place islet xenotransplantation in a strong position to be the ß cell replacement therapy of the future.
Pluripotent Stem Cell derived β cells

Deriving functional β cells from pluripotent stem cells (PSC) may address most limitations of human and animal donor derived β cell replacement, including donor shortage, costly and labour intensive organ procurement and islet isolation procedures and the requirement for immunosuppression. Pluripotent stem cells (PSC) can be cryopreserved and are therefore available off the shelf for directed differentiation into β-like cells. Isolated human islets have been successfully cryopreserved for research but not for clinical use. ESC generation is restricted to donated, in vitro fertilized ova, giving rise to a cell line that can be propagated indefinitely to provide sufficient cells for virtually all medical applications. However, owing to their allogeneic nature, these cells will necessitate protection from immune attack and their embryonic nature poses ethical concerns. The discovery that somatic cells can be reprogrammed to pluripotency (iPSC), has revolutionized the field as it promises to circumvent both the use of embryonic cells and allogeneic rejection.

Previously the only autologous source of PSC was the costly, prospective banking of umbilical cord blood. Deriving β-like cells for transplant from patient derived, autologous iPSC presents a nearly ideal source for β cell replacement therapy. The cost and regulatory burden of individual production of autologous iPSC derived β cells are considered significant barriers to their clinical translation, yet this approach promises immense biological advantages.

The minimal and optimal functional criteria for a β cell replacement product are not clearly defined. Adult islet grafts are stripped of their normal vascularization and innervation, yet retain the complex architecture of islet miniorgans and seem to become revascularized and reinnervated in the liver. It is unknown whether a PSC derived β cell graft can function similarly to an islet graft. The fine-tuned insulin secretion of an intrahepatic islet graft reduces glycaemic variability and mostly eliminates hypoglycaemia. Metabolic studies of islet and pancreas transplant recipients demonstrated that the...
secretory capacity of islet grafts is typically 25-40% \(^{230-232}\), while pancreas grafts restore 60-100% \(^{233,234}\) of the normal insulin secretory capacity in healthy controls. In preclinical and recent onset T1DM, secretory capacity was found to be 25% of normal \(^{235,236}\). Patients with good engraftment and secretory capacity of \(\geq 40\%\), exhibit less metabolic exhaustion \(^{237-239}\) and no functional attrition for the first-year posttransplant \(^{232}\). This indicates that a larger \(\beta\) cell mass improves long term graft survival by reducing metabolic stress on the islet or \(\beta\) cell graft \(^{232,239,240}\). A PSC derived \(\beta\) cell graft can be scaled to fully restore health secretory capacity and thereby may not be subject to the graft attrition over time observed in islet transplantation.

It remains to be seen whether a graft consisting primarily of single \(\beta\) cells (donor or PSC derived) will replicate the intricate functional capacity of the endogenous islet miniorgan or pancreas and islet grafts \(^{217,218,241}\). Current strategies are focused on generating \(\beta\) cells of highest possible purity, either by terminal differentiation of implanted endocrine progenitors in vivo \(^{209,242}\) or transplantation of fully in vitro differentiated \(\beta\) cells \(^{210,212,243}\). In addition, a concerted effort aims to generate a more complex islet like structure from PSCs \(^{244-249}\) including PSC derived \(\delta\) and \(\alpha\) cells (D. Melton and Q. Peterson – personal communication). Thus far, PSC derived \(\beta\)-like cells have been shown to reverse hyperglycemia in diabetic mice and rats \(^{210,250-252}\) as well as preventing hyperglycemia in diabetes prone mice \(^{212}\), but a demonstration in large animals or humans is lacking. The rodent diabetes model may not be a good surrogate for efficacy in humans, as small amounts of static insulin secretion can restore normal fasting blood glucose levels and GTT results in mice \(^{253-256}\). More informative metabolic measurements such as clamp studies, CGM and HbA1c are challenging to do in mice, further limiting the availability of data to predict clinical outcomes \(^{256}\). Therefore, primate studies and clinical trials of nonencapsulated PSC derived \(\beta\)-like cell-clusters are urgently needed. A clinical trial currently in the planning phase may address this question. The Boston Autologous Islet Replacement Program (BAIRT) \(^{257}\) aims to differentiate
autologous iPSC to β-like cells for autologous transplantation in patients with clinical diabetes due to a nonautoimmune loss of endogenous β cell mass (ie, postpancreatectomy for chronic pancreatitis).

