Islet Cell Xenotransplantation: A Serious Look Towards the Clinic

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Abstract

Type I diabetes remains a significant clinical problem in need of a reliable, generally applicable solution. Both whole organ pancreas and islet allotransplantation have been shown to grant patients insulin independence, but organ availability has restricted these procedures to an exceptionally small subset of the diabetic population. Porcine islet xenotransplantation has been pursued as a potential means of overcoming the limits of allotransplantation, and several preclinical studies have achieved near-physiologic function and yearlong survival in clinically relevant pig to primate model systems. These proof-of-concept studies have suggested that xenogeneic islets may be poised for use in clinical trials. In this commentary, we examine recent progress in islet xenotransplantation, with a critical eye towards the gaps between the current state of the art and the state required for appropriate clinical investigation.

Keywords

Introduction

The concept of islet transplantation, with its promise of insulin independence without the morbidity of whole organ pancreas transplantation, has attracted continuous attention for the past 40 years. Indeed, enthusiasm that this procedure would become reproducibly available to patients with type 1 diabetes gained substantially with advances reported by Shapiro, et al at the turn of this century.[1] Since 1999, nearly 900 patients have received islet transplants in North America, Europe, and Australia (www.citregistry.org, personal communication Jan 19, 2014). Through this experience, the therapeutic benefits of islet transplantation have been made evident in a proof-of-concept fashion, with gradual improvements in success over the past decade.[2] Nevertheless, significant barriers to broad implementation of islet allotransplantation remain. These include persistent difficulties in procuring reliable islet preparations, and graft loss due to alloimmune rejection.[3–5] However, organ availability

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remains the dominant shortcoming, eclipsing all other limitations. The numbers make this starkly evident.

Over 1.5 million patients have type I diabetes in the United States, and approximately 30,000 new cases are added yearly. In the past year in the United States, 1169 donor pancreases were recovered out of a potential 6871 deceased donors (OPTN data; Jan 17, 2014). Even if every donor pancreas were recovered and was therapeutic with a single pancreas infusion per recipient (which is not realistic based on current practice), there would still be far too few human organs to establish a broadly applicable treatment. Thus, the need for an alternative tissue source is particularly high in islet transplantation. By general consensus, this burden has fallen onto the stout shoulders of our porcine companions. Pigs mature rapidly, have numerous litters with many offspring, have organs relatively similar in size and physiologic capacity as humans, and produce insulin that is biologically active in humans,[6] thus making them ideal candidates as a tissue source. These physiologic compatibilities have led to the establishment of a pig to primate model as the species-specific pre-clinical testing ground to assess efficacy and safety, and it is in these models that we must look for reasonable estimates of therapeutic success before human trials can commence.

Progressive success in porcine to primate islet xenotransplantation[7–21] has mounted to the point that some have suggested that it is time to seriously consider taking porcine islet xenotransplantation into the clinic.[22–25] After primate studies, particularly those with yearlong survival, the next logical stop on the trajectory is typically early stage human trials. In this commentary, we review the current state of islet xenotransplantation with a focus on critical assessment of the practical hurdles impeding the translation of this approach into a clinical reality. We explicitly ask what is needed to justify asking a person to have pig tissue injected into them with a reasonable expectation of benefit.

Is there an applicable regimen?

Initial studies have clearly demonstrated that transplanted porcine islets can function and provide reasonable glucose control in primates.[7,26] Subsequent work has made it clear that hyperacute rejection, typical seen in primarily vascularized porcine xenografts, is not a barrier in the secondarily host-vascularized islet, making conventional immunosuppression relevant (Table 1). [27,28] Clinically applicable regimens of depletional antibodies, conventional transplant immunosuppressants (e.g. combinations of cyclosporine, tacrolimus, sirolimus, everolimus, and/or MMF), and costimulation blockade have yielded progressively improved xenograft survival and outcomes. However, the regimens generating the best results and leading to long-term survival worthy of clinical consideration, have nevertheless been more intense than those used for any form of routine allotransplantation. It is thus reasonable to assume that unmodified pig tissue will require immunosuppression that is at least, if not more, intensive as that required for allotransplantation.

