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Report from IPITA-TTS Opinion Leaders Meeting on the Future of β-Cell Replacement

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At the time the first pancreas transplant was performed by Kelly and Lillehei in 1966, insulin therapy for diabetes was generally available but administered in a form that is known today as "conventional therapy."[1] In this era, as many as half of all juvenile onset diabetics did not reach the age of 55 years. Early mortality from accelerated cardiovascular disease, renal failure, and hypoglycemia-related events were commonplace. The early low success rate and mortality of pancreas transplantation by comparison were also suboptimal. As will be characterized in the succeeding 8 chapters, the outcome of "best medical therapy" with newer forms of insulin and insulin delivery systems along with dramatically improved outcomes of islet and pancreas transplantation and novel β-cell sources hold great promise for those afflicted.

Among the great strides in diabetes research was the Diabetes Control and Complications Trial (DCCT).[2] This trial,

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published in 1991, showed that intensive insulin therapy when compared with "conventional therapy" dramatically reduced the incidence and progression of the microvascular complications of diabetes, nephropathy, neuropathy, and retinopathy. Thus, with intensive insulin therapy, the mean hemoglobin A1C was improved to 7% compared with 8.3% in the conventional group. The improved microvascular outcomes and measured hemoglobin A1C came at a substantial price, namely, a greatly increased incidence of hypoglycemic events requiring third-party intervention. After 2 decades, the 2 groups have also diverged with respect to mortality; recent reanalysis of the original study groups demonstrates that those individuals who received intensive insulin therapy groups had lower overall mortality.[3]

Current best practice includes the availability of insulin pumps and newer forms of synthetic insulin as well as pharmaceutical agents that augment insulin action. Unfortunately, the widespread application of the therapeutic measures taken in the intensive therapy arm of the DCCT is not the norm. Analysis of data from the 67 centers reporting to the US type 1 diabetes (T1D) exchange shows that even today, more than 20 years after the DCCT, the average hemoglobin A1C for treated patients is 8.3%. Thus, outside of a clinical trial, such as the DCCT, actual practice achieves suboptimal outcomes. A remarkable report of the current state of diabetes care is published in the Journal of the American Medical Association in January 2015. This report shows that in a modern era of diabetes care, mortality remains higher than the general population. For men and women, the life expectancy for those reaching 20 years of age is 11.1 years and 12.9 years less than the general population, respectively.[4] These sobering findings, which have been thoughtfully summarized in an accompanying editorial by Katz, provide a meaningful context for an international conference dedicated to summarize the current state of pancreas and islet transplantation and chart the way forward with an ambitious research agenda.[5] The need for a cure for diabetes through transplantation, stem cell–based therapy, regeneration, newer insulin delivery systems, and devices that warn of hypoglycemia have been brought into sharp focus by these reports which show that the progress in care for diabetics has hit a plateau. Innovation will be required to improve the quality of life and lower morbidity and mortality for those with insulin-requiring diabetes.

Against this backdrop, the International Pancreas and Islet Transplant Association (IPITA), in collaboration with the Transplantation Society (TTS), held a scientific workshop in Oxford England, May 7 to 9, 2014, to review the current status and needed research agenda of 8 current or nascent β-cell replacement therapies: whole organ pancreas transplantation, isolated islet transplantation, artificial pancreas (AP), immunological tolerance, xenotransplantation, encapsulation technologies, β cell regeneration, and stem cell derived β cells. Thirty-two scientists and clinicians representing 4 continents, 7 countries and 29 institutions, with dedicated expertise in these areas were recruited to participate in 8 topical workgroups along with representatives of the NIH (National Institute of Diabetes and Digestive and Kidney Disease, National Institute of Allergy and Infectious Disease), Diabetes Research and Wellness Foundation, the Juvenile Diabetes Research Foundation (JDRF) and industry. In advance of the meeting, the workgroups prepared summaries of their respective topic highlighting the state of their field and the research agenda needed to move the therapy forward to optimal clinical application. Presentation and full group discussion at the meeting generated revised summaries presented in the 8 sections below. These reports are followed by the results of a conference attendee survey examining how the participants view the future of β cell replacement therapies.

ALLOGENEIC PANCREAS TRANSPLANTATION

Current State of the Field

Pancreas transplantation has been available as a cure for diabetes since its first application in 1966. The most common application is simultaneous pancreas-kidney (SPK) transplant for the uremic T1D patients who are free of major surgical risks. Solitary pancreas transplants in the nonuremic diabetic have been largely reserved for patients with brittle diabetes and hypoglycemic unawareness despite best medical therapy or after a successful kidney transplant. Unlike in the European Union where the application of whole-organ pancreas transplantation continues to expand, the number of pancreas transplant cases in the United States has decreased by 35% since the year 2003. The reasons for the decline in the United States are that the number of waitlist patients has decreased and the utilization of donor organ pancreas has declined markedly. The decline in pancreas utilization in the United States followed publication of the pancreas donor risk index (PDRI) which has created an environment of selectivity in the United States.[6] The PDRI has also been validated in the United Kingdom. However, the trend for pancreas utilization in the United Kingdom and Europe is divergent with the US European policies strongly favoring the effective use of regional

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teamwork in pancreas procurement which include policies regarding cryopreservation solutions and the technical details of pancreas procurement. Thus, European-wide policies have led to much better pancreas utilization while achieving excellent results. Thus, although PDRI has been validated on 2 continents, the addition of uniform procurement and preservation policies can further enhance utilization beyond what can be achieved by the high selectivity created by simple reliance on the PDRI alone. Countrywide policies regarding pancreas allocation to islets and pancreas transplantation in Australia have fostered an environment of cooperation and better utilization.

Decreasing waitlist could be attributable to better insulin therapies, reduced enthusiasm for pancreas transplant secondary to data showing limited survival benefit,[7] and better outcomes with islet transplantation.[8] Declining organ utilization followed publication of studies that aggregate risk factors for early graft failure. As such, transplant surgeons are oriented to donor selection strategies that avoid early graft failure using a predictive index. Studies on strategies to increase organ utilization by avoiding early graft failure with active interventions have been scarce over the past decade. Donor selection and intervention studies in pancreas transplant have been largely focused on early outcomes, specifically avoidance of early graft loss from thrombosis and sepsis. Studies of long-term graft survival and the impact of pancreas transplantation on diabetic morbidity and mortality is hampered by the lack of a clear definition of graft failure. Without an internationally accepted endpoint of pancreas transplant function, the literature on the impact of pancreas transplantation on long-term diabetic morbidity can be viewed as observational.

Need for Systematic, Comprehensive Documentation of Pancreas Transplant Outcomes Worldwide

Graft failure criteria cannot be defined clearly unless the goals of pancreas transplant are defined. Is insulin independence the goal of all pancreas transplants? Does partial function of the pancreas benefit the patient in providing stable glycemic control and abrogation of hypoglycemia unawareness? This may be especially relevant for type 2 diabetics (T2D) with high baseline insulin requirements. Further, does partial function provide long-term benefit in incidence or improvement of secondary complications? Should pancreas transplant in total pancreatectomized patients have a dual outcome endpoint for exocrine and endocrine function?

One option would be to have absolute insulin independence as the goal. This would clearly be the most accepted and easily auditable endpoint for all patients. However, when applied across the gamut of patients undergoing transplant, it clearly is restrictive. Another option would be to have different pathways for pancreas transplant with predefined endpoints for each pathway: type 1 with/without hypoglycemia unawareness, T2D nonobese with/without secondary complications, surgical diabetics (postpancreatectomy), and so on. This would require a well thought out algorithm, with clear endpoints in each limb and additional data, such as C-peptide, HbA1C, quantification of insulin requirement, and details on hypoglycemia unawareness and secondary complications to name a few. Currently, none of the abovementioned variables are collected by the United Network for Organ Sharing for the Scientific Registry of Transplant Recipients outcome analysis.

Another important attribute of a system defining organ function endpoints should be verifiability. Currently, pancreas graft failure is self-reported, and because the definition is vague, available data may be regarded as unreliable. Death in most countries can be verified using census or other governmental databases. Insulin use and oral agents can be tracked based on prescription data obtained from third-party payers.[9] Clear auditing guidelines need to be established for any outcome endpoints.

There is opportunity to learn from our islet transplant colleagues in maintaining a detailed database resulting in the ability to look at outcomes with well-defined criteria.[10] If the Collaborative Islet Transplant Registry database were to be linked with the UNOS database for pancreas transplants, both databases would be enhanced with the ability to compare "apples to apples". Granted, the total number of patients is smaller and at least the current funding in the United States is inadequate. Any attempt to increase the granularity of data in pancreas transplantation is met with resistance from the centers largely due to the "unfunded mandate" issue when it comes to data reporting.

Any effort to impact pancreas transplantation positively in the next 10 years will have to start with crystallization of functional endpoints that are widely accepted and verifiable. If achieved, this would lead to a better understanding on the relative impact of pancreas transplants on the burden of diabetes in comparison with other options.

The Research Agenda

Indications for Pancreas Transplantation

The majority of solid organ pancreas transplants are carried out in patients with chronic renal failure associated with T1D. In patients not yet requiring dialysis, the timing of transplantation is based on the anticipated timepoint of needed renal replacement therapy. Typically, a glomerular filtration rate (GFR) of 20 mL/min is accepted as the upper limit of renal function at which it is reasonable to list a patient for a SPK transplant—this is partly driven by the need for equity with respect to other patients competing for donor kidneys.

Assessment for pancreas transplantation is substantially focused on cardiovascular risk—the majority of patients with diabetic renal failure have some significant degree of cardiovascular disease. Units have developed different protocols to assess this, most including some modality of myocardial functional testing (perfusion scintigraphy, echocardiography, cardiopulmonary exercise test, and so on). Research into the predictive ability of these and other factors is needed. Age is a good predictor of cardiovascular disease in this population of patients, and in most patients older than 60 years, the risk-benefit of the combined procedure is judged to be unfavorable. In patients judged to be at a lower risk of perioperative cardiovascular complications, there is consistent circumstantial evidence of the life expectancy benefit of combined pancreas and kidney transplantation.[11]

The place for SPK transplantation in patients with T2D is incompletely defined, although this procedure is carried

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out in increasing numbers by a several units.[12,13] Numerous questions require answers: Does transplantation provide similar long-term benefit as in T1D? Should this be restricted to noninsulin-resistant patients? Should this be restricted to nonobese patients? These and other questions should be addressed by collaborative and carefully designed clinical trials. In patients who do not require renal replacement therapy, the evidence that pancreas transplantation prolongs life is less clear. Recurrent episodes of hypoglycemia, often driven by the desire for tight control and long-duration diabetes, are both life-threatening and highly disruptive to lifestyle, and this is the most widely accepted indication for pancreas transplantation alone. Many patients, however, are not unaware of their hypoglycemia but may nonetheless be very compromised by the complications of diabetes. In particular, patients with rapidly progressive retinal, renal, or neurological complications are often referred for consideration of pancreas transplant alone (PTA).

This creates problems for a number of reasons: first, the outcome of PTA is poorer than that of SPK in nearly all published series, reducing any advantage of normalising pancreatic function. Second, the effect of successful pancreas transplantation with respect to halting the progression or reversing the secondary complications of diabetes has not been convincingly documented in randomized trials. There are many publications based on limited numbers in uncontrolled, observational studies, and the consensus from these shows a beneficial effect; however, publication of positive observation studies may be hampered by publication bias: negative studies are unlikely to be published. However, the quality of evidence is poor, and the risk of publication and other forms of bias considerable. It is clear that a scientifically rigorous approach to this issue is needed to establish the true benefit of PTA beyond reducing hypoglycemia unawareness. Diabetic patients who undergo PTA may contribute greatly to our knowledge of pancreas transplantation: such patients often have less-advanced secondary complications of diabetes, and longitudinal studies in such patients may provide key information as to the effect of pancreas transplantation on the progression of diabetic retinopathy, neuropathy, vasculopathy, and nephropathy. Similar studies in SPK transplant recipients (with established renal failure) are compromised by the very advanced stage of secondary complications—for example, it is hard to measure the effect of pancreas transplantation on retinal disease in a patient who has already received extensive laser therapy.

Another problematic group of patients are those with a significant degree of renal impairment, but not close enough to requiring renal replacement therapy (typically GFR 35 to 45 mL/min) to warrant kidney transplantation. Such patients are not generally eligible for SPK transplant listing and are usually denied PTA listing as well, because of the concern that the effect of calcineurin-inhibitor therapy might accelerate the decline in renal function and bring forward the need for dialysis. Such patients, therefore, are often denied transplantation until such time as their renal function has deteriorated (see above), and often express concern that they are placed at risk of deterioration of nonrenal complications in the meantime. In fact, the degree to which renal function deteriorates under these circumstances is not clear, and some evidence suggests that this might be less than was once thought.[14] This requires further investigation, particularly with the use of non-nephrotoxic immunosuppressive drugs (sirolimus, belatacept), that have not been formally tested in the context of pancreas transplantation.[15,16]

Pancreas Preservation

The majority of pancreas transplant units rely on static cold preservation using University of Wisconsin, histidine-tryptophan-ketoglutarate or Celsior solutions. The majority of published studies suggest no difference between these solutions, although there are 2 publications, which suggest inferior outcomes in histidine-tryptophan-ketoglutarate-preserved organs[17,18] especially with donation after circulatory disease (DCD) organs and longer preservation times.

Cold ischemia time is an important factor in graft outcome, with a hazard ratio of 1.13 in the Donor Risk Analysis of Axelrod et al.[6] The combination of several risk factors (eg, older age, longer cold ischemia time, DCD status) has a substantial impact of the likely outcome of pancreas transplantation. There is clearly a strong argument to develop a means of preservation that reduces the ill effect of longer preservation times.

The use of the "2-layer" method, whereby the organ is suspended at the interface between University of Wisconsin solution and the oxygen carrier perfluorocarbon, is effective in small animal models of pancreas preservation, in the context of islet isolation. In human pancreases, this method also shows a benefit in organs initially preserved in University of Wisconsin solution and those preserved for a prolonged time,[19,20] but has not been subjected to a randomized trial in solid organ transplantation.

Hypothermic machine preservation (HMP) has become increasingly popular in the preservation of marginal and DCD kidneys and is of proven outcome benefit in the context of expanded criteria donor organs. However, there is very little published information in the pancreas. Leeser et al[21] carried out HMP in 4 human pancreases and demonstrated improved islet function after isolation, but there are also concerns that HMP may be damaging to the very fragile endothelium of the pancreas.

Oxygen delivery is increasingly recognized as important in organ preservation, particularly of marginal organs. Experimental work in the porcine pancreas suggests that venous oxygen persufflation at ice temperature effectively improves the viability of the pancreas, and clinical studies are planned.[22]

There is much interest in normothermic machine perfusion in the context of the lung,[22] kidney,[23] and liver.[24] Early attempts to perfuse the pancreas in the same way have been problematic, and perfusions of more than a few hours have not proved successful in experimental models.

Two-layer preservation, persufflation, HMP, and normothermic machine perfusion are all potential targets for clinical research studies, although the technical challenges of machine perfusion (especially normothermic) are not yet sufficiently solved to warrant clinical studies immediately. Key endpoints in such studies would need to include surrogate markers of ischemia-reperfusion injury and reperfusion pancreatitis.

Immunosuppression

Immunosuppression after pancreas transplantation follows a consistent pattern in almost all units. Induction

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therapy is almost universally used, with either nondepleting antibody treatment (basiliximab) or depleting antibody treatment (thymoglobulin or alemtuzumab). There is little systematic evidence as to which of these is better in the context of pancreas transplantation, although there is evidence from kidney transplantation that alemtuzumab is superior to basiliximab as a means of minimizing rejection in low immunological risk patients and that alemtuzumab is equivalent to Thymoglobulin with respect to rejection but may be superior with respect to postoperative infection. There are several publications with respect to alemtuzumab in pancreas transplantation[25-27] but the only reported randomized trial is unpublished.[28] Although there has been no adequately powered randomized trial of induction therapy in pancreas transplantation, it is unlikely that this would provide meaningfully different guidance to that which is emerging from kidney transplant studies.

Steroid avoidance (or sparing) is particularly desirable in pancreas transplantation, and there is evidence from several quarters that the use of alemtuzumab induction enables this to be achieved safely.

Within maintenance therapy, the majority of units use tacrolimus-based immunosuppression; cyclosporine is rarely used as primary therapy although it is used as a secondary therapy in cases of tacrolimus intolerance. The importance of tacrolimus nephrotoxicity (and β-cell toxicity) is debated, and there is a view among some clinicians that the current low tacrolimus level regimens that followed the Symphony study have substantially altered the risk profile of this drug.[29] However, many clinicians believe that the long-term use of calcineurin-inhibitor medication does limit the lifespan of the kidney and that a non-calcineurin inhibitor maintenance regime is desirable. This view is strongly supported by the findings of Budde et al.[30]

Patients with an intermediate level of renal dysfunction pose a specific problem. Such patients may not have a sufficient level of renal function to be able to tolerate safely the incremental deterioration in renal function that my occur with tacrolimus, but do not qualify for a combined pancreas and kidney transplant, being still some years away from the need for dialysis. In this group of patients, trials of novel immunosuppression would be of interest, although the numbers of patients that fulfil the description make the design of a phase 3 trial challenging.

Innovative immunosuppressive regimes might include the use of belatacept and/or sirolimus. Neither drug has the immunosuppressive potency of tacrolimus and would need to be combined in such a way to provide adequate protection from rejection. The use of alemtuzumab may achieve this, or possibly, the use of tacrolimus during the early, high-risk period postoperatively. Kidney transplant trials of sirolimus have generally suggested that this is a drug which may be best introduced at an interval after transplantation. Belatacept has been tested in kidney transplant patients and shown to be nontoxic and well tolerated, albeit with higher rejection rates than cyclosporine. The combination of belatacept and sirolimus has recently been tested,[31] with and without the addition of donor bone marrow (which appeared to have little effect on outcome in this small study). The association with posttransplant lymphoproliferative disorder in Epstein-Barr virus-negative patients is of concern, although this is an uncommon scenario in adults.

Further opportunities will come with the use of cell therapy. Early trials of T regulatory (Treg) cell strategies are now in progress in kidney and liver transplantation, and early-phase studies in the use of mesenchymal stem cells have generated optimism.[32] The pancreas may be a good environment for the phase 2 studies that such strategies will need.

Immune Monitoring and Clinical Assessment of the Failing Pancreas Transplant

Monitoring the graft postoperatively is probably the greatest challenge in pancreas transplantation. Particularly in solitary pancreas transplantation (in which there is no donor-specific kidney to help with graft monitoring), graft surveillance is very subjective—the move away from bladder drainage, although desirable for many reasons, has removed a useful biochemical marker of allograft function. It is very likely that this is an important factor in the higher rate of late graft loss in this group of patients. Trials of immune monitoring are in progress in both kidney and pancreas patients to try to identify an "immunological fingerprint" that predicts imminent rejection.[33]

Other approaches to immune surveillance include endoscopic biopsies—facilitated by placing the graft in a more accessible location with the duodenal anastomosis to the proximal jejunum or duodenum.[34,35] Percutaneous biopsy is carried out by many units, but generally as a diagnostic (ie, confirmatory) test rather than for surveillance.

The hypothesis that portal venous drainage is preferable with respect to the alloimmune response is unproven but may be a viable area for research particularly in the context of more sophisticated methods of diagnosing an early immune response. A small number of units routinely drain the pancreas into the portal venous system, although there is a lack of good evidence for an outcome benefit.[36] The immunologic monitoring for pancreas transplant recipients has largely been directed at conventional monitoring of the alloimmune response. Unfortunately, this monitoring has been ineffective at monitoring the reoccurrence of the autoimmune response. Recipients of pancreas transplants are presumed to be "preimmunized" in terms of having memory autoreactive cells. To design more selective and effective (and possibly milder) immunosuppressive approaches, it is important to establish validated techniques capable of accurately monitoring both alloreactivity and autoreactivity. It is also important to recognize that insulin resistance in the absence of an immune response can contribute to graft failure. Along these lines, standardized metabolic testing will be essential to differentiate between graft failures secondary to insulin resistance versus an autoimmune or alloimmune response to treat reversible causes of endocrine insufficiency. Finally, an accurate assessment of graft failure (resistance versus β-cell loss) will facilitate more accurate definitions of graft function for utilization in registry data.

Pretransplant Screening of the Pancreas Transplant Recipient

The immunologic screening for recipients of SPK transplants, pancreas after kidney transplants, and PTA is essentially directed at the same immunologic work-up as performed for kidney transplantation. This work-up is only directed at the detection of alloantibody, but there is literature that suggests

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that pretransplant work-up looking at markers of autoimmunity should also be considered. The monitoring of the autoimmune response can include the measurement of titers of autoantibodies associated with T1D, including glutamic acid decarboxylase, IA-2, islet-specific glucose-6-phosphatase catalytic subunit-related protein, and ZnT8. It is unclear what clinical relevance these autoantibodies may have as it relates to whole organ transplants. However, there is evidence that the detection of a cellular response against autoantigens before transplantation is predictive of outcome (see below). Prescreening for the autoimmune response before transplantation may have even a higher relevance of the scenario of retransplantation. The immunologic screening before transplantations is an area which needs further investigation. The presence of an autoimmune response before transplantation may guide the choice of immunosuppression. Whether current methods of immunosuppression can block the memory response associated with autoimmunity is uncertain.

Most transplant centers are evolving to the "virtual" crossmatch for screening compatible donors. This requires single-antigen luminex beads to identify relevant anti-major histocompatibility complex antibodies which should be avoided for a given donor. The use of flow cytometry to detect T-cell and B-cell alloreactivity using flow cytometry can also be used, but, for cost reasons, may be limited to highly sensitized recipients. Currently, pretransplant immune monitoring is limited to the standard detection of preformed alloantibody. The role of an additional screen for markers of autoimmunity may provide further guidance with respect to immunosuppressive strategies and their impact on the autoimmune response.