Meanwhile, a first in human, Phase I/II trial is currently being conducted by Viacyte Inc. (clinical trials identifier: NCT02239354), in which human ESCs are implanted at the early differentiation stage of endocrine progenitors. To mitigate the risk of tumor formation and to shield the cells from alloimmune and autoimmune attack, progenitor cells are encased in a macroencapsulation device placed subcutaneously. It has been shown in mice that these progenitor cells functionally mature after transplantation inside these devices. However, the encapsulation device acts as a diffusion barrier, preventing real-time glucose sensing and insulin secretion that is crucial to islet function. Thus, satisfactory metabolic function has not been demonstrated convincingly with encapsulated islets in primates or humans. Viacyte recently obtained IND approval for a next generation product, PEC-direct, permitting vascularization of the encapsulated ESC derived graft. This approach would remove the diffusion barrier, likely to improve metabolic outcomes, while allowing to contain potential neoplasms and to retrieve the graft if necessary. This strategy will require chronic immunosuppression to protect the allogeneic hESC graft from the immune attack that is not blocked by the encapsulation device, limiting its use to the same high risk patient population eligible for islet and pancreas transplants.

A less fibrogenic biomaterial, identified in a large screen, has recently been used to microencapsulate hESC derived β-like cells. The relatively large size of these capsules (1500μm) further reduced fibrosis, but also increases the diffusion barrier. A proportion of transplanted human cells were found to survive for 6 months after intraperitoneal transplantation providing moderate control of fasting blood glucose. However, it is unlikely that the murine results are translatable to the clinic and the intraperitoneal site is of limited clinical use as grafts are not easily retrievable.
Autologous iPSC are not expected to elicit an alloimmune response. While an initial murine study has called this principle into question\textsuperscript{265}, several follow up studies suggest that differentiated progeny of autologous iPS is not rejected\textsuperscript{266,267}. Furthermore, a careful analysis of the immune response to autologous iPSC derived endothelial cells identified a tolerant signature\textsuperscript{268}. In synthesis with studies in clinically relevant NHP models\textsuperscript{269-271} and in humanized mice\textsuperscript{272}, these results point towards a more nuanced picture of iPSC immunogenicity which is dependent on the differentiation stage\textsuperscript{266,268}, reprogramming vectors\textsuperscript{266,268} and the type of differentiated cell\textsuperscript{272,273}. In the first in human trial, using a sheet of autologous iPS derived retinal pigment epithelial cells (RPE) for transplantation, no immune rejection was observed\textsuperscript{274}. The immunogenicity of specific autologous iPS cell products may need to be assessed in (pre)clinical trials to arrive at a nonimmunogenic cell product\textsuperscript{273}.

A barrier common to all modes of β cell replacement in patients with T1DM, is that cells may be subject to recurrent autoimmune attack\textsuperscript{275-277}. The extent to which β cells for replacement resemble the endogenous β cells may render them increasingly susceptible. Therefore a balance of a phenotype that permits mature metabolic function but eludes autoimmune attack could be critical for success\textsuperscript{101,217,218,278}. Engineering non-β cells to produce and release insulin\textsuperscript{278}, and immunomodulation\textsuperscript{279,280} are promising approaches to circumvent or regulate recurrent autoimmunity.