Table 1 lists the immunosuppressive regimens that have been used in these preclinical studies. Most successful studies have used CD154-specific antibodies, agents that are unlikely to be made available for human use in the foreseeable future.[29,30] On the other side

of same the pathway, CD40-specific therapy has also shown efficacy in NHP islet xenotransplantation, albeit to a somewhat lesser degree.[19] Still, even though CD40-specific agents are in clinical trials, their availability for islet transplantation remains years off. Other approaches have been pursued with the expressed intent of eliminating a dependence on CD40-CD154 pathway interruption. The LFA-1-specific antibody efalizumab has been used in NHP allo- and xeno-islet transplantation with reasonable success.[20,31] Very promising clinical results with efalizumab have also been shown to have value in human islet allotransplantation, including successful single donor islet transplantation.[32,33] Sadly and despite these successes in the transplant community, efalizumab was withdrawn from the market as Progressive Multifocal Leukoencephalopathy (PML) became a concern during its primary application for psoriasis.[34] As many studies have demonstrated the clear benefit of these novel therapeutics, without their current availability we are left with essentially suboptimal immunosuppression for xenogeneic islet transplants.

In surveying the entirety of the NHP experience, it is difficult to designate the regimen that is truly ready to be tested. First, no regimen has been reported in which all the agents required are available for a trial. Second, it is not clear that all the drugs in these relatively concentrated regimens are truly synergistic and therefore necessary in combination for a discernable benefit to the xenograft. The NHP is logistically a very difficult setting to dissect out the necessary components of a multimodal regimen. Indeed, even with these agents, many of the NHP studies use potent 3–4 drug regimens that are highly prone to tipping the risk-benefit balance towards insulin dependence and away from an islet xenotransplant trial. Although expense and complexity make it understandable that rigorous studies have not truly defined the regimen of choice, these factors do not actually change the burden of proof required to put a human at risk. Finally, the bulk of the allotransplant literature has strongly suggested that we are on the edge of tolerability with existing multi-drug regimens. When we treated rhesus macaques with belatacept and sirolimus, achieving reasonable alloislet survival, the addition of LFA3-Ig (alefacept) had not improved survival but rather triggered a significantly increased CMV infection rate.[21] Additions to the typical three drug regimens used today in conventional solid organ transplantation generally have signatures of opportunistic infection and off-target side effects, indicating that highly complex regimens such as those being contemplated for islet xenotransplantation will almost certainly have significant morbidity. With even conventional immunosuppression limiting the indication for isolated whole organ pancreas transplantation, we must be highly suspect of proposing substantially more rigorous approaches to achieving xenograft tolerance.

Promising work in novel immune modulating agents has clearly demonstrated that we can improve islet xenotransplantation outcomes relative to historical experimental results, but there remains a critical need for continued drug regimen development if results are to equal or improve on those that have justified islet allotransplantation. Without a regimen of comparable morbidity and efficacy to those used in allotransplantation, it is hard to envision an appropriate choice for a clinical trial. The bar is not determined by what provides the best xenotransplant survival relative to other pre-clinical xenotransplant regimens; it is what meets the requirements for long-term regimen tolerability in patients with type 1 diabetes *relative to all the other available options* for that individual patient.

Is there an established donor islet source?

It is generally accepted that pigs can provide reliable and consistent tissue quality and on-demand availability for clinical use. In addition, the use of pig tissue might reasonably be expected to confer some resistance to recurrent autoimmune destruction in type I diabetics.[28] The rejection of porcine tissue xenografts might also proceed without alloantibody formation, a clear potential advantage over islet allotransplantation.[35] However, the most significant potential benefit of a xenotransplant is the opportunity for genetic modification. This has been suggested to be the primary advantage addressing the issue of over-immunosuppression discussed above, and reports of new genetic modifications are increasingly seen in the literature. Of these, has a candidate pig been identified as the cornerstone for clinical use?