Assessment of the Failing Pancreas Allograft

Pancreatic allograft dysfunction can be a gradual process and is frequently asymptomatic. It can be detected by a gradual escalation in Hgb A1C, or incidental elevations in serum amylase and lipase. Unfortunately, it is frequently discovered by the new onset of hyperglycemia. Early detection is imperative to identify reversible causes of graft dysfunction. Broad consideration for the etiology of dysfunction include: alloimmune rejection, recurrent autoimmunity, insulin resistance (T2D), chronic calcineurin inhibitor toxicity, graft pancreatitis in the absence of rejection, cytomegalovirus, posttransplant lymphoproliferative disorder, and bacterial or fungal infection. An algorithm to distinguish between the above factors requires a standardized clinical assessment. The clinical assessment should be triggered by incidental elevations in serum amylase and lipase, a gradual increase in fasting blood sugars and/or an increasing HgbA1C or hyperglycemia. The assessment should include an ultrasound of the pancreas with Doppler, and potentially magnetic resonance imaging or computed tomography depending on the expertise at the center. Blood cultures and cytomegalovirus cultures should be performed if there is suspicion for an infectious etiology.

Although ultrasonography can provide indirect evidence of acute rejection, its most important application is to guide a percutaneous needle biopsy using an 18 gauge core biopsy needle. Poor visualization of the pancreas secondary to adjacent bowel or patient body habitus may preclude transcutaneous biopsy. Occasionally, a suitable window can be found with CT guidance, and rarely an open/laparoscopic biopsy is required. In the absence of a biopsy, a suspicion for acute rejection can be made based on ultrasonography findings and laboratory findings consistent with rejection (elevated lipase/amylase). Gene signatures associated with rejection have been validated for both kidney and liver transplantation, but not pancreas transplantation.[37] Of equal significance, the rejection "signature" appears before the clinical onset of rejection. Serum markers for rejection in the scenario where a pancreas biopsy is unattainable would be particularly helpful. Unfortunately, these markers need to be validated for pancreas transplantation, and validating gene signatures for both alloimmunity and recurrent autoimmunity should be done. The ability to pick up signals of recurrent autoimmunity as well as the development of a de novo alloimmune response before clinical deterioration would greatly facilitate the management of the pancreas transplant recipient. The availability of tissue greatly facilitates the ability to distinguish between the multiple etiologies leading to pancreatic graft dysfunction. The Drachenberg/Banff guidelines for the diagnosis of rejection were recently updated and will not be reviewed herein.[38] Early recurrent autoimmune disease can be identified by islet-centered lymphocytic inflammation (isletitis), but more frequently late recurrent disease is associated with the absence of insulin producing β cells using immunohistochemical stains.

Immune Monitoring of the Alloimmune and Autoimmune Response

There is an increasing amount of data demonstrating serologic markers of alloimmunity and autoimmunity associated with pancreatic graft dysfunction. Again, it is unclear what the significance of the autoantibodies (GAD, IA-2, IGRP, ZnT8) associated with diabetes is in terms of the development of dysfunction. In islet transplantation, the increase in these titers and epitope spreading was associated with graft loss.[39] Other reports could not find an association between autoantibody and islet graft outcome.[40] With regards to the findings of autoantibodies and pancreas transplantation, Sibley[41] found that there was no evidence of autoimmune recurrence in non-HLA identical recipients and no islet cell autoantibodies. However, Bosi et al[42] found that 9 of 23 pancreas transplant recipients (non-HLA matched) developed ICA antibodies and 7 of 9 went on to develop graft loss with 2 to 35 months after detection. There have been more recent reports of increasing autoantibody titers and epitope spreading in recipients of pancreas transplants that were associated with inferior outcome.[43]

Interestingly, there are an increasing number of reports suggesting that monitoring the cellular response against autoantigens is strongly associated with graft dysfunction. Much of this literature relate to islet allograft survival and will not be reviewed, but is nicely summarized in a review from Abreu and Roep.[44] The assays for monitoring the cellular response to autoantigens have not been standardized, but effective assays that can be validated and performed at multiple laboratories will be an important advancement. The most elegant demonstration of recurrent autoimmune disease after whole-organ pancreas transplantation came from Vendrame and demonstrated the progression of allograft dysfunction associated with the

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development of autoantibodies and a CD4– T-cell response against GAD.[43] These autoreactive cells were isolated from biopsies and detected and sorted using class II tetramers. When these were cotransplanted with human islets into immunodeficient mice, they caused diabetes. In the same report, other CD8+ lymphocytes reactive against IGRP autoantigen were detected using class 1 pentamers. A more recent report from Japan shows similar evidence for recurrent autoimmune disease after SPK transplantation from a DCD donor.[45]

Clearly, a standardized/validated approach to monitoring autoreactive cells with different specificities will be important prognostically and will help guide the immunosuppressive interventions to prevent graft loss after pancreas transplantation. At the same time, these studies may provide insight into the etiology of late graft loss in the absence of an alloimmune response. The tetramer-based assays are compromised by the large volume of blood necessary to detect responsive cells. Other assays using major histocompatibility complex multimers permit direct ex vivo quantification of autoreactive cells and require significantly less blood.[46] This assay, the Diab-Q kit, requires low blood volumes, and its results correlated with clinical outcome in islet recipients. Another monitoring strategy determines the cellular response against overlying peptides of the autoantigen GAD.[47] Although this assay has not been validated, it is attractive in that the assay is not HLA restricted.

Insulin Resistance

Finally, pancreatic graft insufficiency/failure may be related to the development of insulin resistance in the absence of alloimmunity or autoimmunity. In these cases, recipients may have a gradual increase in fasting glucose, or a gradually escalating Hgb A1C. This may be related to weight gain, steroid use, or calcineurin inhibitor toxicity. Because all of these may be reversible, it is important to determine whether increases in fasting glucose and Hgb A1c are related to insulin resistance. The gold standard for determining insulin resistance is a clinical research center-based assessment using the euglycemic-hyperglycemic clamp studies. These studies are labor intensive, and require a stay in a clinical research center, thus are not adapted to office-based assessment of pancreas transplant function. However, the homeostasis model assessment of insulin resistance is gaining widespread use as a result of its simplicity and validity and is based on fasting blood glucose (FBG) and insulin levels.[48] The homeostasis model assessment score correlates well with the euglycemic-hyperglycemic clamp in terms of assessing insulin resistance after pancreas transplantation. The detection of insulin resistance is important, in that it may be reversible by altering immunosuppression, weight loss, addition of oral hyperglycemic agent, or glucagon-like peptide (GLP)-1 agonists.

In summary, a complete clinical assessment of the failing pancreas graft should differentiate between alloimmunity, recurrent autoimmunity, and the development of insulin resistance. Because treatment for each of these etiologies leading to poorer glycemic control requires different strategies, appropriate immunomonitoring and metabolic testing may be able to identify reversible causes for graft dysfunction and loss. Standardization of these noninvasive assays for monitoring the alloimmune and autoimmune responses, as well as metabolic testing for insulin resistance, will provide essential data in terms of early intervention to prevent graft loss. Nonetheless, the validation of noninvasive markers of recurrent autoimmunity as well as metabolic tests for insulin resistance represents a major "gap" in pancreas transplantation. At the same time, validated tests to differentiate between causes for graft insufficiency/failure will be extremely useful in terms of accurately reporting outcomes of pancreas transplantation.

Prospective Randomized Comparison of Pancreas and Islet Transplant Outcomes With Best Medical Therapy

Prolonged insulin independence (47% at 3 years) has been demonstrated in the modern era of islet transplantation.[49] In addition, selected groups have reported 50% or greater 5-year insulin independence rates in islet transplants,[50] thus comparing it favorably to solitary pancreas transplants. This comparison, despite its weaknesses, compels us to strongly consider a prospective randomized trial comparing pancreas and islet transplants.

There are obvious challenges to designing and conducting this trial—when is the right time (pending Food and Drug Administration [FDA] application for registration of islets as a therapeutic agent), what is the design—randomization points and patient counseling. Early randomization could lead to a high patient dropout rate, late randomization may lead to patient apprehension about not knowing whether they would undergo major surgery). Several additional questions arise, particularly reimbursement issues.

Inclusion and exclusion criteria could be controversial. The typical islet recipient (low body mass index, low insulin requirements, early or no secondary complications) is not the typical pancreas transplant recipient. Whether patient selection as currently performed would need to change to enable a clinical trial is an area of uncertainty. Another important consideration is whether the best medical therapy should be compared with both transplant options. Can we get the patients and endocrinologist investigators on board?

Despite these challenges, if this trial can be performed credibly and successfully, it will go a long way in answering key questions that could shape the trajectory of both pancreas and islet transplants in the future. In addition, it will provide a unique opportunity for cross-fertilization and collaborative efforts between investigators that have been previously focused solely in the pancreas or islet transplant fields, as well as the endocrinology community.

Secondary Complications and Mortality

The impact of pancreas transplantation on secondary complications of diabetes has been documented by several investigators. Most notably, in patients with T1D mellitus who did not have uremia and have not received a kidney transplant, pancreas transplantation did not ameliorate established lesions of diabetic nephropathy within 5 years after transplantation, but did so at 10 years posttransplant.[51]

Improvement in motor, sensory, and autonomic indices in patients with diabetic neuropathy after pancreas transplantation has been reported.[52]

At the Manchester Royal Infirmary, 20 SPK transplant recipients were studied before and 6 months after SPK transplantation; these were compared with 15 normal volunteers

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using retinal confocal microscopy: SPK transplant recipients compared with normal controls had a marked decrease in nerve fiber morphometry and nerve fiber length and density dramatically improved.[53]

Thus, the beneficial effect of pancreas transplantation on diabetic retinopathy seems highly logical. However, studies to date do not use strict case-control method comparing transplanted patients versus untransplanted controls. Also, risk factors, such as blood pressure, baseline degree of disease, renal function, and type of immunosuppression, are not controlled.

Does Pancreas Transplantation Protect the Kidney Transplant?

The effect of pancreas after kidney (PAK) on the kidney transplant has been marred by controversy due to lack of clear controlled data. One United States Renal Data System study of PAK in diabetic kidney recipients showed PAK was protective of renal function in all groups, and documented that a GFR between 30 and 39 mL/min was a risk factor for kidney failure after PAK.[54] In a long-term study (5 years) of the efficacy and safety of pancreas transplantation alone, it was shown that in 51 patients with sustained pancreas transplant function, kidney function (serum creatinine and glomerular filtration rate) decreased over time with a slower decline in recipients with pretransplant filtration rate less than 90 mL/min.[55]

Live donor kidney (LDK) transplant alone has been shown to provide a survival advantage in T1D patients compared with deceased donor transplantation. Moreover, recipients of an SPK transplantation had statistically significant patient and kidney graft survivals compared with those T1D patients who received a kidney transplant alone.[56] Interpretation of Young's data is difficult because the donors for those who received a deceased donor kidney (DDK) alone were significantly older than the donors of SPK transplantations. Nevertheless, it appears that kidney survival after multivariate analysis appeared to be superior when a pancreas transplant was performed simultaneously. It has been shown that early pancreas graft failure in SPK transplant recipients is associated with an increased risk for subsequent kidney failure and death.[11] Patients with end-stage renal disease and T2D have been shown to benefit from SPK transplantation in a selected series. A commonality of most of these series is that the T2D recipients were not obese and did not have excessive insulin requirements. Thus, lean, insulinopenic T2D have similar outcomes as T1D recipients of an SPK transplant. Sampaio et al[57] showed that T2D recipients of SPK transplants were not at increased risk for death, kidney failure, or pancreas failure when compared with recipients with T1D.

Attempts to document clear survival benefit after pancreas transplantation have been hampered by limitations and controversy. A large study looking at 4-year survival for transplanted patients versus those on the waitlist suggested a survival benefit in SPK transplantation but a survival disadvantage in PAK and PTA.[7] However, the same population was reanalyzed by a different group showing survival benefit for all groups of pancreas transplants—SPK, PAK, and PTA.[58]

Thus, studies on the effect of pancreas transplantation on patient survival have been affected by the lack of control for renal function, lack of comparability of study groups: SPK versus DDK versus LDK. They have been highly impacted by selection bias (choosing ideal patients for transplantation) and not controlling for the effect of renal function over time. Further, patient risk factors allocated to choice between SPK, DDK, and LDK are not the same.

Proposal for a Randomized Trial in Pancreas Transplantation

Do we need a randomized trial comparing SPK versus kidney transplant alone? There are significant factors that would support the rationale for such a trial. Animal studies of benefit are compelling. Nevertheless, the best medical therapy of diabetes has improved dramatically with progressive reductions in diabetes related mortality and improvement in quality of life. Studies in the past asserting SPK transplant's benefits are poorly controlled especially for renal function. Case selection bias inherent in prior studies can be overcome. Outside the transplant community, pancreas transplantation is not accepted, particularly among endocrinologists. This is further complicated by the fact that the endpoint of pancreas transplant function has never been clearly defined. It is acknowledged that among members of the pancreas transplantation surgical community that pancreas transplantation is effective at reducing mortality and arresting secondary diabetic complications. As such, most would suggest that the time has passed for a randomized trial of SPK transplantation versus kidney transplantation alone. A strategy for analyzing the impact of transplantation-based β-cell replacement on mortality and renal function (as well as other secondary complications) would be for a head-to-head randomized trial of pancreas and islet transplantation. Recent reports of near equivalence in the 5-year graft survival of PTA and islet transplant alone (ITA) make a head to head trial appropriate. Based on these considerations, the following trial is proposed comparing pancreas and islet transplantation. The SPK transplantation could be compared with simultaneous islet kidney (SIK) transplants. In this head-to-head trial, the assumption will be made that no control group receiving a kidney alone is feasible both because the vast majority of the transplant community consider kidney transplant alone inferior to SPK transplantation. Moreover, patient acceptance and knowledge of SPK transplantation is very high. Consequently, enrollment in a trial comparing SPK and SIK transplantations to kidney transplantation alone is likely infeasible. However, nonuremic diabetics receiving either ITA or PTA or previously kidney-transplanted diabetics receiving a islet-after-kidney transplant (IAK) or PAK could be randomized to a third arm of untransplanted controls. In the case of IAK and particularly PAK, there remains significant scientific disagreement regarding the potential benefit of pancreas transplantation on mortality, future renal function, and other secondary complications.

Summary of Research Priorities

(1) Develop carefully designed, well-controlled clinical trials that define the impact of pancreas transplantation on mortality and secondary complications, particularly renal function. Developing validated measures of pancreatic organ function will be required for success.

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(2) The greatest obstacle to growth of pancreas transplantation is low organ utilization rates. Preservation strategies leading to improved early graft survival and function and increased utilization are needed.
(3) Concern about new onset and recurrent renal dysfunction markedly limits growth of pancreas transplantation. Clinical trials of non-nephrotoxic immunosuppression are needed.
(4) Detailed clinical studies verifying the risk of recurrent disease are needed to exclude other causes, such as β-cell exhaustion and alloimmune causes. Refine immunologic detection and prevention strategies against recurrent autoimmunity.

Two parallel randomized trials of whole organ pancreas versus isolated islet transplantation are recommended: (a) SPK versus SIK, and (b) PTA and PAK versus ITA and IAK versus best medical therapy.

ISLET ALLOTRANSPLANTATION

Current State of the Field

Over the last 10 years, islet allotransplantation has developed into an established treatment modality for subjects with T1D complicated by hypoglycemia unawareness, and the procedure is currently reimbursed for this indication in several countries. At present, the primary goal of islet transplantation should be optimal glycemic control without severe hypoglycemia, rather than insulin independence. Importantly, this must be routinely achievable with a single islet infusion. A standardized approach to evaluation of clinical outcomes will be essential for further developments in β-cell replacement.

A recently completed multicenter prospective phase 3 study[59] demonstrated that:

(1) Islets can be manufactured reproducibly at multiple sites using a common manufacturing process.
(2) Independence from exogenous insulin can be achieved in about half of islet recipients at one year from infusion, with 1 or 2 infusions needed.
(3) Glycemic control is excellent even when insulin independence is not achieved.
(4) Hypoglycemia unawareness is treated effectively by islet transplantation, with associated freedom from severe hypoglycemic events.

Islet allotransplantation is also an acceptable therapy for patients with end-stage renal failure and T1D, either simultaneously with or after kidney transplantation.[60] A comparison between islet and pancreas transplantation in combination with a kidney transplant demonstrated achievement of similar HbA1c levels in the 2 groups. Islet recipients were less likely to achieve insulin independence, whereas pancreas recipients had substantially greater procedure-related morbidity.[61]

Because of the limited overall availability of human organ donors, islet allotransplantation is unlikely to provide a cure for all those affected with uncomplicated T1D, unless islet expansion becomes a reality. In addition, "closed-loop-systems" using implantable glucose sensors to control insulin administration may enable good metabolic control without the need for systemic immunosuppression in uncomplicated T1D.[62] However, we fully anticipate that improvements in the outcome of islet transplantation will, within 5 to 10 years, make islet transplantation an appropriate therapy not only for the current indications of hypoglycemia unawareness and grossly unstable glycemic control, but also for all people currently considered eligible for pancreas transplantation. Many of the recent and future advances in islet allotransplantation will benefit clinical islet transplantation overall in the future. This applies whether stem cell or xeno sources are used as the alternative cell source and/or immune tolerance or immunoisolation protocols are used to obviate the need for immunosuppression. To achieve these goals, however, a number of obstacles need to be overcome.

Pancreas Allocation

In most countries, allocation of donor pancreases for whole pancreas transplantation still takes priority over pancreases for islets.[63] Clearly, if reimbursement for islet transplantation is implemented, parity of organ allocation is essential for the islet transplant service to be effectively delivered. The United Kingdom has pioneered a joint pancreas allocation system which is currently being evaluated. It is based on a point system and organs allocated to the matched patient at the top of the joint waiting list, regardless of whether they are listed for whole organ or islets. Overall, it is important that any decision to allocate donors with specific characteristics preferentially to pancreas or islet transplantation should be based on rigorous studies of clinical outcomes.

"Competition" Between Whole Pancreas and Islet Transplant

Whole pancreas and islet transplantation are still often seen as competing therapies.[64] This is not only the case for organ allocation, but also for patient referral and patient selection. Using analysis of stratified outcome data, we need to work toward unified, joint programs of β-cell replacement, with treatments tailored to individual patients, rather than treatments being largely dictated by referral patterns and physician preference. We anticipate that, within 5 to 10 years, islet transplantation will be the preferred therapeutic procedure for β-cell replacement, as a result of the metabolic efficiency and the superior safety profile of the islet in relation to pancreas transplantation. However, in the meantime, it is essential that these 2 different modalities are considered complementary, rather than in direct competition.

Funding and Reimbursement

The encouraging results of islet transplantation over the past decade mean that, from a clinical point of view, islet transplantation can rightly now be considered as a clinical treatment rather than an experimental tool. However, for this transition to be fully realized in terms of islet transplantation becoming a standard therapy worldwide, full reimbursement by health care providers needs to be implemented. Although this has been achieved in a number of countries around the world (eg, United Kingdom, Switzerland, Canada, and so on), this remains an ongoing challenge in many countries including the United States.

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Regulation

Over the last decade, islet isolation has become a highly regulated procedure in most countries. This has added enormously to the cost and complexity of the isolation procedure and as a result has propagated the importance of islet transplant networks, in which 1 or 2 centers isolate human islets within "state of the art" designated isolation facilities for a network of implanting centers.

The degree of regulation of pancreatic islets and the pathway to licensure for an islet product vary in different countries.[65,66] However, all islet isolation and islet transplant teams must work with their regulatory bodies to ensure that islet personnel are closely involved in the development and interpretation of the cell processing regulations, rather than them simply having them imposed from the outside.

The Research Agenda

Pancreas Procurement, Preservation, and Islet Transport

To expand the donor pool, novel strategies of optimized pancreas procurement, pancreas preservation, and islet transport are essential. First, a unified approach to optimal pancreas retrieval during organ procurement is essential, regardless of whether the pancreas is being procured for whole organ or islet transplantation. Indeed, several studies have demonstrated that a dedicated surgical team currently is the most important determinant for the clinical outcome of islet allotransplantation.[67] Second, research into the optimal approach to pancreas preservation, whether persufflation, normothermic perfusion, or perfluorocarbon incubation[68-70] is vital (see previous section on whole organ transplantation). Finally, the stringent regulation associated with human islet isolation described above, means that islet isolation/islet transplant networks will increasingly become the norm. Ideally, each islet isolation facility should support a population of 10 to 20 million. However, for networks to realize their full potential, optimization of islet transport and more efficient systems for islet shipping need to be developed.

Optimization and New Strategies for Islet Isolation

Although great progress has been made in standardization of islet isolation, significant improvements are still needed. The pancreas digestion step, in particular, remains an empiric undertaking, dependent on the relatively haphazard interaction of administered bacterial enzymes with the endogenous enzymes of the donor pancreas. In addition, currently the same techniques are used regardless of the huge variability in human donors. Research efforts should focus on understanding the detailed molecular ultrastructure of the pancreatic islet-exocrine matrix in the full range of donors (age, BMI, and so on)[71] and on developing new, targeted clinical grade enzyme (recombinant) blends that can be used on all available donor pancreases.[72,73] Research should also continue into the selective inhibition of the activated pancreatic enzymes. In parallel, alternative, nonenzymatic technologies for cell separation, for example, photodynamic technologies, and so on, should be investigated.[74,75]

Islet Preconditioning and Culture

The introduction of a pretransplant period of islet culture has been hugely beneficial from a logistical (patient work-up, radiology, and so on), physiological (islet recovery, and so on), and regulatory (functional and safety release criteria) perspectives. However, this period of islet culture also presents an opportunity for novel interventions before islet implantation which could provide exciting opportunities to improve clinical outcomes. Potential strategies include coating with compounds that promote oxygenation and islet engraftment,[76] compounds that reduce instant blood-mediated inflammatory reaction (IBMIR),[77,78] compounds that enable "in vivo" islet imaging,[79,80] compounds that secrete local immunosuppression,[81,82] and compounds inducing protection against hypoxic stress.[83] These approaches are collectively termed "islet preconditioning" and clearly require close collaborations with those in the fields of nanotechnology and tissue engineering. The role of peri-islet "scaffolds" is another area needing extensive research, with exploration of the associated issues of islet-exocrine interactions, for example, paracrine influences, signaling, and so on.[84-86] Moving such developments through regulatory agencies is likely to be complex, because they involve cellular products, devices, and drugs. The issue of commercialization of human cells is also problematic. Scientific international organizations, such as IPITA and TTS, together with patient advocate groups should engage in facilitating discussions with regulatory bodies to make these developments possible.