Finally, there are well-founded concerns about the potential neoplastic transformation of contaminating PSCs and their progeny. Hence, initial clinical trials of stem cell derived β cells will require a transplant site that allows safe graft retrieval, such as a semipermeable membrane placed in a easily retrievable site. Transplant sites suitable for retrievability that have not worked for human islets (ie, subcutaneous), may be more accommodating for PSC derived grafts. Ultimately, the safety of PSC derived β cells may be enhanced by a combination of transplant site\textsuperscript{281-285} and a stringent manufacturing process excluding
remaining pluripotent cells\textsuperscript{286,287}. Encouragingly, several clinical trials using ESC for a variety of diseases are ongoing and no resulting malignancies have been observed to date\textsuperscript{288}.

**Emerging β cell replacement strategies**

As existing technologies evolve and new discoveries are made, some β cell replacement strategies that presently appear out of reach may become feasible. Here, we mention some nascent developments that may reshape our view of β cell replacement in the upcoming decade.

Alternative generation of endocrine pancreatic cells and tissue include regeneration of endogenous β cells, organ bioengineering, synthetic β cells and growing a human pancreas in animals. Numerous potential pharmacologic agents that can induce regeneration of endogenous β cells in mice have been identified but failed to translate to human β cells\textsuperscript{289-291}. It has also been shown that other pancreatic cell types can transdifferentiate to β cells, including α cells\textsuperscript{292} and pancreatic exocrine cells\textsuperscript{293}. Recently, a screening approach has identified an FDA approved drug, Artemisinis, that indirectly targets GABA receptors, to foster α to β cell transdifferentiation\textsuperscript{294}. Both Artemisinis and GABA potently induced neogenesis of β-like cells from ductal epithelium via an intermediate α cell state in human islets, suggesting that this treatment may be translatable\textsuperscript{295}. Reprogramming of exocrine\textsuperscript{293}, gastric\textsuperscript{296} and hepatic cells are other potential β cell sources. Any of these cells may be subject to autoimmune recurrence.

The main goal of β cell replacement can be distilled to real time sensing of glucose levels and other relevant parameters, coupled to regulated insulin secretion with swift physiologic kinetics\textsuperscript{217,297-299}. Several approaches have been undertaken to generate surrogate β cells that harbor some of the intricate physiology of human β cells, from simple ectopic expression of the insulin gene\textsuperscript{300} to harnessing the regulated secretory capacity of non-β, secretory cells \textsuperscript{301}. More recently, HEK293 (human embryonic kidney) cells were transfected
with 2 transgenes to enable glucose sensing and insulin release\textsuperscript{278}. Rational testing and design resulted in an optimized HEK-β cell constitutively expressing a voltage controlled calcium channel and conditional NFAT mediated insulin secretion, enabling glucose stimulated insulin secretion in vitro. The GLP-1 receptor as well as glucose dependent expression of GLP-1 were added to increase sensitivity to food intake in vivo. Transplantation of HEK-β cells into diabetic mice improved fasted blood glucose levels and oral GTT. However, glucose metabolism was not normalized, likely due to the time delay imposed by the transcriptional regulation of insulin secretion\textsuperscript{297} and compounded by the diffusion barrier imposed by encapsulation and insufficient insulin storage\textsuperscript{278,302}. Importantly, a synthetic non-β cell product may evade recognition by autoimmune memory. Synthetic cell design has also been used for the temporal control of cues for directed differentiation\textsuperscript{303} and to improve insulin sensitivity in type 2 diabetes\textsuperscript{304}.