Two immunologic epitopes identified as major barriers to porcine xenotransplantation are galactose-α1,3-galactose (Gal) and N-glycolylneuraminic acid (Neu5Gc), produced by the enzymes α1,3-galactosyltransferase and cytidine monophosphate-N-acetylneuraminic acid hydroxylase respectively.[36] With the advent of transgenic modification of pigs, knockouts of the enzymes responsible for these surface epitopes have been successfully created.[37–40] This has been a significant gain as experience in NHPs with Gal-knockout (Gal-KO) islets versus wild-type islets achieved improved engraftment rates.[18,41] Lutz et al. have elegantly demonstrated production of Gal and Neu5Gc deficient double knockout pigs using zinc finger nucleases, which elicit a significantly decreased in vitro antibody response compared to a Gal-KO standard.[40] Even with this progress, it is still recognized that the porcine glycome presents opportunities to generate more immunologically analogous porcine tissues.[42,43] In addition to knocking out antigenic epitopes, the expression of human proteins such as complement or coagulation regulatory factors may improve engraftment and survival.[17,44,45] New genetic manipulation technologies permit simultaneous gene knockout and integration,[46] as well as the expression of a multitude of genes in one step.[47]

Continued advances in porcine gene modification will inevitably lead to numerous xenograft phenotypes. Each new transgenic porcine tissue must be tested prior to human use with the same rigor as a novel pharmaceutical. These metrics should include confirming viability of the donor line, function of the transgene, and evidence that the gene provides a significant improvement in graft survival when compared to the standard. To date, although there have been many very interesting early glimpses at transgenic pig tissues in the islet arena, there has not been any rigorously controlled studies outside those related to expression of the Gal antigen that clearly demonstrate a salutary effect of a particular transgene.[11,18] Trials to date have involved very small numbers of animals, typically without contemporaneous controls, combined with highly complex regimens that are not truly optimized for clinical translation. Much more work is required. Additionally, there should be assurance that extensive genetic modification does not create unanticipated alterations in the biology of the engrafted cells, particularly as it relates to malignant transformation, before they are approved for use in patients. There will likely be some differences in physiology of porcine islets relative to human islets,[6] and some assurance should exist that genetically modified islets maintain function compatible with human physiology.

Objective measures are required to assess each transgene's real contribution to function and survival before clinical use can begin. Currently, we utilize a dual transplant model to examine short-term differences between distinct islet preparations in vivo.[48] This, we posit, will help choose genetic modifications worthy of the much more resource intensive NHP models assessing long-term survival and function of transgenic islets. It appears reasonably certain that Gal-KO pigs are preferred to wild type animals, and evidence is accumulating for combined Gal-KO/hCD46 transgenic pigs.[17,18] However, the "ideal" xenogeneic tissue source remains undefined. At present, in vivo evidence suggests that genetic modifications may at best only bring pig tissues into the realm of poorly matched allogeneic tissues. At this level, rejection will remain at least as problematic as is the case in allo-islet trials, and immunosuppressive morbidity will be similarly risky. Thus the question then becomes, who would be better suited to be in a xenograft trial over an allograft trial?

Are there eligible patients?

To examine the patients most likely to be appropriate for a xeno-islet trial, it is reasonable to assess the patients approved for allo-islet trials. These are patients in whom exogenous insulin is failing, and for whom the risks of immunosuppression have been sufficiently vetted. The Clinical Islet Transplantation (CIT – www.citisletstudy.org) Consortium is comprised of 13 centers and has served over the past decade as an organization dedicated to rigorously evaluating, accruing and following allo-islet recipients. As with any novel clinical therapy, the threshold for enrollment is very high to minimize patient variability and assess efficacy in a relatively controlled fashion. To develop a clinical trial for islet xenotransplantation, we must at least adhere to the same level of patient scrutiny set forth by allo-islet clinical trials. To begin, we can make two simple assumptions in comparing islet xenotransplantation to allotransplantation:

1. The benefits of xenogeneic islets will be, at best, equivalent to allogeneic islets from a functional standpoint, with most potential benefits related to availability.