Defining the Islet Product

The current approach to establishing identity, potency, and purity of manufactured islet remains crude and variable between centers. Indeed, there is currently no basis for predicting clinical outcomes based on product characterization. Improved measures would facilitate more stringent release criteria as well as enabling meaningful comparisons between centers and for the purposes of rigorous scientific studies.[87]

Strategies to Stratify Recipient in Terms of High Risk Versus Low Risk for Islet Survival/Function

The development and implementation of reliable and accurate methods to stratify recipients according to pretransplant predictors of high risk versus low risk for islet survival/islet function (eg, immunologic and metabolic signatures)[88] should enable a personalized approach to the optimization of clinical outcomes after islet transplantation.

Novel Sites for Implantation

Although the liver is easily accessible and has a number of advantages compared with some other anatomical sites, it is probably not the "ideal site" for islet implantation. Research should continue to focus on identifying alternative implantation sites[89] which will ensure that islet transplantation remains a straightforward, well-tolerated, minimally invasive procedure, but that ensures improved islet graft survival and optimal glycemic control.

"In Vivo" Islet Imaging and Biomarkers for Islet Survival and Rejection

The development and implementation of reliable and accurate methods for "in vivo" islet imaging[90-92] is essential to

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obtain a better understanding of clinical outcomes, that is, islet engraftment, the gradual loss of function, evaluation of the optimal site, and technique for implantation. In addition, identification of a panel of validated biomarkers within the blood would greatly enhance our understanding of islet graft function.

Strategies to Minimize the Need for Systemic Nonspecific Immunosuppression and Eventually Induce Tolerance

Several approaches are supported by strong preclinical results and are close to being introduced in clinical studies. Approaches currently being introduced in clinical studies include[93]: cotransplantation of mesenchymal stem cells (MSCs) or Treg cell; islet pretreatment (anti-HMGB1, islet transduction to facilitate engraftment, and inflammation); islet encapsulation (both macroencapsulation and microencapsulation—see below); recipient treatment using nondepleting monoclonal antibodies (mAbs). Efficient modalities found in experimental studies should be appropriately applied to preclinical study. The obviation of immunosuppression would be the quantum step required to enable islet allotransplantation to be implemented in younger patients, including children. It must be remembered that this remains the ultimate aim of islet transplantation.

Summary of Research Priorities

(1) Optimization of pancreas procurement, pancreas transport, and development of targeted methods for islet isolation to improve functional islet yield to permit routine single-donor insulin independence
(2) Standardization of definition of released islet product to enable accurate comparisons between centers and enable accurate prediction of islet graft outcome.
(3) Development of novel strategies for islet preconditioning to improve islet engraftment and islet graft longevity.
(4) Definition of suitable alternative anatomical sites for islet implantation.
(5) Strategies to minimize or eliminate the need for immunosuppression, enabling the ultimate goal of islet allotransplantation to be reached, that is, the transplantation of children

ISLET XENOTRANSPLANTATION

Current State of the Field

Progress made in human islet transplantation in the past 15 years has made commonplace the restoration of near-normoglycemia, insulin independence, and protection from severe hypoglycemia with a low risk of procedural complications in immunosuppressed T1D recipients.[49] These favorable outcomes can now be achieved in single-donor islet transplant recipients, both with refined induction therapy[94] and calcineurin inhibitor-sparing maintenance immunosuppression.[95,96] Increasing evidence indicates that insulin independence can be maintained in more than 50% of recipients for 5 years[50] and that islet transplants have slow progression of microvascular complications.[97]

Improving human islet allotransplant efficacy and safety outcomes have inspired investigators to develop more widely available cell-based diabetes therapies. Preclinical safety and efficacy data obtained in the last 10 years in the stringent pig-to-nonhuman primate (NHP) islet transplant setting,[98-109] and preliminary safety data obtained in recent pilot clinical trials,[110] suggest that xenogeneic pig islets can possibly be developed into an islet β-cell replacement therapy with broad applicability. However, several hurdles remain to be overcome.

In the preclinical pig-to-NHP model (Table 1), several groups have achieved insulin independence for longer than 180 days with near-physiologic control of fasted and postprandial glucose in a small number of NHP after porcine islet xenotransplantation.[98-103,106-109] Except for 1 report[101] that demonstrated long-term survival of embryonic porcine pancreatic tissue transplanted to the omentum of 2 monkeys immunosuppressed with anti-thymocyte globulin (ATG), anti-IL-2R, anti-CD20, belatacept, everolimus, and FTY720, in all other studies that achieved long-term diabetes reversal, adult islets or neonatal islet cell clusters (NICC) were transplanted intraportally into monkeys in whom the rejection prophylaxis involved induction or both induction and maintenance immunosuppression with CD40-CD154 costimulation pathway blocking antibodies. Although several studies show the efficacy of anti-CD154 antibodies in preventing islet xenograft rejection,[98-100,103,106,107] 1 report indicates that prolonged islet xenograft survival can also be achieved with antagonistic anti-CD40 antibodies.[102]

Genetic engineering of donor pigs mitigates the IBMIR to intraportally transplanted pig islets.[103,111] The IBMIR is a major obstacle to engraftment of intraportal porcine islet xenografts in primates[112]; it is triggered by the contact of isolated islets with blood and causes islet destruction by complement and coagulation activation products and other inflammatory mediators released by recruited neutrophils and monocytes.[113-116] Intraportal transplantation of galactose-α 1,3-galactose (αGal)-deficient NICC from galactosyl transferase knockout (GT-KO) donor pigs[117] increased achievement of insulin independence in rhesus macaques when directly compared with wild-type (WT) NICC.[103] The improved engraftment of αGal-deficient NICC was likely due to reduced antibody and complement binding as well as complement-dependent destruction. Profound IBMIR was also triggered by WT NICC in baboons, with intravascular clotting and graft destruction occurring within hours.[111] In contrast, and without directly targeting coagulation, IBMIR was minimal, and intravascular clotting was not observed in baboons after transplantation of NICC from αGal-deficient porcine donors transgenic for the human complement regulators CD55 and CD59. The extent that the transgenes CD55 and CD59 contributed to the protection of αGal-deficient NICC from IBMIR was not directly addressed. The transgenic expression on adult porcine islets of human CD46, another complement regulatory factor, had little impact on IBMIR but was effective in limiting antibody-mediated rejection.[100]

There is no additional evidence, as of now, of prolonged survival of GM porcine islets in NHP.[118] Xenografts of αGal-deficient NICC in NHP were not protected from eventual cellular rejection.[103] Also, αGal-deficient NICC transgenic for hCD55 and hCD59 underwent cell-mediated rejection within 1 month after transplantation into baboons immunosuppressed with a costimulation blockade-sparing protocol, including ATG, tacrolimus, and MMF.[111] The NICC with islet β cell–specific expression of LEA29Y, a high-affinity variant

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TABLE 1.

Published Studies Demonstrating Prolonged Insulin Independence After Pig Islet Transplantation in NHP With Surgical or Streptozotocin-Induced Diabetes

Donor Islet Tissue	Islet Mass, IEQ/kg	Immunosuppression		Insulin-Independent Graft Survival (range), d	Reference
		Induction	Maintenance		
Wild-type adult pig islets	25 000	α-IL-2R	α-CD154, everolimus, FTY720, ± leflunomide	47, 54, >68, >73, >111, >140, >145, >158, >187	Hering et al, 2006[98]
Wild-type neonatal pig islets	34 000-50 000	α-CD154, α-IL-2R	Belatacept, sirolimus	(30), 76, >86, >147, >169, 194, 344	Cardona K et al, 2006[99] Thompson et al, 2011a[102]
Wild-type neonatal pig islets	50 000	α-CD154, α-LFA-1,	CTLA4-Ig, MMF	Nonengraftment (×4), 137	Thompson et al., 2011b[103]
GT-KO neonatal pig islets	50 000	sTNFR α-CD154, α-LFA-1, STNFR	CTLA4-Ig, MMF	Nonengraftment (×1), 50, 91, 99, 249	
hCD46 transgenic adult islets	85 000-100 000	ATG, methyl-prednisolone, LMW dextran sulfate, prostacyclin	α-CD154, MMF	0, 87, 91, 92, >396	van der Windt et al, 2009[100]
GTKO/hCD46/TFPI^{Ins}/±hCD39^{Ins}/pCTLA4-Ig^{Ins} adult pig islets	50 000-100 000	ATG, methyl-prednisolone, LMW dextran sulfate, aspirin, prostacyclin	α-CD154, MMF	0 (×4), >365	van der Windt et al, 2012 Bottino R et al, 2014[107]
Wild-type adult pig islets	81 000-100 000	α-ICAM-1	α-CD154, sirolimus	>140	Jung et al, 2011[105]
Wild-type adult pig islets	61 000-98 000	CVF, α-ICAM-1	α-CD154 thru day 90, sirolimus	210, 225, 365	Kang et al, 2014[106]
Wild-type adult pig islets	100 000	Factor H, ATG	α-CD154 thru day 90, sirolimus	156 and >256	Kang et al, 2014[106]
Wild-type embryonic pig pancreases (E42)	60 E42 pancreases	ATG, α-IL-2R, α-CD20	Belatacept, everolimus, FTY720	Thru days ~250, ~330	Hecht et al, 2009[101]
Wild-type neonatal pig islets	~50 000	α-IL-2R, α- CD40 Chi220	Belatacept, mycophenolate mofetil	(54), 47, 56, >59, >89, >203	Thompson et al, 2011[102]
Wild-type neonatal pig islets	~50 000	α-IL-2R, α-LFA-1 thru day 35, tacrolimus thru day 56	Belatacept, mycophenolate mofetil	0, 0, 46, 60, 99	Thompson et al, 2012[104]
		LFA-3-Ig thru day 56	Belatacept, mycophenolate mofetil, α-LFA-1 thru day 90	0, 0, 92, 111, 114	
Wild-type adult pig islets	30 000	None	None (macroencapsulation of monolayer of islets on acellular collagen matrix)	140, 140, 161, 168, 196	Dufrane et al, 2010[108]
Wild-type adult pig islets	15 000-62 500	None	None (coencapsulation of islets and MSC on acellular collagen matrix	Up to 224 (3 of 10 > 168)	Veriter et al., 2014[109]

α, anti; ATG, anti-thymocyte globulin; CVF, Cobra venom factor; E42, embryonic day 42; hCD46, human CD46; hCD39, human CD39; IEQ, islet equivalent; Ins, insulin; LFA, lymphocyte function-associated antigen; LMW, low molecular weight; MMF, mycophenolate mofetil; sTNFR, soluble tumor necrosis factor receptor; TFPI, tissue factor pathway inhibitor.

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of the T-cell costimulation inhibitor, CTLA4-Ig, were protected from cell-mediated rejection in humanized mice.[119] Adult porcine islets with expression of CTLA4-Ig, under the control of the porcine insulin gene promoter, have recently been transplanted into NHP; however, the study was not designed to test the immune-protective characteristics of the CTLA4-Ig transgene.[107]

Encapsulation facilitated restoration of insulin independence longer than 180 days in 3 porcine-to-NHP islet transplant studies. Intraperitoneal transplantation of adult pig islets in alginate-polylysine-alginate microcapsules rendered 7 of 9 spontaneously diabetic monkeys insulin-independent for periods ranging from 120 to 804 days with FBG levels in the near-normoglycemic range.[120] Macroencapsulation of adult porcine islets in alginate and transplantation into abdominal subcutaneous tissue as an islet monolayer on an acellular collagen matrix in a macrodevice maintained FBG levels less than 150 mg/dL for 20 to 28 weeks in 5 streptozotocin-diabetic, nonimmunosuppressed cynomolgus monkeys; 2 of the 4 control monkeys that received microencapsulated adult porcine islets under the kidney capsule showed FBG levels less than 150 mg/dL for up to 2 weeks.[108] A subsequent report by the same investigators showed that coencapsulation of islets with MSCs slightly improved oxygenation and neoangiogenesis of subcutaneously placed implants and maintained FBG levels in the near-normal range for up to 32 weeks in nonimmunosuppressed monkeys; however, the cotransplanted MSC did not substantially improve or prolong islet xenograft function.[109]

The first clinical trial of porcine islet xenotransplantation under a comprehensive regulatory framework was performed in New Zealand after the authorization by the Minister of Health under a specific section of the New Zealand Medicines Act, and also after thorough review performed by the New Zealand Medicines and Medical Devices Safety Authority, Medsafe, in consultation with the National Health Research Council and international referees.[110,121,122] This open label, safety, and dose finding phase I/IIa study of microencapsulated neonatal porcine islets, prepared under GMP from designated pathogen-free donor animals and transplanted intraperitoneally in 14 nonimmunosuppressed subjects with unstable T1D, demonstrated the microbiological safety of the tested encapsulated porcine islet product.[123] There was no apparent dose effect of porcine islets, and porcine C-peptide was not measurable in the serum of any of the transplanted subjects.[110] Nevertheless, the reduced frequency of unaware hypoglycemic events, the lower HbA1c levels, and the up to 30% lower daily insulin requirements observed in some of the subjects posttransplant provided indirect and preliminary evidence of islet xenograft function.[110,122] The same encapsulated porcine islet product was subsequently tested at doses of 5000 IE/kg and 10 000 IE/kg in a phase IIa efficacy trial in 8 subjects with T1D and hypoglycemia unawareness in Argentina with authorization by the Minister of Health and approval by the local bioethical committee.[122] This trial confirmed the microbiological safety of the porcine islet product and demonstrated lower insulin requirements, frequency of unaware hypoglycemic events, and HbA1c levels in most subjects compared with pretransplant.

Obstacles to Application of This Therapy

Although substantial progress has been made in islet xenotransplantation, 2 significant obstacles to clinical application remain.

First, a clinically applicable immunosuppressive protocol for preventing rejection of porcine islet xenografts is currently not available.[118] Regimens that protect islet allografts in NHP and humans from rejection, including basiliximab combined with FTY720 and everolimus[124] and antithymocyte globulin combined with tacrolimus and mycophenolate mofetil,[125] fail to facilitate long-term islet xenograft survival in NHPs,[98,111] indicating that either the immunity to islet xenografts is stronger than islet allografts or that additional immune recognition and effector pathways are operative in xenoislet compared with alloislet transplantation. As summarized above, prolonged porcine islet xenograft survival has been achieved with protocols based on CD154-CD40 costimulatory blockade by several groups. However, long-term functional survival exceeding 180 days, the efficacy milestone to be met in 5 or more of 8 NHP before initiating clinical trials according to the consensus statement of the International Xenotransplantation Association,[126,127] has been demonstrated in only a small proportion of transplanted NHPs (1 to 2 of 3 to 7 in several studied cohorts; Table 1).

Lack of more consistent success could be ascribed to failure of the protocol to prevent rejection or to failure of the preclinical model to accommodate the protocol.[128] Therefore, continued understanding and improvement of the preclinical animal model is a critical requirement for documenting long-term success on a consistent basis.

Long-term success has also been precluded by a high proportion of engraftment failure after porcine islet transplantation due to IBMIR and complement-mediated lysis of islets triggered by binding of preformed xenoreactive antibodies. Engraftment of neonatal porcine islets has recently improved considerably with the use of GT-KO donors.[103,111] Early posttransplant loss of adult islets from GT-KO can be very significant,[107] possibly due to the activation of complement by transplanted islets via the alternative pathway.[106] As shown in human islet allotransplantation,[50,129] increasing the proportion of transplanted porcine islets that stably engraft will substantially improve long-term islet graft function.

Several immunotherapeutics with demonstrated efficacy in inhibiting immune responses to porcine islet xenografts are no longer available for clinical investigation in transplantation due to their safety profile. These include in particular anti-CD154 antibodies and also FTY720, anti-LFA-1 antibodies, and LFA-3-Ig. Alternative strategies, such as antagonistic anti-CD40 antibodies, are being investigated; however, none of these investigational antibodies will be available for clinical research in islet xenotransplantation in the very foreseeable future.

The risks of immunotherapeutic protocols being developed for initial pilot clinical trials must not be higher than the risks associated with immunosuppression currently used clinically in organ and islet allotransplantation. For islet xenotransplantation to be applied very broadly in the future, a rejection prophylaxis with a very favorable safety profile will be required. It is reasonable to assume that immunotherapeutic protocols will become increasingly more selective and safe as our understanding of the immunobiology of islet xenotransplantation improves.

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The second major obstacle to broader clinical application of islet xenotransplantation is the high number of designated pathogen-free donor pancreases required for manufacturing a therapeutic patient dose of neonatal porcine and adult pig islets. The minimum number of nonencapsulated pig islets transplanted to achieve normoglycemia in NHP has been 50 000 IEQ/kg or greater for neonatal islets[99,102,103] and 25 000 IEQ/kg or greater for adult islets,[98] though much greater numbers have been transplanted in some studies.[100,106,107] Fewer porcine IEQ are expected to be required per kg body weight of human recipients, whose insulin requirements per kg are 2- to 3-fold lower compared with streptozotocin-diabetic monkeys.[128,130] The exact number of porcine islets required to restore insulin independence in humans with T1D remains uncertain, but assuming a yield of 400 000 IEQ from a good adult porcine pancreas,[131] 3 or more suitable adult pancreases would be required for 1 patient dose. Manufacturing of NICC is much less challenging and costly and islets from neonatal pigs maintain a proliferative capacity after transplantation; however, the number of donor pancreases expected to be required per patient dose with commonly used techniques[132] is considerable. Further improvements in the selection of suitable source pigs and in porcine islet manufacturing will need to be achieved to develop commercially viable adult and of neonatal porcine islet therapy products.

Housing of designated pathogen-free pigs under strict barrier conditions will require significant resources. As the islets will be isolated and cultured, likely for several days, testing of the islets alone (ie, the 'product') for the presence of microorganisms should be sufficient to ensure that no bacteria and fungi will be transmitted, although monitoring of the herd will be required to ensure no viruses are present.[133-135] The requirement of designated pathogen-free porcine donors may therefore not be as stringent for islets compared with organ xenotransplantation. Inevitably, porcine endogenous retroviruses (PERV) will be transplanted with the islets.[136] However, monitoring of humans exposed to various pig tissues and cells has never identified active PERV replication.[137-140] Although national regulatory authorities, for example, the Food and Drug Administration in the United States, will insist on monitoring for PERV, clinical xenotransplantation is unlikely to be precluded on the basis of the presence of PERV alone.[141] Furthermore, if essential, techniques of small interfering RNA could successfully prevent PERV activation after transplantation.[142]

The Research Agenda

The research agenda in nonencapsulated islet xenotransplantation focuses on meeting the key requirements for initiating pilot clinical trials, that is, prevention of IBMIR and prevention of cell-mediated rejection with anti-CD154 sparing and clinically applicable immunosuppression.

The IBMIR is a multifaceted process involving coagulation, complement activation, cytokine and chemokine release, and granulocytes/monocyte infiltration.[115,116] Many different strategies have been investigated. Targeting coagulation using low-molecular weight dextran sulfate alone[114] and in combination with tissue factor pathway inhibitor-transgenic islets,[107] and thrombomodulin[111] has at best mitigated but not prevented IBMIR in preclinical islet xenotransplantation. Strikingly, the use of neonatal islets from GT-KO porcine donors has been more successful,[103,111] pointing to the importance of complement activation via the classical pathway caused by binding of preformed xenoreactive antibodies to islets. It is probable that preformed antibody binding to other (ie, nonGal) antigens will also be an initiating factor in complement activation after porcine islet transplantation in humans. To date, the identity of nonGal antigens on porcine islets has not been fully determined. N-glycolylneuraminic acid is likely to be a target in clinical islet xenotransplantation, though this oligosaccharide is not important in pig-to-NHP islet transplantation because NHP also express it and therefore do not produce natural antibodies against it.[143] Pigs that express neither Gal nor N-glycolylneuraminic acid are now available for preclinical research.[144] Recent evidence demonstrated the contribution of the alternative complement pathway to IBMIR and the mitigation of early loss of adult porcine islets in NHP treated with human factor H.[106] In view of increasing evidence indicating the activation of classical and alternative complement pathways, complement-specific biologics, such as compstatin that inhibit both pathways appear to be promising interventions.[116,145]

For the control of inflammatory cytokines/chemokine axis, biological drugs, such as anti-TNF-α mAb (humira and infliximab), TNF receptor-Ig fusion protein (sTNFR, etanercept), and IL-1β receptor antagonist (anakinra) are available for off-label use.[146] Finally, the CXCR1/2 allosteric inhibitor, reparixin, has been shown to inhibit the infiltration of neutrophils into islet grafts in experimental and clinical islet allotransplantation.[147,148]

An alternative approach to preventing IBMIR would be to place the islets in a site where they are not immediately exposed to blood. The gastric submucosal space has the advantage of being accessible by endoscopy,[149,150] and endoscopic biopsy of the graft may be possible.[150] Other sites are also being explored.[151-153]

Taken together, because the IBMIR is a multifaceted phenomenon, the combination of interventions targeting coagulation, complement activation, and recruitment of neutrophils and monocytes will be required to ameliorate early graft loss and prolong the graft survival in porcine-to-human islet xenotransplantation.

Anti-CD154 antibody-based immunosuppressive protocols achieved long-term survival of WT and genetically modified porcine islets in several NHPs. For the clinical translation of this immunosuppressive protocol, the replacement of anti-CD154 mAb is required due to its associated risk of thromboembolic events.[154] Preventing cell mediated rejection of porcine islets using anti-CD154 antibody-sparing protocols will likely require detailed immune mechanistic studies to determine the precise immune recognition and effector pathways inhibited by anti-CD154 immunotherapy in the above-referenced studies.

Induction therapy with anti-IL2R mAb and maintenance immunosuppression with tacrolimus, sirolimus, and CTLA4-Ig) prevented adult porcine islet xenograft survival on a very consistent basis in a large cohort of monkeys; however, diabetogenic and other side effects associated with this protocol prompted the investigators not to consider clinical development of this protocol (Graham ML and Hering BJ; unpublished).