The concept of blastocyst complementation for the generation of a human pancreas organ in a large animal model has recently cleared several technical hurdles. The aim is to generate a pancreas derived entirely from donor cells by injecting donor PSC into the blastocyst of a host that lacks the ability to generate pancreatic tissue. To this end, an apancreatic pig was generated by disruption of the Pdx-1 gene. Blastocyst complementation with wild-type porcine PSC restored normal pancreas organogenesis\textsuperscript{305}. The same group successfully generated an interspecies mouse pancreas in rats. Transplantation of isolated interspecies islets reversed hyperglycemia in mice under minor immunosuppression\textsuperscript{306}. Evolutionary distance between species precludes the formation of ie, rat-pig chimeras, but a human-pig chimera has recently been generated successfully using human iPSC and wildtype porcine blastocysts\textsuperscript{307}. A serious concern is that human PSC could give rise to chimeric brain or germ cells. However, it has been shown that the forced expression of genes preventing meso and ectodermal fate decisions can direct complementary human cells exclusively to endodermal fate\textsuperscript{308}. Another, elegant approach is the use of cells with limited developmental
potency such as endodermal progenitor cells. However, ESC in a nonnaïve state lack the patency to form chimera as they appear to undergo apoptosis in complemented blastocysts\textsuperscript{309,310}. The expression of Bcl-2 prevented apoptosis in Sox17+ and Nkx2.5+ progenitor cells after blastocyst complementation, enabling region specific, pancreatic chimera\textsuperscript{309,310}. Taken together, these developments open the tantalizing possibility to generate autologous, patient specific human pancreata in pigs that could be used for pancreas or islet transplantation\textsuperscript{311,312}.

Successful decellularization of the pancreas\textsuperscript{313} has fuelled the bioengineering of organs for transplantation\textsuperscript{314}, where these scaffolds are reseeded with allogeneic or autologous PSC derived β cells or donor derived islets to improve their engraftment and function\textsuperscript{313,315-318}. The cellular microarchitecture of pancreatic islets, including vascularization and innervation, has even been constructed using 3D bioprinting of single cells\textsuperscript{319}.

**Conclusion**

The next 10 years will see a continued dependence on pancreas and islet transplantation for selected high-risk patients with T1DM. However, rapid progress in the areas of xenogeneic islet transplantation, stem cell derived β cells or other emerging β cell sources will ultimately replace the limited sources of adult human tissue. Although xenotransplants were previously thought to be compromised by both immune destruction and zoonotic infections, the success of porcine islets transplanted into diabetic nonhuman primates\textsuperscript{119,120} and multiplex gene editing to reduce immunogenicity and infectious risk\textsuperscript{60,159,320} may help overcome these barriers. Similarly, protocols to derive β-like cells from either embryonic and iPSC sources continue to improve in generating fully functional cells in vitro\textsuperscript{210-212,321}. The generation of fully differentiated β-like cells and other strategies may decrease the risk of neoplastic transformation following transplantation. Finally, the
continued development of strategies to protect β cell grafts from recurrent autoimmunity and alloimmunity\textsuperscript{159,279,280,322} as well as immunotherapy for T1DM may assist in making β cell replacement therapy possible for all people with diabetes (Table 1).

In the meantime, closed loop, mono or bihormonal artificial pancreata are expected to further improve glycaemic control and reduce the burden of diabetes management\textsuperscript{323}. Several closed loop algorithms have been tested in clinical trials in both inpatient and at-home settings. A meta-analysis of these trials confirmed that study subjects had an increase in time spent in glucose target range and reduced HbA1c values, compared to open loop diabetes management and greater improvement with bihormonal systems\textsuperscript{323}. However, while these artificial pancreata efficiently reduced glucose variability in the steady state, food intake and the multifactorial influences of daily activity on glycemia were not controlled to the extent of control that a successful islet transplant provides\textsuperscript{78,324-328}. These shortcomings are expected, given that these artificial pancreas systems rely solely on the delayed measurement of glucose levels and are limited by the pharmacokinetics of exogenous insulin\textsuperscript{327}. Considerable cost of this technology, including the ongoing expense of disposable supplies will likely limit access to this therapy. Nonetheless we expect that artificial pancreata will fill the gap for low-risk T1DM patients until a safe and efficient β cell replacement is ready for prime time.