2. The risks related to immunosuppression will, at best, be equivalent to allogeneic islet transplantation, with most data suggesting that the intensity of immunosuppression will be incrementally greater.

Enrollment into an experimental trial demands that patients are selected specifically for the potential therapeutic advantage set by the investigators with a clear understanding that the benefits outweigh the known, and potentially for unknown, risks *for the individual patient*. Islet allotransplantation has established a rigorous standard for application in the type I diabetic population, and a similar standard will need to be followed in islet xenotransplantation. Based on current patient selection criteria, the vast majority of patients with type I diabetes are not considered candidates for islet transplantation (Table 2). As of December 2013, the CIT Consortium enrolled 445 patients into their studies, 128 of whom have met criteria for an islet cell transplant after screening. Therefore, from an already limited number of patients with type I diabetes, investigators are tasked to find a suitable number who would qualify for a xenogeneic trial based on those rigorous standards, but simultaneously not be enrolled in or better served by an islet allotransplantation trial. Additionally, by the assumptions we have discussed, these patients would not benefit more

from an islet xenotransplant trial, except for an instance to forgo a prolonged wait for a donor organ, and would be subjected to potentially greater risk from an immunosuppressive standpoint. As such, justification for such a trial and the accrual of patients would be a challenge; this may be largely limited to candidates with substantial temporal needs (e.g. immediate threats to life without reversal of diabetes that cannot be achieved through exogenous insulin or an immediate islet or pancreas allograft).

The 2009 International Xenotransplant Association (IXA) consensus statement addresses this issue by focusing on patients with significant hypoglycemic unawareness.[23] In these patients where the life-threatening risk of hypoglycemic unawareness may be offset by an islet transplant, if an allotransplant is not readily available then they may be considered for a readily available xenotransplant. Another potential group for treatment proposed by the IXA uses the example set by islet after kidney transplantation; recipients of a kidney allograft could potentially undergo a concomitant xenogeneic islet transplant.[24,49] The basis for this justification is that patients will already be receiving immunosuppression and undergoing strict follow-up similar to any other transplant recipient. However this narrows the scope of treatable patients even further. Again in referring to the CIT experience thus far, of 72 patients enrolled in islet after kidney transplantation, only 24 were eligible for an islet transplant.

A particular setting that may favor islet xenotransplantation is when the kidney recipient has hypoglycemic unawareness *and* is broadly allosensitized.[49] However, as previously discussed, we still have not fulfilled the preclinical basis to maintain contemporary regimens for renal transplant immunosuppression and cross apply it to an islet xenograft with confidence. It has also been noted that these patients likely experience a greater impact from their comorbidities on the basis on them even needing β-cell and renal transplantation, and this may portend poorer outcomes in general and not provide a solid clinical testing model. The layering of an experimental therapy on top of a renal transplant may even put the allograft at risk if the xenograft or immunosuppression were to present unexpected problems, endangering the patient further. Even if we achieve some level of engraftment, we may only be sensitizing vulnerable patients to a potential long term cure via future porcine xenografts if we are not applying an ideal regimen for long term engraftment. The central purpose of an islet xenotransplant trial is to assess the possibility of a safe, effective, reliable treatment for type I diabetes. As we continue to discuss the standards and practices for such a trial, justification in patient enrollment should take the priority of safety over efficacy in order to establish the appropriate ethical foundation. The targeted treatment population should have the fewest variables that could contribute to a poor outcome, not only in terms of the trial, but more primarily for recipient health.

Are there other options with more favorable risk-benefit ratios?