Whether antagonistic anti-CD40 mAb can substitute for anti-CD154 mAb in preventing islet xenograft rejection is

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currently unknown, it is conceivable that anti-CD40 mAbs are less effective than anti-CD154 mAbs because they fail to mediate Fc-dependent depletion of activated T cells,[155] and they fail to block the interaction of CD154+ T cells with monocytes, macrophages, and neutrophils expressing the integrin Mac-1 as an alternative pathway for CD154-mediated inflammation.[156] To date, the efficacy of only 1 antagonistic anti-CD40 mAb in preventing porcine islet xenograft rejection in NHP has been reported.[102] Currently available antagonistic anti-CD40mAbs proven to be effective in islet allotransplant models include chi220,[157] 3A8,[158] 2C10R4,[159] and ASKP1240 (4D11).[160] The replacement of anti-CD154 mAb with anti-CD40 in a protocol which achieved long-term islet xenograft graft survival in NHP using ATG induction combined with anti-CD154 mAb and sirolimus maintenance failed to show similar efficacy (Park CG et al, unpublished).

Additional studies exploring the efficacy and mechanisms of action of presumably more potent antagonistic anti-CD40 mAb, such as 2C10R4 and ASKP1240, used for induction and maintenance therapy alone and in combination with other immunotherapeutics remain to be performed. We suggest, therefore, that an anti-CD40mAb–based regimen will prove effective when neonatal islets from genetically engineered pigs are transplanted. Anti-CD40mAb-based regimens have shown efficacy in xenotransplantation of hearts and kidneys from genetically engineered (eg, GT-KO/CD46) pigs in baboons.[161,162] Accordingly, future studies of anti-CD40 mAb should also address the survival of islets from genetically modified donors.

Additional immune intervention can be harnessed, such as negative vaccination using ethylenecarbodiimide-fixed donor apoptotic cells[163] and thymus cotransplantation[164] to reduce the immune response or induce tolerance against xenoantigens. Pig islet transplantation may be enhanced by the cotransplantation of mesenchymal stem cells (of either recipient or donor origin)[165,166] or donor Sertoli cells.[167,168] Both cell types may facilitate revascularization of the islets, reduce the inflammatory response, and provide immunoprotection.

What Is the Potential for the Treatment of Diabetes?

A number of key requirements for performing additional clinical trials of porcine islet products have already been met. First, the regulatory framework established by national health authorities (including but not limited to the US FDA and European Medicines Evaluation Agency) and the recommendations made by International Xenotransplantation Association and the World Health Organization provide a safe and suitable framework for conducting clinical trials of investigational porcine islet products in T1D. Second, a surveillance and safety program has been developed to detect, measure, manage, report, and respond to infectious diseases caused by known infectious agents and, possibly, previously unknown or unexpected pathogens in individual recipients of pig tissues. Finally, suitable, designated pathogen-free, WT source pigs have been generated for planned pilot clinical trials. However, other key requirements remain to be met, including first and foremost the development of a safe and consistently effective rejection prophylaxis and the development of a commercially viable porcine islet product. Accordingly, the significance of islet xenotransplantation in the care of patients with diabetes in the next 10 years will be determined by progress made in these 2 areas. Equally important in the determination of the future significance of islet xenotransplantation will be the progress made in the development of the AP and stem cell-derived sources of islet cells.

Summary of Research Priorities

(1) Prevention of IBMIR. This will likely require a multifaceted approach including targeting coagulation, compliment, inflammatory cytokines/chemokines and granulocytes-monocytes.
2. Development of effective and clinically acceptable antirejection regimens. An important current focus is on the targeting CD40-CD40L interactions with anti-CD40 antibodies.

ISLET ENCAPSULATION—AN ONGOING DEVELOPMENT CHALLENGE

Current State of the Field

Over 40 years of islet encapsulation research has failed to provide an approved clinical product despite many encapsulation approaches and efforts, including several clinical trials. This IPITA effort is critical to focus on future research goals and objectives that have the promise to achieve a successful clinical encapsulated islet product in as short a time as possible. A major review of encapsulated islet efforts has recently been published which describes the history and accomplishments of research and development of islet encapsulation as part of an Advanced Drug Delivery Reviews issue entitled "Cell Encapsulation and Drug Delivery."[169,170] There have been 2 major types of encapsulating devices: macrodevices and microencapsulation.

Macrodevices

Macrodevices seek to confine the total transplanted cell volume within a single, confined device. The appeal of this approach is that the implant is easily transplanted and retrieved. The primary challenge of this approach, however, is that, when avascular, it is plagued with inefficient nutrient and product delivery.

Early Extravascular Diffusion Devices

The origin of these extravascular diffusion devices began with the work of Algire, Prehn, and Weaver (1948-1959) who originated a planar diffusion device for the purpose of studying the mechanisms of immune rejection of cells and tissues. In the process of their successful research, they defined membrane biocompatibility, host cell membrane overgrowth, delays in immune rejection of encapsulated tissues, and prevention of allograft rejection, but not xenograft rejection. After the development of hollow fiber technology for renal dialysis, Amicon hollow fibers became the target of inserting islets inside to use as diffusion devices. The majority of these studies were performed by Wm. Chick who reported that their long-term results were limited by host membrane overgrowth. However, this problem for hollow fibers was overcome by the use by the cytotherapeutics team of tubular devices with altered membrane materials in 1985 to 1995 that enabled encapsulated islet implant success in rodent models. A noncurative clinical trial was published with subcutaneous implants of this device in 3 types of human recipients:

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nondiabetic, T1D, and T2D. This study demonstrated the recovery of viable and functional human islets after several weeks of implanted islet allografts. However, the low packing density reduced the clinical interest in this device type due to the large volume of encapsulated islet hollow fibers that would have been required for a curative clinical trial. Because this trial was performed without the use of immunosuppression, it is important to note that 2 of the 9 recipients developed donor antigen sensitization. This is a potential risk for diabetic patients receiving encapsulated islets without immunosuppression because this could increase their risk of a positive crossmatch if they ever required a kidney transplant.

Current Extravascular Diffusion Devices

The next device approach returned to a planar device design by Baxter Healthcare in the early 1990s to develop a device for their future gene therapy products. It was a well-designed flat sheet device with a central islet chamber and a tubular loading port. Although it worked well in rodents, once an alginate matrix was used to prevent cell clumping and necrosis, results in large animals showed less robust capillary ingrowth in the outer walls of the polyester outer coat. It became known as the Theracyte device after it was sold by Baxter to Theracyte. Currently, this device type is the first choice for 2 companies looking for a diffusion device to encapsulate human embryonic stem cell (hESC)–derived islets that will assure these cells cannot escape from the device. Both companies, Viacyte and Betalogics, are making separate modifications to this device type to meet their needs for this newly developed insulin-producing cell (IPC) source.

Current Direct Oxygenation of Extravascular Diffusion Device

Reviews of many results of encapsulated islet implants reveal that the major acute cause of encapsulated islet death is hypoxia. It takes too long for new capillary development and ingrowth to keep the freshly implanted, encapsulated oxygen-requiring islets from dying. Since 2005, β O_2 has been developing many ways to provide direct oxygen delivery to the encapsulated islets through peripheral connections to their implanted device. These β-O_2 studies have been successful in rodents and more recently in large animals. The first individual patient trial for this device showed persistent islet graft function in the chamber for 10 months with regulated insulin secretion and preserved islet morphology without immunosuppression.[171] Ongoing clinical trials are planned for the near future.

Intravascular Diffusion Device

In the 1980s and 1990s, the WR Grace company joined in a research venture with Biohybrid to develop an intravascular device to eliminate the acute loss of islet mass from hypoxia as well as continuously supply oxygen to the functional encapsulated islets. After many designs, they developed the "Hockey Puck" device that perfused arterial blood flow through tubing around which the islets were implanted within the device. This device demonstrated the longest duration of efficacy for a macrodevice both with islet allografts and xenografts in diabetic dog recipients to date. The FDA was reviewing the potential to initiate clinical trials with this device when disaster struck this model by the unexpected disconnection of the carotid artery cannulae to the device resulting in exsanguination of the diabetes-cured canine recipients. This complication closed the program, and except for a few repeats with different approaches, this type of device has not continued forward for any clinical applications.

Intravascular Ultrafiltration Device

Although a limited number of investigators have tried this approach, the concept is excellent for the islets because it not only provides continuous oxygenation to the islets but also eliminates the problems of diffusion of insulin from the device. Even though the in vitro results were excellent and the early rodent in vivo results were promising, this lead has not been followed since the 1980s.

Microdevices

Alginate Microcapsules as Islet Diffusion Devices

Alginate and similar hydrogels that formed into microcapsules have produced hundreds of publications with multiple successes in rodent models of diabetes, but remain with limitations for achieving significance for large animals and human clinical trials. The standard islet encapsulating alginate microcapsules are produced by droplets of sodium alginate mixed with islets into a bath of $CaCl_2$ or $BaCl_2$ that rapidly crosslinks to form the capsule containing the islet. Standard alginate microcapsules are 500 to 1000 microns in diameter with a significant percentage of the capsules not containing islets. Because gravity pulls on the islet with its higher density than the alginate during the drop formation and falling, many of the islets tend to sink within the droplet so when it is cross-linked, a portion of the islets is on the edge of the capsule, not adequately protected from immune attack. These large capsules have a very small percentage of their volume as islet so a potential clinical dose of alginate-encapsulated islets is very large, even for the peritoneal cavity. Multiple methods have been developed to make the alginate capsules smaller by reducing the surface tension of the droplet, such as vibrating the droplet needle or using an air knife. Connecting the needle with direct current to the calcium bath provides an electrostatic condition that also results in smaller capsules. However, all of these methods to make smaller alginate capsules by reducing the surface tension should be replaced with the more recent methods of making small capsules by the use of microfluidics. Another basic problem is that the pore size is very open so that a second layer of polyamine or similar substances are coated on the surface. However, because this second coat makes the biocompatibility worse, a third coat of alginate is required. The vast majority of published encapsulated islet results use alginate-encapsulated islets. They have been implanted successfully in rodents, to a lesser degree in large animals as well as humans. Currently, Living Cell Technologies has been conducting clinical trials of alginate-encapsulated porcine islet transplantation in different countries with several collaborators. Ongoing results have not yet achieved the degree of success desired.[110]

Alginate Alternatives

To address these problems, investigators have been working on making Minimal Volume Capsules of alginate as

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demonstrated most successfully by the Calafiore group. Other modifications are to replace alginate with agarose as was done by Iwata's group. Due to slower crosslinking, they moved to an emulsion approach that results in centered islets with smaller capsule volumes. Although these results appeared promising in rodents, this approach has not been picked up by other groups. Taylor Wang has developed multicomponent islet coatings that also center the islet and have shown good results in rodents. Large animal studies appear promising for this unique microcapsule.

PEG Conformal Coatings

Jeff Hubbell working with Novocell developed an interfacial polymerization formed by radicals that causes a thin coating of poly(ethylene) glycol (PEG) to form on the surface of each islet outward to a desired thickness. Rodent implants were excellent, but failed in translation to large animals due to bioincompatible reactions and islet losses that required development of new forms of PEG that were biocompatible. The PEG-coated baboon islets were successfully implanted subcutaneously into diabetic baboons with normoglycemia maintained up to 2 years without immunosuppression or insulin. A GLP study in diabetic baboons was performed, successfully leading to the approval of a ;phase I/II clinical trial in T1D recipients. However, when only partial function was achieved in the first 2 recipients, the trials were closed. This PEG technology has been taken to a second generation by Hubbell who is now collaborating with Converge, a biotech start up in collaboration with the Diabetes Research Institute with early rodent and large animal results that are promising.

Nanoscale or Layer-by-Layer Islet Coatings

The Iwata group has been developing even thinner islet coatings with different materials, such as PEG-lipid coatings with poly(vinyl alcohol) alginate up to 10 layers thick on each islet with early promising rodent results. More recently, they are trying to place layers of living cells, such as chondrocytes on the surface of islets, to protect them from immune destruction.

Novel Thermoreversible Islet Coatings From a Glucose Polymer

Alex Gorkovenko has produced a novel polymer of glucose that is a thermal-responsive gel that is both biocompatible and has programmable biodegradability. In collaboration with Prodo Labs, a new islet encapsulation technology is being developed with encapsulated islet size peaking at 250 microns and centralization of the islets with few empties. In vitro studies show excellent glucose-stimulated insulin release results with initial implants into diabetic mice showing graft function.

Corporate Involvement in the Development of Islet Encapsulation

From 1980 to current, there have been over 40 corporations involved in developing encapsulated islets into a clinical product. The companies range in size from small startups to large corporations, such as Baxter, Amicon, WR Grace, Metabolex, Gore Medical, and Johnson& Johnson. The specifics of these efforts are documented in the Advanced Drug Delivery Reviews article.[170] There are currently 11 corporations actively involved developing islet encapsulation technology.

The Research Agenda

With a vision to translating islet encapsulation to the clinic within a short timeline (5 years), the goals are modest, with the convergence of a short-course immunosuppressive therapy and a focus on allogeneic islet sources. Once the potential of microencapsulation as a strong tool for mitigating immunological attack is demonstrated, broader goals seeking to expand the cell source and/or eliminate immunosuppression completely can be explored.

Alginate Encapsulated Human Islets With Modest Immunosuppression

Because of the ongoing problems with alginate encapsulation, a minimum volume capsule with modest immunosuppression should be targeted as a likely candidate to demonstrate clinical efficacy within this time frame. Previous approaches sought to gain it all without the use of immunosuppression. A first step to the clinic would be to define a minimal immunosuppression approach that permits islet graft function while reducing the burden of totally relying on the coating to protect the encapsulated islets. Given what limited information is known about the capacity of encapsulation to mitigate immune rejection in a patient that will express both alloimmunological and autoimmunological responses, the first step to the clinic would be to combine either low-dose or short-course immunotherapy with encapsulation to define if this combination could have a synergistic effect. Yet, this minimal approach may create new problems if the alginate coating is too large to implant into the portal vein. Even if it is sufficiently small for the implants, it brings the additional burden of alginate bulk in the portal vein with repeated implants. This research would require minimal volume capsules because allogeneic islets are initiated in diabetic large animals to determine the risks and benefits before implanting into diabetic humans. Although previous clinical trials used the intraperitoneal site, the optimal encapsulated islet implant site needs to continue as a research target.

Screening New Biomaterials With Low Innate Immune Activation Propensity

The current alginate/agarose polymers suffer from the lack of specific control properties and purification. Several new and unique biomaterials are becoming available which can better meet these requirements and should be moved rapidly to large animal studies once biocompatibility is demonstrated for each candidate.

Nonalginate New Polymers

Several of these new polymers under development need to be tested for biocompatibility and toxicity with islets followed by islet functional testing. If these are minimal, they need to be fast tracked into islet functional testing and implanted. Some of these include Alginate-N3 hydrogel, extracellular matrix microspheres, and extracellular motifs.

Microfabrication Approaches to Decrease Capsule Sizes: Many of these choices can result in smaller capsule sizes

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increasing nutrient/waste diffusion and functional responsiveness as well as be more amenable to intrahepatic sites.

Bioactive Polymers to Enhance Vascularization and Engraftment

These include known items such as vascular endothelial growth factor or other methods of encouraging rapid new vessel formation at the time of implant. It also could include methods of prevascularization of devices to establish a vascular response before implanting the islets within the device, if it can be loaded after implant. It also could include the incorporation of VEGF or other stimulants of new vessel growth within the device that are designed to elute from the device for a period.

Combination of Encapsulation and Local Immunosuppression

The use of systemic immunosuppression is to be avoided if possible. However, localized immunosuppression adjacent to or from within the encapsulating device may be sufficient to protect the islets. Polymers could be used to provide controlled release for local immunosuppression. This approach could also include the addition of human MSCs within the device. Alternatively, the use of a genetically modified islet source, such as transgenic pigs expressing LEA294, a high affinity variant of T-cell costimulating inhibitor CTLA4-Ig, may provide local immunosuppression for encapsulated islet transplants.

Alginate Encapsulated Porcine Islets With Full immunosuppression Either Simultaneous or After Kidney Transplant

Alginate is insufficient to protect xenograft islets from immune attack so these devices will always require immunosuppression. The allograft immunosuppression is unable to protect unencapsulated xenografts but the combination encapsulation and allograft immunosuppression may be effective in xenografts. The focus of this approach is to increase the potential donor islet pool using xenografts without excessively increasing immunosuppression over that needed for allografts.

PEG Encapsulated Human Islets With Immunosuppression

Although the first-generation PEG patent protection was abandoned by Novocell, the second-generation PEG technology should be tested in humans initially with immunosuppression. Although there are different surface modifications that can reduce inflammation, the safety profile for PEG is better than alginates. The combination of PEG and immunosuppression has been shown to increase early engraftment.

Oxygen Delivering Macrocapsules Without Immunosuppression

Delivery of oxygen gas into the device is under development by β O_2 which may shift the primary cause of encapsulated islet graft failure from hypoxia to an immune cause. The clinical proof of principle for macro-oxygenation needs to be completed. In addition, microencapsulation of perfluorocarbon or oxygen-generating materials has been accomplished and need to be considered for these applications either inside of the islet device or in close proximity.

Key Methodologies Not Being Used

Intravascular Macrodevice Approach

Although this approach may seem too risky as evidenced by the previous attempts in diabetic dogs, there have been extensive advances from product development in vascular grafts and shunts because those studies were completed. A new approach with modern techniques and shunt materials needs to be considered. The risk of blood interactions should be reduced by new polymers and intravascular device designs.

Combination of Immunosuppression and Encapsulation

(a) Systemic low-dose or altered dose or additional drug—In the past, encapsulation approaches have typically attempted an "all or nothing" approach to the use of immunosuppressive drugs. As such, limited preclinical, and no clinical, studies have explored the potential synergistic effects of immunosuppressive reagents and encapsulation. Multiple approaches could be evaluated, such as systemic low dose/altered dose/alternative drugs. Further, when encapsulated islets are placed within alternative sites, avenues for local delivery of drugs are highly feasible. Drugs could be eluted from the encapsulation material itself or from eluting materials placed around the implants.
(b) Local delivery of drugs from encapsulating materials—The addition of different types of drugs that can be eluted or released from the encapsulating materials need to be defined and tested to avoid the systemic complications of immunosuppression. This is especially true for treating patients with diabetes because they need islet replacement to prevent the complications from reaching an end stage rather than waiting to perform a transplant after the complications have already done significant damage.
(c) Macrodevice approaches other than theracyte extravascular device—The design of this device with polyester as its outside supporting structures makes it very hard to use without ingrowth of the device into the recipient. Either coating the polyester or replacing it will increase the value of this type of device.
(d) Convergence of vascularized implant site with encapsulated islets—New ways of combining these 2 requirements need to be focused on to develop novel ways of achieving this goal. Examples include modification of the local environment of the device with bioactive surfaces, localized drug delivery, and in situ oxygen generation. Alternatively, the site could be prepared before implanting the islets so oxygenation no longer is a restriction after implant.
(e) Reduced capsule volumes and thickness—The days of injecting large volume alginate or other types of capsules need to be over because these large volumes are not clinically relevant. The focus should only be on minimal volume capsules that can be achieved with different approaches already defined.

Novel Encapsulation Materials

New Hydrogels

(1) Glucose polymer—This new polymer has many opportunities to be further modified with different types of additions to the basic structure of the polymer.
(2) Synthetic materials—Examine PEGs or other novel hydrogels or responsive and bioactive materials for islet coating technologies.

Define the Optimal Biomarkers and Assays for Predicting Islet Survival and Function and Understanding Causes of

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Failure—The current approaches are often missing mechanistic evaluations that are critically needed to understand the progress and predict the next studies to be completed.

Areas of Controversy and Impediments

Optimal Implant Site

(a) Unique considerations for encapsulated islets—There is a need to compare the unencapsulated islet implant requirements with the unique requirements for encapsulated islets for each implant site under consideration. The specific items below are left in question form for those areas that have not been firmly established.
(b) Intrahepatic site—There certainly is clinical evidence that intraportal vein encapsulated islet implants can cause portal hypertension, liver impairment, hemorrhage, and death, although on a very limited basis. Portal pressures should be routinely measured during the infusion as is done for unencapsulated islets. Yet, there are several critical questions that have not been answered. What is the volume of encapsulated islets that the human liver can handle for a single encapsulated islet implant yet avoid portal hypertension? Can the encapsulation material degrade sufficiently fast enough to complete the next implant and still be protective of the encapsulated islets? What is the time course over which repeated intraportal encapsulated islet implants can safely be performed?
(c) Intraperitoneal Site—Because the human pelvis is shaped as a centrifuge tube when sitting or standing, injected islets that do not stick to the abdominal tissues fall to the bottom of the pelvis, compact there, and undergo hypoxic death rather quickly. Can one make the capsules more sticky to prevent their dropping to the pelvis while at the same time not have this increased stickiness lead to capsule fibrosis?
(d) Omental site—The omental site has advantages in that a variety of pouches can be created that can entrap the islets that can also lead to vascularization. Yet, will the capsules stay within the omentum itself and gain vascularization or does a pouch have to be created to hold them and does vascularization have to be induced?
(e) Intestinal or gastric submucosa site—Encapsulated animal islets have been injected in both the intestine and gastric submucosa. Can the encapsulated human islets be injected into these sites and gain sufficient vascularization without compromising the integrity of the intestine or stomach while maintaining long-term functional survival?
(f) Subcutaneous site—The subcutaneous site has shown promise in animal models. One human study has suggested that long term insulin injections before encapsulated implants lead to sufficient subcutaneous scarring that insulin transport away from the implant site is delayed and reduced.[170] How normal functioning will the encapsulated islets be in the face of extraportal circulation? How fast will the insulin delivery out of this site be in the face of chronic scarring from years of insulin injections? How severe will the local fibrosis and fat formation become in these subcutaneous implant sites done in the face of insulin injection scarring?

Type of Device in Terms of Islet Retrieval and Replacement

(a) All-in and all-out device type—This device is designed to be implanted with an islet load and then easily removed completely when the islets need to be replaced. Thus, it cannot cause the surrounding tissues to grow into the device. Can such a device be readily removed with the expended islets and readily replaced without damage to the patient at that site? Such a curative device may require 200 cm^2 surface area as a minimum and perhaps even 2 to 3 times for a curative response with the required numbers of encapsulated islets. What sites are amenable to these requirements for this type of device? How will this approach deal with the immediately postimplant islet hypoxic losses?
(b) Flush and reload device type—This type of device is designed to be permanently implanted into the recipient and as such requires the ability to flush out the old islets and reload fresh islets whenever required. When the islets need to be replaced, what design characteristics are required to maintain the implanted device within the host yet still permit effective removal of the expended islets by simply flushing them out and then loading the new islets into the implanted device? How does one assure the prevention of encapsulated islets becoming stuck to each other preventing their flushing?
(c) Combination of all-in/all-out and flush/reload devices—Experimental designs may require some combination of these very basic requirements to be successful in readily achieving removal and replacement of islet loads in these recipients.