The upcoming decade will be an exciting time as we move forward with clinical trials utilizing cutting edge technologies with the aim to provide a treatment for the millions of patients worldwide suffering from diabetes. Patients with diabetes are highly motivated to participate in clinical trials which offer the potential to relieve them of the burden of achieving tight control using exogenous insulin therapy. For that reason, it is the obligation of the medical and scientific community to assure scientific rigor and ultimate patient safety as we move forward with these novel strategies.
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<table>
<thead>
<tr>
<th>β cell replacement therapy</th>
<th>Pancreas</th>
<th>Allogeneic/autologous islet</th>
<th>Stem cell derived β cells</th>
<th>Xenogeneic (porcine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) ‘on demand’, unlimited source of β cells</td>
<td>☒ No marginal graft use slightly extends donor pool; Future chimeric pigs with human pancreata.</td>
<td>☒ No marginal graft use maximises available donor pool.</td>
<td>☑ Yes</td>
<td>☑ Yes*</td>
</tr>
<tr>
<td>(2) Consistent and standardized, high quality β cell replacement therapy</td>
<td>☑ Yes organ reconditioning used to improve marginal grafts.</td>
<td>☑ Yes</td>
<td>☑ Yes</td>
<td>☑ Yes</td>
</tr>
<tr>
<td>(3) Opportunity for genetic modification</td>
<td>Limited no extended culture period. Chimeric pigs with human pancreata amenable to modification.</td>
<td>☑ Yes limited culture period - allows for in vitro manipulation before transplant.</td>
<td>☑ Yes</td>
<td>☑ Yes</td>
</tr>
<tr>
<td>(4) Potential to avoid recurrent autoimmunity</td>
<td>☒ No</td>
<td>☒ No</td>
<td>☑ Maybe</td>
<td>☑ Maybe</td>
</tr>
<tr>
<td>(5) Potential to prevent allogeneic sensitization</td>
<td>☒ No</td>
<td>☒ No</td>
<td>☑ Yes autologous iPSC-or ESC-derived β cells</td>
<td>☑ Yes</td>
</tr>
<tr>
<td>(6) Avoid amyloid deposition</td>
<td>☒ No</td>
<td>☒ No</td>
<td>☑ Limited no long-term data addressing amyloid toxicity</td>
<td>☑ Maybe</td>
</tr>
<tr>
<td>(7) Demonstrated clinical insulin independence for &gt; 1 year</td>
<td>☑ Yes</td>
<td>☑ Yes</td>
<td>☐ No</td>
<td>☐ No</td>
</tr>
<tr>
<td>(8) Reported reversal of</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>diabetic complications in humans</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
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<td>----------------------------------</td>
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</tr>
<tr>
<td>(9) Potential risks to recipient</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>- invasive surgery/ peri-cardiovascular risk</td>
<td>- infections disease transmission</td>
<td>- Neoplasia</td>
<td>- PERV</td>
<td></td>
</tr>
<tr>
<td>- immunosuppression side effects</td>
<td>- immunosuppression side effects</td>
<td>- For ESCs</td>
<td>- immunosuppression side effects</td>
<td></td>
</tr>
<tr>
<td>(10) Immunological barrier to overcome</td>
<td>High – Allogeneic immunity and recurrent autoimmunity, amyloid deposition.</td>
<td>Medium (autologous) to High (allogeneic) – Recurrent autoimmunity and amyloid deposition effect both autologous and allogeneic modalities.</td>
<td>Medium (iPSC) to High (ESC) - ESC are allogenic cells; iPSC can be autologous but still face recurrent autoimmunity; both affected by amyloid.</td>
<td>High to Very high – xenogeneic barrier (genetic engineering aims to dismantle). But evidence suggests a lack of recurrent autoimmunity and unaffected by amyloid.</td>
</tr>
</tbody>
</table>

\(\times\) = no; \(\checkmark\) = yes; iPSC (induced pluripotent stem cell); ESC (embryonic stem cell); PERV (porcine endogenous retrovirus).