In the development of any novel treatment, an understanding of the potential therapeutic benefit is always balanced by a similar understanding of the potential risks. The evidence is clear that physiologic islet replacement can be achieved with a porcine xenotransplant, yet understanding the aspects of porcine islet xenotransplantation that could potentially harm patients is paramount. Despite similar porcine and human insulin homology and the

regulated glucose control achieved in primate models, there is some discrepancy at the biochemical level via glucose sensitivity and subsequent insulin response by the islets themselves.[50,51] Thus remains the question of how well porcine islets will function when introduced to human physiology when the time comes, as our only comparison has been in primates.[6] Second, we have not yet determined the optimal donor age for porcine islet isolation to most closely parallel a human equivalent and provide the best outcomes in procurement, logistics, and function.[52] Third, zoonoses continue to remain at least a theoretical concern for xenotransplantation, and islets are not an exception.[53,54] The porcine endogenous retrovirus (PERV) has been the most extensively studied in this regard,[55–58] with in vitro and mouse models demonstrating infection across the species barrier.[59,60] However, research into viral mechanisms has already determined several treatment targets.[61–63] In the 1950s, contamination of Salk's polio vaccine production with the initially unnoticed simian virus 40 (SV40) unintentionally lead to the prevalence of human BK and JC viruses we see today.[64] This example of intended good leading to inadvertent harm is a lesson to heed when approaching xenotransplantation — what we don't know may still hurt us. Despite lacking in vivo evidence of PERV transmission into a human recipient, PERV or another infectious agent may still pose a threat to our investigating vulnerable immunosuppressed patients. In design of a clinical trial, these are all questions that we will need to approach our patients with, in order for them to fully appreciate the risks in their participation. Our NHP models have given us substantial evidence and insight into therapeutic benefits of porcine xenotransplantation, however the issues that may arise with pig-to-human islet xenotransplantation should also be scrutinized with the same rigor.

The risk-benefit assessment is not limited to a comparison with persistent diabetes or allotransplantation alone. Rather, it also requires an individual assessment of all available therapies, specifically the numerous biotechnologies designed for insulin replacement therapy that have emerged during the past decade, and for which patients being considered for islet xenotransplantation may also be eligible. The concept of the artificial pancreas has continued to evolve since the introduction of the insulin pump in the 1980s. With advanced continuous glucose monitoring and continual improvement of insulin dosing algorithms, these systems offer a significant improvement in glucose control without the morbidities of a transplant.[65,66] Cell scaffold biomaterials have also emerged as microenvironments for islets, providing oxygen and nutrients in the critical early stages of implantation. [67] Some of these materials can incorporate immune modulating agents or growth factors to improve survival and mitigate immune rejection.[68] Investigation into encapsulation technology has also continued; it remains attractive to create a shell around islets theoretically protecting them from immunologic factors with simultaneously allowing diffusion of necessities such as oxygen, nutrients, and smaller proteins.[69] Preclinical NHP studies implanting encapsulated islets have demonstrated long-term function even without immunosuppression.[70,71] Macroencapsulation of islets into hollow fiber scaffolds can be implanted intra- or extravascularly with the same benefits of immune isolation.[66] A recent report described an extravascular macroencapsulation chamber implanted without immunosuppression resulting in improved glycemic control and viable islets on explant after 10 months.[72] Although many of these technologies focus on human islet allotransplantation, the source for islets in these devices and systems may very well be the pigs we are working

with.[73] A New Zealand trial using encapsulated porcine islets has had promising initial results with forthcoming data highly anticipated.[74] None of these technologies are proven to date, but all are making headway with similar speed to xenotransplantation. For example, clinical trials for the totally artificial pancreas are underway (www.artificialpancreasproject.com).

These innovations hold similar potential for near-physiologic function with advantages that tissue transplantation, allo- or xeno-geneic, may not be able to provide. As the continuum of treatments expands, each modality should not be viewed as the therapeutic standard for a specific disease, but rather as a viable option after weighing the potential risks to an individual with objective justification for its benefits. Islet xenotransplantation is one of many treatments for type I diabetes transitioning to the clinic, and the as each modality contains a unique set of risks and benefits, rigorous evaluation of available options is necessary to determine the best outcome for individual patients.