Enhanced Oxygenation Requirements

There are 2 problems with lack of oxygen for encapsulated islets: (a) immediately after implantation a significant number of islets die of hypoxia as it takes several days for new blood vessels to grow and (b) unless islets have sufficient oxygenation, they cannot maximally produce their required insulin. So, by definition, any encapsulation system that chronically limits oxygenation levels of the β cells will have a marginal outcome.

(a) Acute Postimplant Requirement × 2 to 4 Weeks

Direct Oxygen Delivery—β O_2 is directly supplying free oxygen on a regular basis to the encapsulated, implanted islets that will presumably be required for the life of the islets within this type of device.

VEGF before or acutely with islet—While both approaches need to be tested, it is clear that VEGF added without any hypoxic tissue targets will not lead to effective vascularization because there is nothing for the new vessels to attach to, and thus they will disappear. So timing of treating the device without the islets has to be closely determined. In addition, adding the VEGF at the time of implant will not by itself help much because the islets will die off before sufficient quantities of new vessels can grow out.

Vascularization of device before islets—There are other ways than adding VEGF to the device to grow new vessels before implanting the islets, such as maintained factor release over time and others. These need to be explored to see if they can benefit islets survival.

Devices Containing Microencapsulated Oxygen—Microencapsulated oxygen is currently available. Yet, it is not being used because some believe that it will only stay oxygenated for a few hours after implant that is not long enough to protect the islets beyond acutely. However, with the oxygen carrier encapsulated, it cannot escape to the lungs and will remain in the implant site still binding the oxygen. When it has released all of its oxygen after implant, one needs to test whether simply breathing 100% oxygen for several minutes a day will replenish the oxygen

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carrier with sufficient fresh oxygen binding to the carrier located with the islets to maintain their function.

(b) Chronic Oxygen Requirement

Adequate oxygen availability is required for normal islet function. With restricted oxygen, the encapsulated islets may remain viable but cannot function. Easily replenished chronic oxygen supply with oxygen carriers with islets is required as long as islet function is required.

Islet Functional Capabilities

Encapsulated versus nonencapsulated islets—For each specific type of islet encapsulation approach developed, what limitations on islet function are the result of the encapsulation process, considering both acute and chronic changes?

Duration of effective treatment for a long-term product—There certainly is a difference in postimplant success for islet autografts and allografts or xenografts. How efficiently do the encapsulated islets continue to function at what percent of their normal capacity both acutely and long term? What is the functional duration of the encapsulated islets functioning at what level of normal responsiveness?

Strategy for islet replacement—To avoid being a simple demonstration using a fraction of the needed islet mass, a strategy for islet full replacement by encapsulated islets is required with current technology in which normoglycemia can be fully attributed to the encapsulated graft. What are the criteria for replacement of encapsulated islets? What is the priority of encapsulated islet recipients to receive additional doses of islets over those who have not received an islet implant? What is the role of donor sensitization in determining which patients get the next doses of encapsulated islets in cases where low or no immunosuppression is required?

Sticky islet coatings—Sticky islet coatings increase the host response for vascularization but also increase the fibroblast and immune attack. Is there a method to get just the right kind of islet coating stickiness? Does host reactivity to the device reduce over time?

Insulin delivery to portal vein or systemic vein—Encapsulated islet implants into the portal vein have been limited by capsule size and breakdown rates. What are the requirements for islets in terms of long term function if they deliver insulin through the portal vein or do not? Does this site of delivery have any effect on delayed function leading to reactive hypoglycemia?

Speculation of Potential Therapeutic Utility in 5 to 10 Years

(1) There will be multiple types of encapsulated islet approaches under clinical trials permitting direct comparisons of different types.
(2) Novel methods of required oxygenation of encapsulated islets will be obtained.
(3) Combination of islet encapsulation with local immunosuppression delivery will greatly reduce risk of systemic immunosuppression expanding procedure from limited kidney transplant recipients to widespread application.
(4) Successful human islet expansion will reduce the need for more immune challenging porcine islet xenografts.
(5) Due to these improvements, encapsulated islet therapy will be expanded to people with significant T2D as well as meeting demands of people with T1D.

Summary of Research Priorities

(1) Conduct a preliminary trial of alginate encapsulated islet allotransplantation with a short-course or low-dose immunosuppression. Type 1 diabetics who have already received a renal transplant would be candidates because they are already obligated to chronic immunosuppression.
(2) Development of improved biocompatible encapsulation materials and capsule designs.
(3) Define new approaches to gain oxygen delivery to encapsulated islets to improve both early engraftment and long-term survival.
(4) Define optimal transplant sites that have adequate capacity/surface area and that circumvent the differences of the intraportal and intraperitoneal sites.

AP AND INSULIN DELIVERY SYSTEMS

Current State of the Field

New treatments for T1D may be suited to different clinical situations and stages of disease—it is unlikely that 1 form of treatment will be best for all patients in the foreseeable future. Diabetes evolves over years from diagnosis when significant endogenous insulin secretion still exists to established disease with barely any endogenous insulin (C-peptide negative). This progression is associated with increasing lability and difficulty in achieving glycemic control and increasing prevalence of hypoglycemia unawareness. Hypoglycemia unawareness in most cases results from physiological adjustment to recurrent hypoglycemia with reduced counter-regulatory hormone production and reduced autonomic symptom response. It does not result from structural autonomic neuropathy. The frequency of severe hypoglycemic episodes is strongly correlated with unawareness. This is a major clinical challenge in people with established diabetes and has become the usual indication for pancreas or islet transplantation alone (in the absence of a kidney transplant). Overnight hypoglycemia is also a significant problem and is the major cause of the "dead-in-bed" syndrome.

One possible treatment—the AP—can be traced back decades to studies demonstrating the possibility of external blood glucose (BG) regulation using intravenous BG measurement and infusion of insulin and glucose.[172,173] With the establishment of subcutaneous insulin delivery[174,175] and minimally invasive continuous glucose monitoring (CGM)[176] as viable diabetes technologies, increasing academic and industrial effort was focused on the development of closed-loop systems using CGM coupled with a subcutaneous insulin pump. In 2004, the Advanced Insulin Infusion using a Control Loop project based in Cambridge reported the first promising results[177]; in 2006, the JDRF Artificial Pancreas Project was initiated; in 2008, the US National Institutes of Health launched an AP initiative; and in 2010, the European AP@Home consortium was established. A roadmap toward a viable AP was accepted, which included sequential steps beginning with automated mitigation of hypoglycemia and progressing through control-to-range and control-to-target toward fully automated, possibly multihormonal, AP.[178] By 2010, the AP became a global research topic engaging physicians and engineers in an unprecedented collaboration. Key milestones of this development are described in recent reviews.[179-182]

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ADVANTAGES	KEY CHALLENGES
Potential cumulative effect of improved control; in particular improved average glycemia, increased time in range, and reduced time in hypoglycemia down to a minimum.	Subcutaneous delay and variability of insulin absorption and insulin action,[183,184] CGM inaccuracy, noise, and loss of sensor signal.[185,186]
Can be available on consumer electronics (eg, smartphone); durable and potentially upgradeable.	Patient-friendly user interface[187]; AP network quality and connectivity.[181]
Close to clinical reality.	Cost—consumables approximately US $4000/y. Still will require some human intervention.
Reduced demand on patient for close monitoring of glucose values and computing of insulin doses, particularly overnight.	Loss of insulin infusion [cannula failure] and fault detection[188]; automated handling of meals and exercise[179]; will still have alarms requiring human intervention.
No immunosuppression.	Insulin is still infused subcutaneously, ie, the wrong place, resulting in inferior insulin action for the foreseeable future. Multiple devices and sites may be difficult for some.
Can manage overnight [basal control] safely.	Ability to cope with meals, exercise and illness needs to be tested. Fault detection needs to be robust.

In those with early T1D (with preserved insulin secretion; C-peptide positive), it is possible that the combination of residual β cell function and closed loop insulin delivery might be safe and deliver very tight metabolic control. This may have both short- and long-term benefits. In established diabetes, closed loop systems may reduce or prevent overnight hypoglycemia. It remains to be tested whether in longstanding diabetes closed loop delivery can restore hypoglycemia awareness to the same extent that can be achieved by pancreas or islet transplantation. It is also possible that if used widely and early enough, the reduction in major hypoglycemia, especially prolonged nocturnal hypoglycemia that occurs frequently with conventional treatment, may be enough to reduce over time the number of people suffering from problematic hypoglycemia unawareness.

Current technology has shown that AP is able to improve average glycemic control and simultaneously reduce moderate hypoglycemia; thus, it is likely but untested that the AP would be able to prevent severe hypoglycemia known to cause seizures or loss of consciousness. Ongoing AP trials aim to reduce mild hypoglycemia enough to allow consistent restoration of hypoglycemia awareness similarly to current studies of islet transplantation designed to include patients with hypoglycemia unawareness experiencing frequent disruptive severe hypoglycemic events. There are still technological problems, such as accuracy of the glucose sensor, network connectivity between the devices comprising the AP system, and reliability of the insulin pump. Most of these problems are currently being mitigated by algorithm development, and technology improvements that are likely to bring the AP to mainstream use in the not too distant future.[181,182] The table below summarizes certain advantages of, and challenges to, AP systems:

Subcutaneous and implantable insulin pumps (CSII, CIPII)—Continuous subcutaneous insulin infusion (CSII) relies on providing basal and bolus insulin using insulin analogs.[189] Structured education programs teach patients to adjust insulin doses based on premeal BG levels and carbohydrate intake and can achieve target glucose control with lower rates of hypoglycemia. Forty percent of the patients referred to the UK Dose Adjustment for Normal Eating program have impaired awareness of hypoglycemia, which is restored in 40% of those 1 year after UK Dose Adjustment for Normal Eating.[190] Meta-analysis shows that insulin pump therapy can provide a 4-fold reduction in the rate of severe hypoglycemia events.[191] Continuous intraperitoneal insulin infusion (CIPII) with an implantable pump is available in a few countries. In a randomized, cross-over study with CSII in a group of patients with a baseline HbA1c approximately 8.2%, the use of CIPII was associated with less severe hypoglycemia, although baseline rates were low, with no difference in improvement of HbA1c approximately 0.5%.[192] In a second study of similar design in a group of patients with a baseline HbA1c approximately 8.6%, CIPII was associated with a 0.76% reduction in HbA1c compared with CSII with no difference in time spent in the hypoglycemia range or rate of hypoglycemia events.[193] However, the need for operative placement and reoperation on average every few years to address complications, such as battery replacement, catheter occlusion, pump dysfunction, pain, and infection, have limited broader CIPII application.[194]

CGM is available in some countries. Since the advent of CGM technology,[195-197] significant progress has been made toward versatile and reliable CGM devices that not only monitor BG day and night but also provide feedback to the patient, such as alarms when BG reaches preset low or high levels. A number of studies have documented the benefits of CGM[176,198-201] and charted guidelines for its clinical use[202,203] and its future as a precursor to closed-loop control.[204,205] Although many studies have excluded patients with problematic hypoglycemia, the largest trial of CGM use to date showed a 0.5% improvement in HbA1c from a baseline of 7% or greater with no increase in hypoglycemia for patients 25 years or older who most often used their glucose sensor during at least 6 or 7 days each week. Another study demonstrated that CGM in children and adults with a HbA1c less than 7.5% was associated with a 0.27% improvement in HbA1c and less time in hypoglycemia.[201] In a retrospective clinic-based analysis, use of CGM in a cohort of patients with problematic hypoglycemia at baseline was associated with a reduction in severe hypoglycemia episodes from 8 to 1 per year, an improvement in HbA1c from 8.1% to 7.6%, but no change in hypoglycemia awareness.[206]

Low glucose suspend (LGS) is considered a precursor to closed-loop because of the information exchange between CGM and the insulin pump. Two randomized control trials, which were enriched to different levels with

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hypoglycemia, showed significant reductions in hypoglycemia: automation to simulate pancreatic insulin response showed a 38% reduction in nocturnal hypoglycemia compared to CGM alone without increasing HbA1c.[207] In the second randomized control trial, LGS versus CSII showed a significant reduction in severe hypoglycemia in patients with impaired awareness of hypoglycemia that was associated with less time spent in the hypoglycemia range, and again no change in HbA1c; however, the number of severe hypoglycemia events was rather low at baseline and the patients are rather young with short disease duration.[208]

Closed Loop Control:

Algorithms

The central part of any AP system is a control algorithm receiving CGM and CSII information and computing insulin microdoses which are sent for delivery to the insulin pump at short time intervals, typically every 5 to 15 minutes. Various types of control algorithms were introduced ranging from relatively straightforward proportional-integral-derivative controllers[209] to complex model-predictive algorithms,[184] and empirical logic approaches.[210] Essential for the design of a closed-loop algorithm is a modular structure that defines the control action[211]:

(1) Safety and prevention of hypoglycemia ranging from straightforward LGS to sophisticated model-based hypoglycemia safety systems[212];
(2) Control-to-range (known also as treat-to-range) which mitigates both hypoglycemia and hyperglycemia and aims the maintenance of BG levels within a certain range (eg, 4-10 mmol/l)[213];
(3) Control-to-target which aims near-normalization of glycemic control mimicking the pancreas action in health, which always tends to bring glucose homeostasis to a set point (eg, 5-6 mmol/L).

AP Technology

Forty years ago, the AP was a refrigerator-size device (eg, the Biostator[172]). The first inpatient closed-loop control studies that began 8 years ago used AP systems based on laptop computers wired to CGM and insulin pump.[209,214-217] More recent studies took laptop-based systems to the bedside of patients in overnight summer camp[218] and home trials.[219] However, such systems were unsuited for everyday free-living use, and a different design approach was needed to bring the AP truly home.[220] In 2011, the first portable AP system was developed at the University of Virginia using an Android smartphone as a computational platform and was tested in 2 pilot trials of outpatient AP done simultaneously in Padova, Italy and Montpellier, France.[221] These first studies demonstrated that inexpensive consumer electronics devices are capable of running closed-loop control, and therefore smartphone-based systems may be 1 possible approach to an affordable AP in the near future.[181,182] The system can be used as individual modules as well as a fully integrated device. For example, the system can operate in a sensor-only mode providing extension of the CGM receiver functionality, predictive alarms and capabilities for remote monitoring of the patient over the internet; or the system can work as a pump "companion" providing wireless capabilities and basal/bolus advice to the patient.

Clinical Trials

Extensive inpatient clinical studies were conducted in 2008 to 2012 using increasingly more sophisticated approaches: manual adjustment of insulin delivery according to control algorithm recommendations,[214,215] overnight-only control,[216] automated day-and-night closed-loop,[217] dual-hormone control using glucagon as an additional control mechanism,[222] personalized control algorithms,[223] and monitoring of additional input signals to the control algorithm (eg, heart rate) during exercise.[224] These studies demonstrated the superiority of closed-loop control over standard insulin pump therapy in terms of improved average glycemia, improved time within target range, and reduced hypoglycemia. Similar results were achieved by different control algorithms as demonstrated by a comparative study.[225] As noted above, the transition of the AP to ambulatory use began in 2011 and since then studies have been conducted in controlled outpatient settings (eg, hotel, guest house),[226,227] summer camps for children with diabetes,[218,228] and patients' homes.[229] Typically, studies that used laptop-based wired systems were restricted to overnight bedside AP use,[218,219,229] whereas studies using wireless portable systems were full-time, day and night.[226,227] In particular, the smartphone-based system mentioned above logged over 70 days and 200 nights of closed-loop control so far.[226-228] A number of clinical protocols are under way with progressively longer duration and relaxed setting transitioning to long-term AP trials at home. Preliminary data were reported at the 2014 session of Advanced Technologies and Treatment for Diabetes in Vienna from studies at Cambridge, United Kingdom, and the University of Virginia, showing significant improvement of glucose control overnight.

Pancreas transplantation represents the gold standard β-cell replacement therapy. Islet transplantation is emerging as a minimally invasive alternative to pancreas transplantation that can provide near-normal glycemic control while ameliorating problematic hypoglycemia. As a benchmark, successful pancreas transplantation fully normalizes glucose homeostasis, normalizing HbA1c while abolishing hypoglycemia, as evidenced by a CGM study in recipients of SPK transplantation documenting a mean glucose 5.6 ± 0.4 mmol/L and 96% of time spent between 3.3 and 7.8 mmol/L not different from 5.5 ± 0.4 mmol/L and 99% in a control group of healthy volunteers. In a study of T1D patients selected for successful SPK transplant (HbA1c ~5.2%), IAK (HbA1c ~5.5%) or CIPII (HbA1c ~7.1%), glycemic control assessed by CGM demonstrated mean glucose of 5.4 ± 1.1, 5.8 ± 0.8, and 7.8 ± 1.7 mg/dL, respectively, with significantly less glucose variability in both transplant groups and no (SPK) to minimal (IAK) time spent in the hypoglycemia range compared with significantly greater number and duration of events with CIPII.[230] Another nonrandomized study compared patients with a baseline HbA1c approximately 8.3% consecutively treated with either islet transplantation or CIPII and followed up or 3 years, and similarly demonstrated superior glycemic control (HbA1c ~6.6 vs. 8.1% at 3 years) and reduction in hypoglycemia with the cellular replacement therapy; however, adverse events occurred 4 times more often with islet cell compared with CIPII therapy.[231] In contrast, current use of AP components, including insulin pumps, CGM, and LGS, can improve, but not normalize, glycemia. Although it is tempting to apply such technology to those experiencing problems with hypoglycemia, information from this population is limited. In

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addition, inaccuracy of glucose sensors and variability in subcutaneously delivered insulin absorption and action still limit the application of AP technology. However, closed-loop control, albeit imperfect when compared to cellular replacement, has the potential to become widespread by virtue of its accessibility, plentiful supplies (as opposed to limited supply of islets), ease of initiation without surgical intervention, and no need for immunosuppression.

The Research Agenda

Metrics

The field of islet transplantation has developed quantitative measures of glycemic lability and hypoglycemia severity that ensure proper identification of those patients with longstanding C-peptide–negative disease at greatest risk for severe hypoglycemia[231,232]; these tools have been used to assess CGM[233] and should be further used to assess LGS and the emerging components of the AP system. Sophisticated analysis of glycemic control is possible with large amounts of sensor data uploaded to the Cloud for remote analysis.[232] Comparison of different technologies will be improved if the same metrics are applied to all, whereas at this stage, different measures of glycemic control are used.

AP Technology

The transition of the AP to everyday diabetes therapy use is contingent upon seamless concerted work of a device network encompassing the patient. Achieving reliable system operation is possible through 2 distinct routes:

(1) CGM-insulin pump communication and all control algorithms are being implemented in the insulin pump. This "traditional" approach is currently adopted by industry, for example, Medtronic, Animas, and Roche. Advantages include straightforward system integration and simultaneous testing of all system components. Disadvantages are increased system cost, limited flexibility to use devices interchangeably and select the best components from different manufacturers, slow life cycle of the technology (typically 4-5 years), and potentially slower adoption of new control approaches.
(2) Use of consumer electronics whenever possible. Advantages include:

- Contemporary smartphones are: readily available and inexpensive, computationally capable of running closed-loop control, wirelessly connectable to CGM and insulin pumps and capable of broadband communication with a central location for remote monitoring and safety supervision, and no current insulin pump offers all of these capabilities.
- The technological life cycle of a smartphone is months, as opposed to years for insulin pumps; thus use of consumer electronics allows easier hardware updates and keeping up with contemporary user-interface appearance and device form factor.
- Psychological studies show that many patients (particularly children and teenagers) are reluctant to use their insulin pump in public, missing boluses and slipping into poor glycemic control when privacy is limited (eg, during school days). However, no one is embarrassed to use a smartphone, and that may be a key to better patient engagement and better glucose control.
- Disadvantages include difficulties with system integration and regulatory approval.

Bioartificial pancreas

A few years ago, we speculated that it would become possible to combine cellular and mechanical insulin replacement in a single unified treatment strategy.[234] Specifically, limited-volume islet infusion can be used to initiate, but not complete the process of β-cell replacement, partially mitigating hyperglycemia and restoring the counter-regulatory responses to hypoglycemia. Additional insulin can be then delivered by a closed-loop controller. Such a combination therapy could alleviate the problem of limited islet supply and at the same time facilitate the work of the mechanical AP by aiding the control algorithm with partially biologically restored glucose control. Although such combination therapies have not been attempted, primarily because the AP is still not ready for long-term use, randomized controlled clinical trials could be planned to compare cellular versus artificial insulin replacement therapies, as well as combination "bioartificial" approaches in terms of effectiveness, cost, and availability of the treatment. It could be hypothesized that: (i) limited islet transplantation and/or regeneration would partially restore β-cell function resulting in reduced glucose variability; (ii) artificial closed-loop would then deliver additional insulin, further reducing glucose variability; (iii) in turn, reduced glucose variability would exert less stress on the transplanted β cells, thereby increasing their longevity. However, the addition of the costs and risks of 2 new technologies may reduce the feasibility of this approach.

Whether a bioartificial pancreas is feasible or not, it emphasizes that AP and cell-based technologies for glycemic control have the potential to be used together, not only separately. Treatment for people with T1D should include consideration of these new technologies as well as conventional therapy. It is reasonable to consider testing AP in patients early in the course of uncomplicated T1D when glucose counter-regulatory defenses remain intact, thus minimizing the risk for severe hypoglycemia. For patients with established (C-peptide negative) T1D experiencing frequent severe hypoglycemia despite best medical management, islet transplantation appears the most promising alternative to a whole pancreas transplant.

Summary of Research Priorities

(1) Assessment of state of the art AP technology with standardized measures of glycemic lability and hypoglycemic severity developed by the islet transplant field.
(2) Full incorporation of consumer electronics (smartphone technology) to allow remote monitoring/supervision, opportunity for frequent hardware and software updates, and to negate the psychological stigma of in public pump use.
(3) Consider assessment of combine AP-islet transplant therapy to address the limited islet supply and need for multiple islet doses and perhaps limit β cell stress, thereby improving islet performance and longevity.

IMMUNE TOLERANCE FOR ISLET AUTOIMMUNITY AND ALLOIMMUNITY

Current State of the Field

There are no currently widely available, safe, and extensively validated approaches for establishing tolerance for islet

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transplantation in humans. Although a large number of approaches seem to be efficacious in rodent models, most have failed to translate into success in primate or porcine large animal models. Some success has been achieved in humans in renal transplant protocols which require low-risk donors and recipients, extensive immunosuppression, and components of hematopoietic chimerism. Moving forward, there are several principles to be taken into account. First, when assessing tolerance for islets and because of the unique characteristics of different immune responses (eg, memory, cross reactivity, number of reactive clones, and cells), it is very important to separately measure and assess responses to autoantigens and to alloantigens. Depending on future potential, responses to xenoantigens will also have to be separately addressed. Second, it is important to have measures not only of islet function and injury but also measures of immune reactivity and immune regulation to prospectively monitor recipients. Third, it is clear from murine studies that there are many components to immunity, and that tolerance is achieved only by targeting the distinct arms of the immune response. Broadly speaking, innate B-cell and T-cell responses must be controlled to induce and maintain tolerance. It is also clear that tolerance is achieved not only by preventing these distinct responses but also by generating regulatory phenomena. Fourth, it is clear from murine studies that the most robust tolerance for both autoimmunity and alloimmunity is achieved in protocols that incorporate some form of hematopoietic stem cell chimerism along with immune regulation.[235,236] Chimerism successfully prevents the B- and T-cell components of adaptive immunity while simultaneously generating a variety of suppressor and regulatory mechanisms (eg, anergy, deletion, suppressor cells).[237]

Current clinical studies define various approaches that hold some promise for tolerance induction or provide a guide for approaches that do not work (Tables 2 and 3). The ONE Study (www.onestudy.org) is currently validating procedures for generating suppressive, Treg and mesenchymal stem cells that can be grown and manipulated. Although there is currently no information yet as to efficacy of these regulatory cells in transplantation, safety studies have been performed in patients with T1D. Low dose IL-2 has been shown in human clinical trials to increase peripheral Treg cell and ameliorate autoimmune HCV-induced vasculitis.[238,239]

Current T1D clinical prevention and intervention trials may define lead assays, drugs, and procedures for overcoming autoimmunity at early or late stages (Tables 2 and 3). A number of observations, failed trials, and/or validation studies have yielded important and surprising results that provide extremely important guidelines for translation of information to tolerance trials and for interpreting putatively interesting signals in the preclinical literature.

Grant et al[240] reported on the results of an independent laboratory's tests of novel agents to prevent or reverse T1D in the nonobese diabetic mouse, diabetes prone BB rat, and multiple autoimmune disease–prone rat models. Methods were developed to mimic human clinical trials, including prescreening, randomization, blinding, and improved glycemic care of the animals. Agents were selected by an NIDDK appointed independent review panel. Agents selected to prevent diabetes at later stages of progression were: a STAT4 antagonist (DT22669), α 1 anti-trypsin, celastrol, and a macrophage inflammatory factor inhibitor (ISO-092). Agents tested for reversal of established T1D were: α 1 anti-trypsin, tolerogenic peptides (Tregitopes), and a long-acting formulation of GLP-1 (PGC-GLP-1). None of these agents prevented or reversed T1D, whereas the positive control interventions were effective: anti-CD3 reversed diabetes in the NOD mouse, dexamethasone prevented diabetes induction in the multiple autoimmune disease–prone rat, and cyclosporine prevented diabetes in the BBDP rat. This important study highlights the limitations of much of the primary rodent literature and strongly demonstrates that stringent confirmatory testing will be required in rodent models before translation to large animal or human experimentation.

Sarikonda et al[241] reported that combination therapy with anti-CD20 and either oral insulin or proinsulin does not protect hyperglycemic NOD mice, but the combination with proinsulin offers limited efficacy in T1D prevention. The ITN, TrialNet, and NHP studies listed in Tables 2 and 3 demonstrate many outright failures or very limited signals for OKT3 (anti-CD3ε), thymoglobulin (polyclonal antithymocyte globulin), α 1 antitrypsin, oral insulin, rituximab (anti-CD20), daclizumab (anti-IL-2Rα), abatacept (CTLA4-Ig), alefacept (LFA3-Ig), and canakinumab (anti-IL-1β). Although these results are obviously disappointing, they also show what does not work so that nonproductive avenues of research are no longer pursued.

The current state of the art does not define how best to plan the sequence of preclinical or clinical trials for interventions and markers. For example, should NOD or humanized mice be used to evaluated treatments for the autoimmune component? What is the best model to provide supporting data before initiating clinical trials? Should we first validate biomarkers and then go to clinical trials, versus validate an approach that achieves tolerance in primates first? If clinical trials are contemplated, who is best suited to which therapy? How is safety defined in clinical tolerance trials, especially for treatment of autoimmunity and alloimmunity in T1D, where alternative treatments (ie, semisynthetic insulins, closed loop insulin pumps) are rapidly improving? Should trials first be performed in transplant populations with larger numbers of patients (ie, renal allografting)?

It is important to realize that immunologic interventions can likely be enhanced by other nonimmunologic approaches in islet transplantation. Thus, there are multiple opportunities for cross-fertilization with technologies and approaches from the other workgroups. Encapsulation techniques may obviate the need either to measure immunity or to develop novel immune or tolerance techniques. It is conceivable that a perfect encapsulation system will allow long-term graft survival with conventional immunosuppression accompanied by immunosuppression minimization and weaning. Although xenografts present a large series of novel antigens to which it is currently very difficult to provide adequate immunosuppression, encapsulation may overcome these problems by masking antigens and/or protecting from effector mechanisms of inflammation and rejection. The β-cell regeneration and stem cell technologies may overcome some toxic effects of conventional or novel immunosuppression. Likewise, enlarging and continually replacing the β cell mass may also overcome immune reactivity and help to generate exhaustion in antigen-reactive clones of T cells and B cells. Conceivably, the combination of encapsulation, regeneration, stem cells, and xenotransplant along with conventional immunosuppression

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TABLE 2.
ITN Studies

Title of Trial	Enrollment	Launch Date	Completion Date
ITN005CT (NIS001)	Target: 36	08/07/2001	August 30, 2010
Islet transplantation in type 1 diabetic patients using the Edmonton	Final: 36		Primary results published in NEJM 2006.
Protocol of steroid-free immunosuppression PI: James Shapiro	Closed		
ITN007AI	Target: 81	September 18, 2003	October 2007
Phase II multiple dose treatment of T1D with hOKT3γ1 (Ala-Ala)	Final: 10		Results in Clin Immunology 2009
PI: Kevan Herold—trial results show some preservation of C-peptide.	Closed		
Diabetes 62:3766-3774, 2013			
Diabetes 62:3901-3908, 2013			
ITN017AI	Target: 8-12	July 01, 2004	May 2007 (sign off on final statistical report)
Evaluation of tolerability, safety and pharmacokinetics of hOKT3γ1	Final: 6		LPLV = December 14, 2005
(Ala-Ala) in Participants with T1D			
PI: Kevan Herold	Closed		
ITN027AI AbATE	Target: 81	September 02, 2005	In follow-up;
Phase II multiple dose treatment of T1D with hOKT3γI (Ala-Ala)	Final: 83		LPLV: April 2011
(teplizumab) PI: Kevan Herold	Enrollment Closed		Study results in Diabetes 2013
ITN028AI START	Target: 66	September 10, 2007	Ongoing;
Trial of thymoglobulin for treatment of new onset type 1 PI:	Final: 58		LPLV: July 15, 2013
Stephen Gitelman—trial results show no effect:	Enrollment		Primary study results in the Lancet D&E 2013
www.thelancet.com/diabetes-endocrinology Published online	Closed		
August 28, 2013 http://dx.doi.org/10.1016/S2213-8587(13)70065-2			
ITN041AI RETAIN	Target: 16	November 15, 2010	Ongoing;
Effect of intravenous α-1 antitrypsin on preserving β-cell function in	Final: 17		LPLV: July 2013
new-onset T1D. Part Ia	Enrollment		
PIs: Gordon Weir, Terry Strom	Closed		
ITN041AI	Target: 16	June 2013	Planned;
Effect of intravenous α-1 antitrypsin on preserving β-cell function in	Planned to open to enrollment 06/2013		LPLV: November 2015
new-onset T1D. Part Ib			
PIs: Gordon Weir, Terry Strom			
ITN041AI	Target: 66	October 2014	Planned;
Effect of intravenous α-1 antitrypsin on preserving β-cell function in	To date: 0		LPLV: January 2018
new-onset T1D. Part II			
PIs: Gordon Weir, Terry Strom	On hold until analysis of part I completed		
ITN045AI	Target: 66	March 2011	Ongoing;
Inducing remission in new onset T1D with alefacept (Amevive)	Final: 49		LPLV: April 2014
PI: Mark Rigby—trial results show some preservation of C-peptide	Enrollment		Primary study results in the Lancet D&E 2013
www.thelancet.com/diabetes-endocrinology Published online September 23,	Closed		
2013 http://dx.doi.org/10.1016/S2213-8587(13)70111-6			
ITN504AI ABBA	Target: 380	November 2004	Analysis underway;

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TABLE 2. (Continued)

Title of Trial	Enrollment	Launch Date	Completion Date
Cytokine production in children with preclinical and clinical type I insulin dependent diabetes mellitus PI: Jorma Ilonen	Final: 298 Enrollment Closed		March 2013 Results published in Journal of Autoimmunity 2010 Results published in Diabetes 2014 (Innate immune activity is detected before seroconversion in children with HLA-conferred T1D susceptibility and a type I interferon transcriptional signature precedes autoimmunity in children genetically at-risk of T1D.)
ITN504AI phase II Cytokine production in children with pre-clinical and clinical type 1 insulin-dependent diabetes mellitus PI: Jorma Ilonen	Target: 250 Final: 261 Enrollment Closed	October 2007	Analysis underway; March 2013
ITN508AI Comparison of T cell assays-T1DM consortium (Pilot II) PI: Kevan Herold, Gerald Nepom, Jeffrey Bluestone, Paul Lehmann, Peter Heeger, Jerry Palmer, H. Michael Dosch	Target: 60 (30 healthy control, 30 T1DM pts) Final: 46	April 2003	Completed Results published in Diabetes 2006.
ITN058 AI EXTEND: Preserving β-cell function with tocilizumab in new-onset T1D PI: Carla Greenbaum	Target: 66	In development	Planned November 2019
ITN040CT EXIST: islet transplant follow-up	7 of the original 36 participants	May 2001.	April 30, 2014 (end of long term FU)
ITN012AI autoantigen vaccination in T1DM Autoantigen vaccination in newly diagnosed human T1DM PI: Tihamer Orban	Target: 12 Final: closed	V1.0 February 13, 2002	2007 Closed (production issues)
ITN018AI IL-2 RAPA	Target: 10 Final: 9 Closed	FPFV = September 2, 2005	LPLV = October 2010 Study results in Diabetes 2012

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TABLE 3.
TrialNet Studies

Title	Enrollment	Launch	Completion
Effects of oral insulin in relatives of individuals with T1D in the diabetes prevention trial—type 1	Target: 372 Final: 372	1994	2003
"Pathway to prevention" natural history study of the development of T1D (screening, follow-up of at-risk)	Ongoing Screening: 114 879 At risk: 3334	2004	Ongoing
New-onset T1D—Mycophenolate Mofetil/Daclizumab Clinical Trial	Target: 120 Final: 126	2004	Enrollment: 2007 Outcome: 2009
Effects of rituximab on the progression of T1D in new-onset subjects	Target: 66 Final: 87	2006	Enrollment: 2007 Outcome: 2008
Oral insulin for prevention of diabetes in relatives at risk for T1D (Testing prediction from DPT-1 Oral Trial)	Target: N/A* To date: 240 (1° stratum) 350 (all strata)	2007	Enrollment: 2015 Outcome: 2017
Nutritional intervention to prevent T1D—pilot trial	Target: 90 To date: 123	2006	Enrollment: 2008 Outcome: 2009
Effects of CTLA-4 Ig (abatacept) on the progression of T1D in new onset subjects	Target: 108 Final: 112	2008	Enrollment: 2009 Outcome: 2011
Effects of CTLA-4 Ig (Abatacept) for Prevention of Glucose tolerance in relatives at risk for T1D	Target: 206 To date: 4	2013	Enrollment: 2017 Outcome: 2019
Effects of canakinumab on the progression of T1D in new onset subjects	Target: 66 Final: 71	2010	Enrollment: 2011 Outcome: 2012
Anti-CD3 (teplizumab) for prevention of diabetes in relatives at risk for T1D	Target: N/A* To date: 26	2011	Enrollment: 2016 Outcome: 2018
Long-Term Investigative Follow-Up Trial (LIFT)	Target: TBD	2012	Ongoing
Trichuris Suis Ova (TSO) to prevent epitope spreading in people at risk for T1D	Target: TBD	2013	TBD

and monitoring may achieve the goal of long-term islet function with ease of monitoring plus minimal toxicity.

Obstacles to Application of This Therapy

There are many unsolved immune barriers imposed by the lack of markers for diagnosing recurrence, rejection, tolerance; infections which stimulate innate immunity and tissue inflammation; cross-reactive or antigen-specific memory T cells and memory B cells for which there is no effective immunosuppression; and hematopoietic stem cell engraftment without graft vs host disease using nontoxic conditioning. Overcoming these barriers will require effective and validated biomarkers for immune monitoring, effective anti-inflammatory immunosuppression which targets innate and adaptive immunity without toxicity, effective immunosuppression for memory responses, and far more reliable and less toxic procedures for achieving bone marrow chimerism.

There are many unsolved nonimmunologic barriers which include limitations to islet mass and quality; lack of specific, sensitive, and reliable islet imaging or functional monitoring; and islet toxicity imposed by many immunosuppressive and immunomodulatory drugs and procedures. Overcoming these barriers will require progress in islet isolation, regeneration and stem cells; validated measures and markers of islet mass and function; and novel immunosuppressive regimens that obviate the need for islet toxic agents.

The Research Agenda

There are several approaches which could achieve maturity within the next few years and be ready for large scale clinical trials. As noted above, hematopoietic stem cell chimerism is validated for proof of concept in rodent, large animal, and human studies. Additional approaches to enhance this modality may include vascularized bone marrow and/or intrabone marrow injection of hematopoietic stem cells to improve the establishment and durability of chimerism. Rodent studies[242] achieving durable chimerism by adding expanded recipient Treg cells and human studies safely achieving transient chimerism[243,244] provide proof of concept that the combination of Treg cells plus nonmyeloablative bone marrow transplantation has great potential to achieve durable and safe chimerism.

The T1D early-onset and preventive studies outlined above may soon point the way to therapies that are easily adapted to islet transplantation. We can expect results over the next few years, although there are no currently validated therapies or prime candidates.

Biomarkers are critically required to move the field forward. A technology that will dramatically advance the field is a validated assay or biomarker that effectively yields highly predictive data for immunosuppression and tolerance on the one hand, and antigen reactivity on the other hand. Assays based on T cell ELISpot, serum DSA, circulating plasma β-cell DNA, or peripheral blood mononuclear cell profiling by FACS or RT-PCR have been shown at times to be strongly associated with important immune events and outcomes.[245,246] There are interesting hints that B cells or NK cells are markers or mechanistically related to tolerance. However, the associations are not so strong that that these tests are highly predictive or actionable, and none of these tests or combinations of these tests have areas under the curve greater than 0.95, which is required for a reliable clinical test. Array platforms that measure DNA simple nucleotide polymorphisms, messenger RNA, microRNA, proteins, metabolites, or microbiota

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are all available and have all been proposed as possible hypothesis generating approaches in small clinical studies. None are validated in clinical transplantation. For autoimmunity, biomarkers may have to be validated in the T1D studies and extended to islets. For alloimmunity, biomarkers may have to be validated in larger studies in bone marrow transplant and kidney or liver transplants before attempting to translate to islets.

Specific targeting of T and B memory cells to prevent recurrent autoimmune disease and chronic ongoing rejection is required. No current agents have been shown to be clinical efficacious in this regard. Although Alefacept did have some effects on memory by peripheral lymphocyte subset analysis, it was unable to have a clinical impact on memory responses in kidney clinical trials and it is clear that all other currently approved drugs are not effective in safely controlling memory responses. Success and validation may first have to come from bone marrow transplant, kidney, or liver transplants and then translated to islets.

Encapsulation technologies and novel sites of transplantation may mitigate issues related to islet quality, islet quantity, islet regeneration, tolerance, innate immunity, and memory responses. The experimental approaches to site and to encapsulation are relatively advanced and may provide the ability to move forward rapidly.

It is clear that a combination of several approaches outlined above must be considered.[247] Combinatorial approaches will undoubtedly create significant contractual, regulatory, and safety hurdles.

What Is the Potential for the Treatment of Diabetes

It is noteworthy that minimal or minimized immunosuppression is widely used and potentially ready to implement in islet transplantation. A regimen consisting of transient steroids, transient anti-inflammatory (eg, TNF-α blocker), CNI-free, mTORi-free or transient, and costimulatory blockade (Belatacept) has some proof of concept. An S1P1 modulator (Fingolimod) could be added to this regimen. A major barrier to implementing this approach is lack of validated biomarkers for islet function and immune monitoring. Additionally, it may be argued that any protocol relying on long-term immunosuppression, even at low levels, could only be applied in patients with life-threatening complications of diabetes, such as hypoglycemic unawareness. Given the excellent standard of care for diabetes that is currently available without transplantation, the application of transplantation to broader diabetic populations will only be ethically feasible when approaches that do not require long-term immunosuppression, such as tolerance or encapsulation, are successful and safe.

The Treg cell therapy will likely be part of a combination therapy approach. In particular, mixed chimerism induced and sustained by Treg cells plus nonmyeloablative conditioning may be one of the most likely approaches to be tried in islet transplantation. As progress is made in Treg cell therapy and nonmyeloablative conditioning in other areas (eg, autoimmunity, cancer), those results may be translated to islets.

If substantial progress is made in stem cells, encapsulation, β-cell regeneration, isolation, imaging, biomarkers, or xenotransplantation, each of these areas will synergize for the induction and maintenance of tolerance.

Summary of Research Priorities

(1) The recent success of chimeric tolerance in renal transplantation potentially sets the stage for application to islets. The use of protocols with high-level donor chimerism may simultaneously achieve allotolerance and rid the host of their native autoimmune prone T-cell repertoire.
(2) Costimulation blockade with simultaneous targeting of CD28-B7 and CD40-CD40L remains a scientifically attractive approach. New CD40 targeting agents in conjunction with the recently approved anti-B7 belatacept may permit such testing in the near future.
(3) Trials of innovative regulatory cell based approaches (Treg cells) are also attractive in that it may be possible to interrupt in parallel auto and alloimmunity with precise antigen specificity.

STEM CELLS AS A SOURCE FOR β CELLS

Current State of the Field

Diabetes is a debilitating disease characterized by a chronic inability to normalize BG levels. Transplanting cadaveric pancreata or isolated pancreatic islets can restore glucose homeostasis, but organ demand outstrips supply and donor quality contributes to short-term complications. Consequently, there is significant interest in alternative sources of IPCs. To overcome the limitations of currently available therapies, research efforts have focused intensively on generating functional β cells or endocrine cell clusters from stem cells. A variety of stem cell types have been considered as potential future sources of transplantable β cells which include human pluripotent stem cells (hPSCs), such as hESCs and human-induced pluripotent stem cells (hiPSCs), mesenchymal stem cells generally isolated from bone marrow or cord blood, stem cells isolated from adult tissues, or directly reprogrammed somatic cells. This review will focus on pluripotent stem cell (PSC) and reprogrammed somatic cell sources. Although the concept of reprogramming somatic cells directly into β-like cells is in its infancy, recent exciting progress in the PSC field has led to refined protocols yielding highly enriched populations of monohormonal insulin-secreting cells and the initiation of pilot clinical trials.

Pluripotent Stem Cells

The PSCs are characterized by 2 features: their ability to differentiate to any of 3 somatic cell lineages (ectoderm, endoderm, or mesoderm), and their ability to replicate indefinitely in a stable pluripotent state.[248-250] Because PSCs can evade senescence in culture, they provide an unlimited supply of cells suitable to meet the demand for a replacement cell therapy.[249] The PSCs can be directed to differentiate to a variety of specific lineages with relatively high efficiency.[251,252] The hESCs were first isolated and cultured by Thomson using inherently variable mouse fibroblast feeder cells and serum supplemented media.[253] Significant improvements now allow derivations and stable extended culture under defined xeno-free conditions yielding cells with a normal genetic karyotype suitable for cell banking and clinical applications.[254-256]

A second pluripotent cell source originates from somatic cells which can be reprogrammed to a pluripotent state through a process first identified by Shinya Yamanaka and Sir John Gurdon.[257-259] Reprogramming can be accomplished by forced expression of a combination of transcription factors (eg, OCT4, SOX2, KLF4, and cMYC or OCT4, SOX2,

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NANOG, and LIN28), a process initially reported using retroviral transfection techniques.[258,259] Subsequent studies showed that reprogramming could be achieved using nonintegrating episomal vectors based on Epstein-Barr virus or Sendai virus delivery systems[260-263] and, more recently, with RNA- or protein-based methods.[264,265] Many human cell types have now been reprogrammed to iPSCs, for example, adult and embryonic fibroblasts, mononuclear peripheral blood cells, T cells, islet cells, pancreatic acinar cells, and hair follicle cells, among others. Human iPSCs have been derived from patients with a variety of different diseases including diabetes.[266-268]

Direct Reprogramming

Rather than generating stem cells from somatic cells and subsequently differentiating them to the desired lineage, attempts have been made to circumvent this process and induce direct reprogramming of somatic cells to β cells, either in vivo or in vitro. In this method, enforced activation of key pancreatic transcription factors, usually in combinations, is used to drastically alter the program of expressed genes thereby leading to a dramatic change in phenotype, often across typical lineage boundaries.[269] Such an approach has been attempted in several different cell types, such as pancreatic acinar cells,[270-272] hepatocytes,[273] and fibroblasts.[274] Although somatic cell reprogramming to pancreatic lineages involves epigenetic conversion, it is not known whether cells transform directly into β cells, or whether they first revert to a multipotent state and then redifferentiate toward β cells (ie, 2-step process) or whether certain cell types are more efficiently reprogrammed. Also, whether such conversions result in stable, robustly functional β cells remains to be determined.

Conversion of hPSCs to β Cells

The overall preclinical research goal of the field is to achieve efficient derivation of a functional β cell mass in vitro that is able to rapidly and reliably cure diabetes in mice, both longstanding gold standard preclinical diabetes assays. To achieve in vitro conversion of hPSCs to β cells, a highly productive approach has proven to be the application of knowledge gleaned from developmental biology studies. However, most information about pancreas development has been obtained from organisms, such as frogs, chickens, zebrafish, and mice, and it is well known that aspects of pancreas development and islet biology differ between humans and lower organisms.[275-277] Because of this, refinement of differentiation protocols has progressed through a combination of strategies that include both rational design and empiric testing of developmentally important effector molecules and monitoring expression of key transcription factors. These endeavors have also been complemented by an increasing understanding of the epigenetic landscape and transcriptional profile of human pancreas development and fully differentiated β cells.

In vivo pancreatic development is a complex process involving sequential lineage restriction steps to form a composite, well-vascularized endodermally derived organ consisting of acinar, ductal, and endocrine tissues.[278-280] Based on principles of vertebrate development, a multistep model of β-cell formation from stem cells in vitro has been proposed,[281] attempting to recapitulate sequential stages of in vivo development including gastrulation, endoderm specification, gut-tube morphogenesis and organ budding from the gut tube, and finally organ-specific cellular differentiation within the organ bud. This multistep model has since provided a foundation for methods and protocols applicable to pluripotent stem cell differentiation. A full description of pancreas development and the evolution of β cell in vitro differentiation protocols over the last decade is beyond the scope of this review, and the reader is referred to the following references: Hosoya et al, Alexander and Stainier, Clements et al, and Shen.[282-285]

Rapid progress is being made to improve and refine in vitro differentiation protocols to achieve monohormonal β-like cells that express key maturity markers and exhibit robust glucose-stimulated insulin secretory responses. Many believe that a more mature differentiated cell will be more advantageous therapeutically as well as provide a tool to facilitate studying β-cell pathophysiology and testing of novel pharmaceuticals. Until recently, in vitro–derived IPCs largely exhibited a polyhormonal phenotype[282,283,286-289] and most likely corresponded to cells of the primary endocrine transition observed in murine and human development,[290,291] which ultimately do not give rise to adult β cells. Instead, adult β cells are thought to arise from a distinct developmental event, the secondary transition, marked by transient expression of neurogenin-3 (Ngn-3). Although the precise reasons for incomplete differentiation under some conditions are still unclear, it is worth noting that immature phenotypes are also observed when other lineages, such as blood, cardiac, and neural cells, are derived from PSCs. Polyhormonal endocrine cells appear to have reduced levels of, or lack, important β-cell transcription factors, such as PDX1, NKX6.1, and MAFA,[286,287] and in some cases, key β-cell transcription factors, such as PAX4 and ARX, are misexpressed compared with adult human β cells in vivo.[286,287] From a functional viewpoint, immature hPSC-derived insulin-positive cells appear to express reduced levels of other important genes including potassium channels, proconvertases, Zinc transporters, islet-associated polypeptide (IAPP), and urocortin3 relative to adult β cells.[40] Thus, the expression of these markers was essential for screening conditions that yielded more mature functional β cells in vitro.[292]

Several groups have recently reported improved differentiation protocols, which achieve monohormonal β cells and better in vitro functionality, promising to finally remove this longstanding roadblock.[293-295] Interestingly, different protocols were used by each of these groups yet there were some commonalities. By extending the culture period, including 3-dimensional suspension culture and exposure to ALK5iII, Shh inhibitors (SANT1 or KAAD cyclopamine), γ secretase inhibitors to inhibit Notch signaling, and thyroid hormone (T3), these three groups were able to enhance glucose-stimulated insulin secretion (GSIS) in vitro. However, Rezania et al[292] showed that their cells exhibited dampened secretory characteristics in perifusion assays compared with human islets. Furthermore, although similar results in GSIS assays were achieved with hPSC-derived β cells and human islets, the degree of variability from preparation to preparation of hPSC-derived β cells was surprisingly high.[292,294] Importantly, the insulin-positive cells displayed a monohormonal phenotype with improved expression of key β-cell signature genes. Although there were many differences in the culture

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conditions among these recent reports, it is still not entirely clear whether all of the components in each protocol are necessary and whether the makeup of the cell populations derived by the different protocols is the same. Nevertheless, these current reports demonstrate rapid progress in the field toward achieving a more physiologically functional β-like cell in vitro from hPSCs.

Critical progress in efficiently reversing diabetes in mice was also reported this past year.[292-294] Until recently, when hPSC-derived pancreatic tissues were transplanted into immunodeficient mice, either under the kidney capsule or into the epididymal fat pad or in an immunoisolation device in the subcutaneous space, the graft did not regulate glucose or produce secreted C-peptide immediately.[296-384] Instead, the graft appeared to mature over many[6,13-20] weeks to form functional pancreatic tissue in a time frame similar to the in vivo maturation of human fetal pancreas.[300] The mature graft ultimately did contain islet-like structures comprised of α (glucagon), β (insulin), δ (somatostatin), ghrelin, and pancreatic polypeptide hormone-producing cells and is competent to maintain glucose homeostasis in mice made diabetic with alloxan or streptozotocin despite imperfect insulin secretory kinetics of hPSC-derived endocrine grafts.[301] Efficient in vivo endocrine maturation appeared to be dependent on a sufficient number of Nkx6.1 + PDX1+ pancreatic progenitor cells (PPCs) in the transplanted population,[298] and if/how the cells were encapsulated.[301] Although the delayed correction of chemically induced diabetes by hPSC derivatives represented an important preclinical milestone, there remained great interest in accelerating maturation in vivo. Indeed, 2 studies have now reported more rapid (2 weeks and 6 weeks) reversal of murine diabetes after transplanting more mature cells under the kidney capsule in *Akita* and streptozotocin models, respectively.[292,294]

Now that the derivation of hPSC-β-like cells in vitro exhibiting improved physiological and phenotypic characteristics of adult, mature β cells resulting in more rapid cure of diabetes in mice has been achieved, the next questions likely to arise are: what accounts for the GSIS variability and dampened insulin secretory kinetics in perifusion assays and can this be improved? Will a macroencapsulation device provide a suitable environment for mature cells as it does for maturing progenitors? Other questions concern the delivery of these cells to patients: What cell population is the best to transplant? Are progenitors sufficient to transplant or is a terminally differentiated functional population better? Perhaps a mixed or hybrid population is a better choice? Is a mixture of endocrine cell types necessary or beneficial and to what degree, or is it sufficient to transplant a graft solely composed of β cells? Finally, and perhaps ideally, can one derive/engineer a renewable, yet fully differentiated β cell from stem cells? Many of these questions should be addressed in preclinical animal models.

Reports of pancreatic lineage differentiation and β-like cell formation in vitro have come from many laboratories with many different cell lines, using a variety of culture protocols. However, few protocols have been compared head to head; even fewer have directly compared multiple different cell lines in parallel. Thus, which line and which protocol provides optimal pancreatic β cell differentiation have not been determined prospectively. Given genetic and epigenetic differences of different PSC lines, it is not surprising that different cell lines can behave dissimilarly under the same conditions.

Analyses of nonpancreatic cell types within differentiation cultures, regardless of the protocol, have been limited. These nonpancreatic cell types, including but not limited to undifferentiated cells, could potentially inhibit or enhance ongoing differentiation. The retention of undifferentiated cells through later culture stages raises the specter of teratoma formation after transplantation. Most culture protocols published to date generate heterogeneous populations with some unwanted cells, which brings up several questions: how pure does the population need to be? How precisely defined does the cellular product need to be before it is deemed suitable for therapeutic use? Are certain unwanted cell types acceptable while others are not?

Regardless of whether transplantation of fully functional β cells or PPCs is the therapeutic platform, both strategies would benefit from technology that modifies the immunogenicity of the graft, or induces host immunological tolerance, or protects the graft from host alloimmune and autoimmune responses. Ongoing efforts are addressing this need through use of immunoisolation devices, modified stem cells,[302] advanced immunosuppression protocols,[303] tolerogenic strategies, or using syngeneic hiPSCs. Macroencapsulation approaches are particularly attractive because they have the benefit of graft cell containment, reducing the risk of excessive growth or the spread of cells with teratoma-forming potential as well as limiting possible alloimmune and autoimmune damages.

Obstacles to Application

The achievement this past year of improved in vitro GSIS and more rapid reversal of diabetes in mice was a major milestone. Yet, many clinicians may still view the variable GSIS results and subnormal insulin secretory kinetics in perifusion assays that have been reported as a substantial obstacle to widespread application of this technology. Why has this milestone been so hard to achieve and how will we know when we have achieved it? The field would benefit from a consensus agreement as to what are benchmark phenotypic and functional characteristics of an in vitro hPSC-derived β cell. Most would agree that at the very minimum the following should be achieved: (i) a consistent, reproducible stimulation index without secretagogues of at least 2 to 3, and (ii) immediate or near immediate reversal of diabetes in mice with a normal glucose tolerance curve and stimulated C-peptide release in response to IV or PO glucose, which is eliminated if the graft is removed. A more stringent definition might include results of a panel of phenotypic markers, GSIS including secretagogues, estimation of proinsulin-insulin ratios, and insulin secretion kinetics derived from perifusion assays that mirror human islets. Additionally, determining physiological responses at the single-cell level may be valuable, given the possible heterogeneity of the insulin + cell populations. A comprehensive analysis of the resulting β-cell population will benchmark the degree of functionality achieved and facilitate comparisons between cells produced using different protocols. If not transplanting a functional β cell, how long will patients be willing to wait before they are able to eliminate insulin therapy—or how long will a transplant be allowed to persist before it is deemed a failure? Moreover, many clinical events could thwart in vivo maturation and the development of functionality, such as rejection, recurrent autoimmunity, or toxic immunosuppressant medications.

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An important part of preclinical evaluation of a possible therapy is to understand the immune responses to hESC/iPSC-derived PPCs or β cells in immunocompetent hosts and to devise ways to protect cells from alloimmunity and autoimmunity. To date, these clinically relevant questions have not been addressed in depth. Although encapsulation as a transplant delivery system may demonstrate efficacy, it is also possible that graft damage will still occur because many current devices do not effectively exclude cytokines. More studies evaluating host alloimmune and autoimmune responses to encapsulated hESC-derived β cells are needed. In an ideal scenario, customized patient-specific iPSC lines may obviate the need for immunosuppression. However, existing data are unclear about whether syngeneic iPSC progeny would be destroyed after transplantation[304,305] and studies to date do not address this question specifically for pancreatic lineages. Furthermore, it is currently unknown whether syngeneic grafts derived from Type I diabetes mellitus patients will indeed elicit immune responses or be susceptible to recurrence of autoimmunity. Thus, more work needs to be done to clarify and better characterize the anticipated immune responses to syngeneic and allogeneic hPSC-PPC or β cell grafts. Moreover, based on the absence of autoimmunity in Type 2 diabetes mellitus patients, it may be reasonable to first test syngeneic iPSC-derived β cell transplants in this population.

Teratoma formation, or malignant transformation of a teratoma into a teratocarcinoma, is a concern of any proposed hPSC-based therapy. With teratomas reported in the context of a number of current in vitro pancreatic differentiation methods, effective, safe, simple, and inexpensive methods to prevent or limit teratoma formation are needed. Furthermore, it will be necessary to quantify the risk for a given cellular graft in order to assess its risk-versus-benefit ratio. Therefore, developing a predictive, quantitative assay in which to test a cell product would benefit the field. Such an assay could be an in vivo assay, such as the injection into the hind limb of an immunodeficient mouse similar to what is currently used to determine pluripotency of cell lines. However, this method is neither quantitative nor rapid. A rapid, relatively high throughput in vitro assay would represent a more ideal method, if available. The PluriTest assay may prove to be valuable for this purpose.[306,307] Ultimately, a fully terminally differentiated purified cell population may have an extremely low teratoma risk profile, but determining this preclinically for a given cell population would have merit. Macroencapsulation may very well be the least expensive and most effective "teratoma prevention" method currently available, but improved, less fibrogenic, more proangiogenic, and cytokine-excluding encapsulation methods may be needed. Other methods could require genetic manipulation of the cells before differentiation and transplantation; however, such methods may carry their own attendant risks.

Current differentiation protocols use large quantities of expensive growth factors. Can these be replaced with less expensive small molecules that signal through the same receptors and produce the same biological results? An example of this is the use of LDN193189, a bone morphogenic protein antagonist instead of Noggin in recent studies.[292,294] High-throughput screens for small molecules which can replace expensive growth factors may not only allow the differentiation process to be less expensive but also more efficient. Additional optimization will be needed to scale up the differentiation protocols to generate large numbers of β cells to meet the demands of millions of diabetes patients.

Finally, regulatory considerations will present significant challenges in this area. The use of cadaveric islets in transplantation is already regulated in the United States as a manufactured cell product. Stem cell-derived β-cell products will likely be regulated similarly by most regulatory agencies. Additional use of encapsulation devices, scaffolds, other supporting cells, angiogenic agents, or immune modulatory factors will render the therapy as a combination product. There will be complicated preclinical data packages for these combination products depending on the perceived risks of the individual components and their combinations. Genetically modified lines may be subject to additional regulatory requirements. Academic researchers and industry stakeholders will have to work closely with regulatory reviewers to manage reasonable amounts of preclinical work to justify clinical trials. In addition, to use a cell line clinically, it is critical that cell lines be generated under current Good Manufacturing Processes (cGMP). Durruthy-Durruthy et al[308] recently published on a rapid and efficient conversion of integration-free human iPSCs and suggested strategies to convert to cGMP culture conditions. Currently, there are numerous groups developing cGMP iPSC lines (Cellular Dynamics International, National Institutes of Health, Riken Institute, Roslin Institute).

Direct Reprogramming of Somatic Cells to β Cells

Another strategy for generation of IPCs in vivo or in vitro involves reprogramming adult cells directly to a pancreatic cell lineage. A number of groups have demonstrated that β cells may be generated from non–β cells through ectopic or artificially induced gene expression of a single transcription factor (*Pdx1*) alone or with other pancreatic genes. Ectopic expression of PDX1 is sufficient to convert fetal α-cells to β-cells in vivo and can promote functional insulin expression in mouse liver.[309,310] Combinatorial transfections of the transcription factor genes *Pdx1, MafA*, and *Ngn3* can reprogram murine pancreatic exocrine tissue or liver to form functional β-cells in vivo, and the resulting cells appear competent to rescue animals from hyperglycemia.[270,311,312] Ectopic *Pdx1* expression can also induce fate conversion in vitro, as demonstrated in the case of cultured keratinocytes converted to a pancreatic β-cell fate.[313] These aggregate results suggest that ectopic expression of *Pdx1*, alone or with other genes, may provide an alternative means of generating functional β-cells. Recently, Zhu et al[274] reported on the direct reprogramming of murine fibroblasts to definitive endoderm by a transient expression of pluripotency reprogramming factors in conjunction with a unique combination of small molecules and growth factors. They then followed a differentiation protocol to develop pancreatic progenitors that reversed diabetes in a rodent model. On the other hand, human pancreatic duct reprogramming may be improved by Ngn-3 overexpression, inhibiting Delta-notch signaling and coexpressing Myt1.[314] These data demonstrate how the plasticity of adult cells, in particular those of related lineage, might be harnessed to produce β cell phenotypes.

Many challenges on the road to clinical application remain as this strategy is relatively young. The reproducibility and functionality of resulting cells still need to be rigorously

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tested. Can the efficiency of an in vivo reprogramming approach be increased to achieve a suitable β-cell mass for replenishment of lost β cells in large animals or humans? Moreover, the long-term safety of reprogrammed cells is not known. An advantage of direct reprogramming is that cells would be syngeneic to the prospective recipient; yet, a potential disadvantage is that autoimmunity may limit the emergence of new β cells in T1DM patients. Nonetheless, recent data provide significant encouragement that a direct reprogramming approach could 1 day generate new β-like cells ex vivo, or in a patient.

The Research Agenda

Refined and improved differentiation protocols are needed to achieve consistently efficient yields of β cells in vitro which exhibit normal insulin secretion kinetics. A better understanding of the signaling molecules regulating the later stages of endocrine cell specification, delamination from the epithelium, formation of islet cell clusters, and achievement of functional GSIS during in vivo development would greatly aid the field in achieving the milestone of robust functionality in vitro. The field would undoubtedly benefit from having a central core laboratory for comparisons of cells generated by different protocols. Putting different cell populations from a variety of laboratories and companies through a variety of functional assays such as static incubation assays, perifusion assays, gene and protein expression assays, and mouse transplant assays, and so on to directly compare cells would be a valuable endeavor for the stem cell community.

Identifying and isolating derived β-like cells for investigation would benefit from availability of advanced reporter lines. A human ESC *Insulin-eGFP* reporter line generated by Micallef et al[315] has been used in several studies to characterize insulin-positive cell expression profiles and physiology.[316,317] However, most in vitro differentiation conditions generate insulin + glucagon + polyhormonal cells. Thus, it would be beneficial if additional reporters such as for *Glucagon, PDX1*, or *Nkx6.1* could be engineered into the same cell lines. Cell lines such as these would aid in isolating various cell populations for further characterization and transplantation. Additionally, engineering antibiotic resistance genes into the *insulin, PDX1*, and other relevant gene loci could facilitate reducing the heterogeneity and improving the purity of the resulting populations. Efficient genome editing methods are now available to accomplish this; however, they remain costly and labor intensive.[318-320]

The current assay for pluripotency and estimating teratoma risk is time consuming and expensive. An improved predictive assay would support the field by providing a reliable, rapid, sensitive, and quantitative measure of teratoma risk. Such an assay would benefit preclinical studies anticipating investigational new drug submission. Another "safety net" approach might involve developing hPSC lines that contain suicide genes tagged to pluripotency or progenitor gene promoters which may be valuable for ensuring removal of undifferentiated or partially differentiated cells thereby reducing the risk of teratoma formation.

To date in preclinical studies, stem cell β-like derivatives have primarily been functionally tested in mouse models of chemically induced diabetes, as a proof of principle. Few other models have been tested because of the paucity of these diabetes models existing on a suitable immunodeficient background. Therefore, having readily available additional diabetes murine immunodeficient models would benefit the field. Additionally, more robust humanized murine models as well as large animal models would also be advantageous to studying the immunogenicity and dosing strategies of the stem cell-derived β-cell populations.

Human iPSCs provide a valuable tool for disease modeling[268,321-325] and several hiPSC lines have been generated from patients with T1D and T2D.[266,267,326] From a scientific and disease modeling point of view, it would be useful to have hiPSC lines from a variety of types of diabetes including monogenic diabetes to take advantage of these possible in vitro disease models. Repositories could be established and then these models would need to be characterized and validated. The combination of data from genome wide association studies, genome editing and hPSC-derived β cells, provide a powerful set of tools to study the genetics of various forms of diabetes and mechanisms of pancreas development.

Safety is a potential issue for virally derived hiPSC lines intended to be used clinically that relate to genomic perturbations and disease transmission. It is hoped that nonintegrating methods of reprogramming would provide a satisfactory safety profile, but whether this is the case after transplantation of iPSC progeny needs to be rigorously tested. Safety concerns regarding oncogene expression, viral immunogenicity, and genetic instability of hiPSCs may be averted with further improvements for derivation and expansion, making this a feasible cell source for future therapeutic purposes.[327]

Human PSC lines that possess characteristics that thwart or downregulate adaptive and/or innate immune responses may be valuable cell lines for clinical application. For example, Rong et al[302] expressed molecules which blocked costimulatory signals and downregulated immune responses to hESC progeny. Other strategies might involve modifying HLA antigen expression on hPSCs or expressing proteins that promote regulatory T cells.

Direct reprogramming usually involves adding pancreatic transcription factor genes analogous to the addition of pluripotency genes for stem cell reprogramming. However, the process of reprogramming with integrating lentiviral vectors can alter the genome leading to malignant transformation or reduced differentiation. Although nonintegrating vectors exist, those containing pancreatic transcription factors are not widely available for use.

There is a need for better cell delivery devices taking advantage of material science, 3-dimensional culture, nanotechnology, matrix biology, improved oxygen delivery platforms, and better encapsulation technology. Many encapsulation methods currently induce fibrosis after implantation in large animals despite promising results in rodents. Materials and devices that reduce the fibrogenic foreign body host response while still allowing macromolecular nutrients to bathe cells and excluding immune cells, antibodies and ideally cytokines would be a valuable transplant vehicle. Combining platforms could provide a more physiological environment for growth, differentiation, and survival of the cells long term.

Although islets can function in vivo after transplant as long as they are well vascularized, challenges still exist with identifying an optimal site for islets and β-cell grafts derived from hPSCs. Ideally, one would prefer to transplant allogeneic islets (or human stem cell-derived endocrine cells) into patients into a site that is safe, accessible with minimal risk,

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and optimal from the point of view of allowing adequate volume, is not highly immunogenic, is well vascularized, and is free from instant blood-mediated inflammation. Considering these requirements, the venous sac may prove a suitable transplant site for allogeneic islets in human, but only preclinical studies have been conducted to date.[328] Lymph nodes and the omentum have also been shown to be potential sites for islet transplantation.[329,330] Ultimately, additional bioengineering approaches may be needed to incorporate scaffolds and ECM molecules to construct well-vascularized tissue from single cells. Several studies have made headway in making devices or scaffolds for appropriate islet cell engraftment.[331-333] A clinical study is currently underway to study whether a subcutaneously implanted, prevascularized scaffold may be suitable for islet transplantation (http://clinicaltrials.gov/ct2/show/NCT01652911), and this has clear relevance for stem cell-derived cells. Further studies in this arena and approvals through the FDA will undoubtedly inform future related studies with stem cell-derived β cells.

Summary and Speculation on Therapeutic Impact

Rapid progress and expansion of knowledge continues to characterize the field of deriving β cells from stem cells which began approximately 15 years ago, at which time the transcriptional network of pancreas development and nature of human definitive endoderm was entirely unknown. We have come a long way since then. Yet, the majority of research remains in the preclinical realm focused on optimizing methods for the in vitro conversion of stem cells or somatic cells to high yield, functional cells that resemble adult human islets/β cells. Given the complexity of the developmental processes scientists are trying to mimic, it is not surprising that this has proven such a difficult task. Nonetheless, real progress is occurring, and there is tremendous anticipation that physiologically normal adult β-like cells will be achieved in the very near future. Even without achieving this in vitro milestone, companies, such as ViaCyte, Inc (http://viacyte.com), are moving ahead with pilot clinical trials. ViaCyte, Inc has proposed a phase I safety and dosage trial combining an hESC-derived pancreatic progenitor cell product delivered in a macroencapsulation device and transplanted into T1DM recipients. How this trial and others like it unfold will significantly affect the public's and investor's impressions of the field's potential. The results will either embolden others to propose additional clinical pilot trials or send the field back to the drawing board. Still, many unanswered questions need to be addressed and new technologies devised to support the responsible development of the field. Given the steady progress and new innovations the field has witnessed over the last several years, many are confident that the existing challenges will ultimately be overcome and that an effective and safe stem cell-based β cell replacement therapy will emerge in the coming decade.

Summary of Research Priorities

(1) Recent reports highlight progress in achieving refined differentiation protocols for driving human pluripotent stem cells to β-like cells with improved physiological function and greater capacity for more rapid correction diabetes in mice. However, further work is needed to understand the reason for why these cells still do not exhibit normal stimulus-secretion coupling or dynamic insulin release in perfusion assays.
(2) Additional studies evaluating host alloimmune and autoimmune responses to encapsulated and unencapsulated human pluripotent stem cell-derived β cells, in both the syngeneic and allogeneic settings, are needed.
(3) The stem cell-derived β cell therapy field would benefit by testing strategies incorporating new encapsulation technologies, novel cellular deliver methods and sites, and innovative tissue engineering approaches.
(4) Further experimental work is needed to study the ability to directly reprogram somatic cells into β-like cells and assess their function in vitro and in animal models.
(5) Teratoma formation is a key safety issue with the potential therapeutic application of human pluripotent stem cell-derived β cells. Studies which define this risk and assays that better predict this risk would advance the field.

β CELL REGENERATION FROM PROLIFERATION AND NEOGENESIS

Current State of the Field

There is a great need to find sources of β cells that can be used to replenish those that have been lost in diabetes. This commentary focuses on the potential of the pancreas to regenerate β cells that can reverse the diabetic state. There are reasons to be optimistic that new β cells can be generated by proliferation of existing β cells and by neogenesis, the production of new islet cells from non-islet cells in the pancreas or other organs.

The purpose of this short commentary to discuss the potential for β cell regeneration in the human pancreas that could be exploited to replenish the β-cell deficit of people with both T1D or T2D. The major questions being addressed are whether β`-cell replication can be significantly enhanced and whether there are cells in the endocrine pancreas or other organs that can serve as precursors for the formation of new β cells.

Is There Significant β Cell Turnover in the Adult Human Pancreas?

Some have argued that virtually all of one's β cells develop by the end of young adulthood and that no new β cells appear during later adult life in humans.[334-336] One argument against significant human β-cell turnover comes from negative studies using in vivo thymidine analog incorporation and radiocarbon dating.[334] Despite these conclusions, other data support the presence of some level of β-cell turnover. For example, we know there is a constant loss of β cells as evidenced by staining for terminal deoxynucleotidyl transferase dUTP nick end labeling[337] and other makers of death, yet β cell mass is well maintained for decades.[338,339] For this reason, there must be some generation of new β cells to keep up with cell loss. Moreover, evidence suggests some expansion of β-cell mass in obesity and pregnancy.[337,340] The assumption that the process is slow is supported by studies quantifying the accumulation of lipofucsin, a marker of aging, in a very high percentage of β cells.[341]

Evidence for β Cell Replication in Adult Human Pancreas

Much of the evidence that β cells do not replicate comes from studies done on pancreases obtained at autopsy or from cadaver donors.[335,342] The most commonly used tool for assessing β-cell replication is Ki67, but it is well known in studies of cancer pathology that the numbers of mitotic

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figures and Ki67 positivity fall with warm and cold ischemia.[343,344] This has also been recently demonstrated with mouse and pig pancreases subjected to autopsy conditions and evaluated with Ki67.[345] These findings support the likelihood that the negligible replication rates found with Ki67 have led to erroneous conclusions. They also fit with the finding that Ki67 positivity in β cells can be found in fresh surgical specimens of pancreas and in human islets transplanted under the kidney capsule of immunodeficient mice.[346] The actual rate of β-cell birth from replication is very difficult to estimate because Ki67 positivity is not necessarily equated with the generation of new cells. However, if β cells in adult humans have 0.4% Ki67 positivity, and Ki67 positivity lasts 12 hours, and if there were no neogenesis or apoptosis, β-cell mass could more than double in less than a year. However, Ki67 positivity can be found in cells that do not divide but are arrested in cycle. It can also be associated with DNA damage and apoptosis.[347] Although many unknowns remain, there is now good evidence that there is some capacity for regeneration of β cells from replication in adult human pancreases.

Evidence for β Cell Neogenesis in Adult Human Pancreas

Islet neogenesis in rodents and humans remains a controversial topic.[348,349] Lineage tracing experiments in mice have provided mixed results, and it is unlikely that similar studies can be done with human tissue. The evidence in support of neogenesis remains circumstantial, some of it stronger than others. For example, the presence of insulin-stained cells in the duct epithelium and the finding of increased numbers of single and small clumps of β cells in human pregnancy and in other situations may be suggestive but are hardly definitive.[340,350,351] One cannot be certain that these small clumps of β cells did not arise from replication of a few existing cells.

However, finding cells in the duct epithelium that costain for insulin and cytokeratin 19 carry more weight[350] because they suggest a dynamic process. In addition, pancreatic intraductal neoplasms are rarely seen before age 35 years, but are found in 60% of non-neoplastic pancreases by age 45 years and in 75% by age 55 years.[352] Pancreatic intraductal neoplasms can frequently contain significant numbers of islet hormone-positive cells.

Is There Evidence That β Cell Growth can be Stimulated in the Adult Pancreas?

The β cell mass is modestly increased by 30% to 50% in insulin-resistant obese human subjects.[337,339] Another example of increased β-cell mass is the normal human pregnancy.[340] Increased β-cell mass and high circulating GLP-1 levels have been seen in some subjects after bariatric surgery[353]; however, the cause-and-effect relationship between the two has not been established. On the negative side, subjects with T2D have been treated for years with GLP-1 agonists and dipeptidyl peptidase-4 inhibitors, yet no evidence for increased β cell functional mass has emerged after drug treatment was stopped.[354]

Patients with longstanding (over 50 years) T1D can routinely be found to have some β cells in their autopsied pancreases.[355] The question of whether these cells are resistant to immune killing has not been answered. These could be a subset of uniquely strong β cells that survived from childhood, but a more attractive hypothesis is that new β cells are generated continuously from neogenesis and then killed by autoimmunity. This possibility is supported by the presence of islet cell antibodies in these subjects, by the finding of many single and small clusters of β cells in the pancreases, which suggest neogenesis, and by the observation of some lymphocytes in the few remaining islets of these pancreases.

What Causes β Cell Death?

At least some β cells have a limited life span with an apoptotic death. Perhaps some cells live for decades, although other die early for unknown reasons. One possibility is that islets and most other organs have a natural remodeling process, such that cell birth and apoptosis serve as mechanisms to facilitate structural change.

In the development of both T1D and T2D, there has been much speculation about what might cause an increased rate of cell death other than immune destruction.[356] Although usually discussed as being important for T2D, the same problems must be faced by the residual β cells of T1D. The reality is that we have little idea about which processes are the most important pathways to death, but attention has focused on the following candidates:

(a) Glucose toxicity (glucotoxicity): Clearly, hyperglycemia, even in the range of just impaired glucose tolerance, has a deleterious influence upon β cell function, most notably acute GSIS. There is currently considerable interest in the dedifferentiation of β cells that occurs in a hyperglycemic environment.[356,357] Some of these phenotypic changes are presumably responsible for dysfunctional insulin secretion. Importantly, insulin secretion rapidly returns to normal in T2D shortly after normoglycemia is restored by bariatric surgery.[358] Little is known about the molecular mechanisms through which glucotoxicity might cause β cell death. The descriptive term "overwork" is often used and it would be helpful to have this concept better defined.

(b) Lipotoxicity and glucolipotoxicity: Almost all of the evidence supporting the presence of lipotoxicity or glucolipotoxicity comes from in vitro experiments in which free fatty acid such as palmitate are added to isolated islets or β cell lines. Palmitate and other free fatty acids certainly have toxic effects, and as such can be useful to examine stress and death pathways. However, do FFAs exert adverse effects on β cells in real life? As of yet, the evidence that this occurs in vivo in human or animal diabetes is sparse and unconvincing.[359,360] Moreover, it is difficult to find correlations between β cell dysfunction and FFA levels as subjects develop diabetes, whereas the correlation between rising glucose levels is very strong.[361]

(c) ER stress: There are good reasons to think that ER stress from the secretory demands of insulin resistance and hyperglycemia leads to the demise of some β cells[362] but we do not have good markers that can follow the process.

(d) Oxidative stress (ROS): Again, there are many reasons to think that oxygen radicals could cause cell death resulting from β cell "overwork" or the challenges of the diabetic environment,[363] but this process also lacks markers.

(e) Amyloid: Amyloid deposits are found in many of the islets of people with T2D but there are also few in normoglycemic obese subjects with insulin resistance. The amyloid deposits are formed by fibrils of IAPP and a strong case has been made the initially formed IAPP oligomers can damage membranes and cause cell death.[364,365]

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The Research Agenda

Can We Significantly Increase β-Cell Mass by Stimulating β-Cell Replication Either In Vivo or In Vitro?

Much has been learned about the cell cycle mechanisms in murine and human β cells.[366-368] In addition, various compounds that can stimulate β-cell replication have been identified by high-throughput screening.[369,370] There has been a recent identification of betatrophin, which is thought to be a factor secreted by insulin-resistant livers that can stimulate β cell replication.[371] Its mechanism of action has not yet been defined. With all of these works, the differences between mice and humans must be carefully defined because β-cell turnover in mice is far higher than that in humans. Research on this topic remains active and promising.

It would be ideal if an intervention could be identified that could stimulate β-cell replication with no side effects just enough to restore that β-cell mass to normal. However, it is hard to imagine that such a safe-specific drug with definable activity will be developed in the near future. It should be more feasible to find a way to enhance replication of β cells from isolated islets in vitro, whereupon the cell product could be carefully characterized and then could be transplanted.

What Are The prospects for Significantly Increasing β Cell Mass by Stimulating Neogenesis Either In Vivo or In Vitro?

In Vivo Possibilities

Similar to the situation with replication, an effective in vivo treatment to effectively induce neogenesis will probably be very difficult to develop. However, a striking recent result found that in alloxan diabetic mice significant recovery of functional β-cell mass from terminally differentiated acinar cells could be induced by short-term growth factor therapy (epidermal growth factor and ciliary neurotrophic factor), thus providing potential avenues for drug development.[372] Another notable finding is that pancreatic exocrine cells can be reprogrammed to become β-like cells by injections into mouse pancreas of adenoviruses carrying 3 transcription factors, Ngn-3, Pdx-1, and MafA, which are important for β cell development and maintenance.[373]

In Vitro Possibilities

An increasing number of investigators are focusing on ways to generate β cells from exocrine cells in vitro. The first encouraging result was a demonstration in 2000 that cultured human duct cells covered with Matrigel could produce new islet cells,[374] a result that was confirmed shortly thereafter.[375] There continues to be a lot of work exploring the potential of converting pancreatic duct cells,[374,376] centroacinar cells,[377] or acinar cells[372] to new islet cells and even fully formed islets.

To obtain sufficient numbers of islet cells from exocrine cells, it will be necessary to expand the exocrine cells in vitro. One approach is to exploit natural branching morphogenesis to create organoids in tissue culture (481). Another is to expand exocrine or even islet cells in tissue culture through the process of epithelial-mesenchymal transition and then use various differentiation factors to produce islet cells that might be used for transplantation.[379]

Among recent examples of progress, the Clevers group found a way to activate duct cells to express Lgr5, a marker for adult stem cells.[380] These have a propensity to develop into organoids, which allows expansion, and then when transplanted with fetal pancreas into recipient mouse kidneys can form islet cells. The group of Kim[381] was able to expand purified CD133+ human duct cells into epithelial spheres and then reprogram them with adenoviruses carrying Ngn-3, Pdx-1, MafA, and Pax6 into human IPCs. A similar approach has been used by the group of Docherty.[376]

Islet Cell Plasticity

There has been considerable interest in the possibility that 1 type of islet cell can be converted to another. Leading the way, the Herrera group used lineage tracing to show that after severe β-cell ablation with diphtheria toxin, some of the residual α cells could assume a β-cell phenotype.[382] With genetic engineering, the group of Collombat showed that expression of Pax4 in α cells led to the conversion of these cells to β cells.[383] Another example comes from lineage tracing studies with diabetic *Foxo1* knockout mice, in which conversion for α cells to β cells was demonstrated.[357] A major question is whether such conversion happens naturally to any meaningful extent. A study using extreme ablation with streptozotocin found no evidence for such regeneration.[384] The more important question is whether this is a pathway that can be exploited for therapeutic purposes. Although challenging, there are enough α cells to hope that conversion and expansion might provide enough β cells to provide therapeutic benefits.

CONCLUSIONS

Much work is now being done to find a new source of β cells that can be used to replenish the β-cell deficit of diabetes. There is well-justified excitement about progress in converting hESCs and hiPSCs to β cells. However, there are also impressive advances in finding ways to exploit the regenerative potential of cells in the adult human pancreas. It is essential that these and other promising avenues be intensively evaluated.

Summary of Research Priorities

(1) Evidence is presented that there is a slow rate of β cell turnover in the human adult pancreas, occurring from replication of existing β cells and the birth of new β cells through neogenesis. The potential of exploiting this for clinical application is being explored by many laboratories.
(2) There is also a slow rate of β-cell death in the adult human pancreas occurring through the processes of apoptosis and necrosis.
(3) There is evidence that the rate of β cell death is increased in T2D, and the contributing mechanisms are thought to include endoplasmic reticulum stress, toxic amyloid oligomers, oxidative injury, and the ill-defined processes of overwork and glucose toxicity.

Report of Meeting Survey

The quest for a safe and efficacious form of β-cell replacement is at a unique juncture in its history with multiple therapies with either existing or potential application that will in the coming years compete for a place in the care of patients with T1D and potentially T2D. To gain a sense from the expert group, we assembled in Oxford as to their opinion of what the field of β-cell replacement would look like in the

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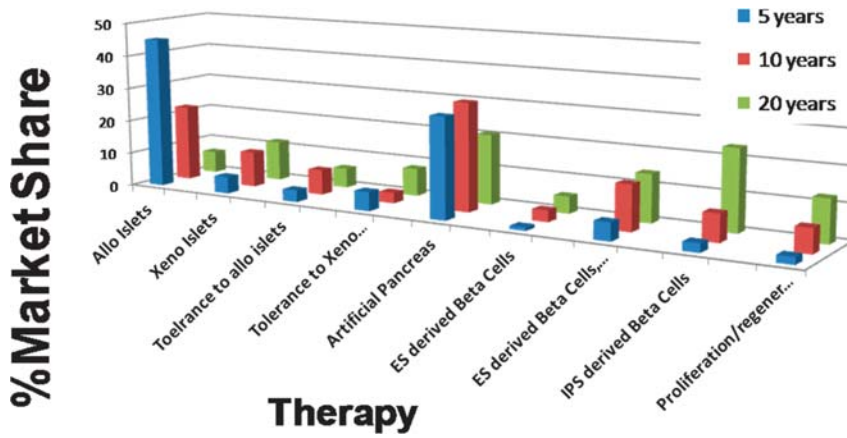

FIGURE 1. Expected changes in β cell replacement over time. before the conclusion of the conference, a web based survey was completed by the participants seeking their individual opinion as to what portion of the β-cell replacement market would be captured by the existing and potential therapies discussed at the meeting. For each of the time horizons of 5, 10, and 20 years, respondents allocated predicted market share between 9 potential therapeutic options. The graph shows the average market share awarded to specific therapies at 5, 10, and 20 years.

future, we conducted a survey on the final day of the meeting. The survey asked each participant to assign their predicted market share at 5, 10, and 20 years in the future of clinical β cell replacement activities to each of the therapies we discussed: allogeneic islets, xenogeneic islets (encapsulated or not), tolerance for allogeneic islets, tolerance for xenogeneic islets, AP, ES-derived β cells, encapsulated ES-derived β cells, IP-derived β cells, and β-cell regeneration from proliferation or neogenesis. For each timepoint, each survey participant assigned 100% of market share among these therapies.

The results in Figure 1 reveal that at 5 years hence, our panel believes that the market will be dominated by isolated allogeneic islets and the AP with anticipated minor contributions from IPs and ES-derived β cells, xeno islets, and tolerance. About 50% of respondents predicted no or negligible contributions from tolerance to xeno islets, endogenous islet regeneration/proliferation and IPs derived β cells. At 10 years, the most significant increase was in the predicted role of encapsulated ES-derived β cells and further expansion of reliance on AP. There were also minor increases in xeno islets, tolerance to allo islets, IP-derived β cells, and continued significant predicted activity in allo islets. By 20 years, hence, our experts predicted market domination by IP-derived β cells and AP with contraction of allo-islet activity and modest expansion of β cell proliferation/regeneration. The survey has obvious limitations including a small sample size and pool of respondents selected for meeting participation based on expertise in a given areas that likely carries with it an associated bias.

Summary

Biologic or biomechanical therapy capable of replacing the β cell mass has the potential to positively impact the health and well being of millions of people with insulin-dependent diabetes. Research in this area stands at a pivotal moment at which a number of viable strategies exist or are under development. Broad application depends on achieving both technical and financial feasibility. The ultimate goal of a "true cure," in which diabetic individuals achieve euglycemia with a single procedure associated with minimal risk, without long-term toxic drugs, and unfettered by external devices and/or frequent monitoring, appears to still be some years away. However, dramatic progress has been achieved toward the more proximate objectives of improved glycemic control and elimination of hypoglycemia and long-term vascular complications.

Long-term whole organ pancreas and isolated islet results have improved significantly over the last decade, with the latter now approaching the success of the former in insulin independence rates at the 5-year mark. It seems likely that allogeneic pancreas and islet transplantation will remain a treatment of choice for the foreseeable future in kidney recipients already obligated to lifelong immunosuppression until a more complete and permanent restoration of euglycemia is available. Nascent tolerance promoting protocols could aid in improving the risk-to-benefit balance for both islets and whole organ pancreas. With the present supply of transplantable pancreases used optimally, no more than 13% of the annual incident cases of T1D can be cured. In practical terms though, today, fewer than 5% of the annual incident cases are transplanted. The reality of the limited supply of deceased donor organs ultimately constrains the impact of islet and pancreas transplantation and compels researchers to press forward to develop broader strategies such as the AP, xenogeneic islets, and stem cell-derived β cells for which the supply will be limitless; in these areas, recent progress has been most impressive.

The AP continues to be refined with more sophisticated delivery algorithms, improved sensors and exploration of mobile device control. For xenogeneic islets, dramatic progress is evident in the long-term survival of porcine islets in primates using genetically modified donors and/or improved biologic immunosuppressants. Microencapsulation and macroencapsulation devices that exclude direct immunity by physical means may further aid in fostering xenogeneic islet graft survival but will likely find their primary place in the containment and protection of early versions of stem cell–derived allogeneic β cells. Deriving functional β cells from stem cells has experienced the most celebrated recent advances. Improved differentiation protocols that permit large scale/unlimited production of IPCs are now available, and although "normal" β-cell function has not yet been achieved, the ever quickening pace of progress suggests they are not far off. Importantly, this therapeutic modality will ultimately need to confront the likely requirement for a containment device

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and the need to be retransplanted periodically. These blemishes notwithstanding, the tremendous perceived potential of the approach for clinical application is evident in the huge venture capital investment that was rapidly garnered after the report of the most recent advance in embryonic stem cell differentiation into proper β cells. Consistent with the informal survey we conducted, iPS-derived β cells, which currently suffer from regulatory hurdles and the lack of a viable business model, and the seemingly more remote regeneration of native β cells, may offer the ultimate chance for a personalized true cure of insulin-dependent diabetes by avoidance of barrier devices and toxic immunosuppressive drugs.

The research agenda we have detailed is designed to facilitate full exploration of the potential of each proposed β-cell replacement solution so the optimal therapy is advanced as quickly as possible. Success in this endeavor will require broad and deep financial support from philanthropic (JDRF, Diabetes Research and Wellness Foundation, ADA, and so on) and public funding agencies worldwide; the investment needed is large but the potential reward will be profound. It is imperative that high impact, scientifically sound approaches are not overwhelmed by industry, private, or venture capital-supported priorities just because they hold a more lucrative near-term business model; scientific merit should dictate the course. The adherence of the historical funding agencies to traditional peer-reviewed methodology will be the incubator of novel approaches. This is a rapidly evolving landscape, and new data and novel ideas may radically divert the path forward. However, the diverse recent progress is tangible and undeniable, and the next decade is bound to witness a fascinating unfolding of competing solutions to cure insulin-dependent diabetes.

Our assessment of the data presented creates the opportunity for IPITA/TTS to endorse the following broad agenda for specific support by the peer-reviewed agencies.

(1) Allogeneic islet transplantation using novel strategies to facilitate engraftment, enhance graft longevity and ultimately gain immunosuppression-free survival in adult and pediatric patients.
(2) Xenogeneic islet-based approaches with and without encapsulation.
(3) Stem cell–based therapy of diabetes.
(4) Regeneration based therapy.
(5) Mobile device–based control of glucose sensing-insulin delivery: AP.

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