Conclusion

The xenotransplantation of porcine islets into NHPs has demonstrated that porcine islets can render a primate insulin-independent. Islets from pigs can engraft, survive, and restore some level of glucose-sensitive insulin production. Although one might assume that the stage is now set to for a clinical trial, numerous substantial barriers remain when considering the reality of an ethically conducted clinical trial. As the field approaches the reality of translation, it must begin to view the current state of xenotransplantation not as it compares to previous experimental results (a comparison that is quite favorable), but rather to the exceptionally high bar set for all therapeutics seeking approval. The community's enthusiasm for this major step must be contained within the same reserve of reality facing any novel therapy. There are still risks, many defined and certainly some yet to be defined, of porcine xenotransplantation into humans. With this in mind, enthusiasm must not outweigh judgment, and investigators must acknowledge the substantial responsibility involved in asking a human to undergo experimentation. This includes real assessment of the alternative therapeutic options, with each given due consideration as a potential solution, and earnest appraisal of the risks of life long immunosuppression and exposure to xenogeneic tissues relative to dependency on exogenous insulin. Islet cells may still be the first "organ" to be transplanted across the species barrier for humans, but it should be done when the pieces are set: a defined regimen made up of available agents that has been shown to work; a defined donor source that has been shown to be modified based on controlled comparative studies; and a target patient population in which a xenogeneic islet is a better option than all the available alternatives.

Acknowledgments

Abbreviations

NHP	nonhuman primate
Gal	galactose-α1,3-galactose
Neu5Gc	N-glycolylneuraminic acid
Gal-KO	Gal-knockout
PERV	Porcine Endogenous Retrovirus
CIT	Clinical Islet Transplantation Consortium
IXA	International Xenotransplant Association
SV40	Simian Virus 40

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Table 1

Preclinical experience of porcine islet xenotransplantation in diabetic nonhuman primates

Immunosuppression that is not currently available for human use has been highlighted. All but one study with considerably prolonged survival (>100 days) use at least 4 agents.

Porcine donor	Recipient species	Transplant Site	Immunosuppression	Maximum graft survival	Reference
Adult	Baboon (n=3)	Intraportal	ATG +Cyclosporine+Aza	<2 days	Buhler et al.[7]
Adult	Rhesus (n=6)	Intraportal	None	>3 days	Kirchhof et al.[8]
Adult	Cyno (n=12)	Intraportal	Group I: anti-IL-2R, FTY720, everolimus, anti-CD154 Group II: anti-IL-2R, FTY720, everolimus, anti-CD154 Group III: anti-IL-2R, FTY720, everolimus, anti-CD154, leflunomide	45 days >187 days >158 days	Hering et al.[10]
Neonatal	Rhesus (n=9)	Intraportal	Group I: no immunosuppression Group II: anti-IL-2R, anti-CD154, belatacept, sirolimus	5 days > 260 days	Cardona et al.[9]
Adult	Cyno (n=3)	Intraportal	ATG, anti-CD154, MMF (Gal-KO/hCD46 transgenic)	>100 days	Ayares et al.[11]
Neonatal	Rhesus (n=6)	Intraportal	anti-IL-2R, anti-CD154, belatacept, sirolimus	>187 days	Russell et al.[14]
Neonatal	Rhesus (n=5)	Intraportal	anti-CD154, anti-CD25, belatacept, sirolimus	>76 days	Cardona et al.[12]
Adult	Cyno (n=10)	Intraportal	Group I (n=2): ATG, anti-CD20, tacrolimus, sirolimus Group II (n=4): ATG, anti-CD154, MMF, cobra venom factor Group III (n=1): ATG, anti-CD154, MMF, dextran sulfate	< 5 days > 58 days (partial) 3 days	Rood et al.[13]
Adult	Cyno (n=9)	Intraportal	Group III (n=1): ATG, anti-CD154, MMF, dextran sulfate	>60 days (partial)	Casu et al.[15]
Embryonic	Cyno (n=3)	Omentum	ATG, anti-IL-2R, anti-CD20, CTLA-4Ig, FTY720, everolimus	>393 days	Hecht et al.[16]
Adult	Cyno (n=9)	Intraportal	Group I (n=4): ATG, anti-CD154, MMF, dextran sulfate (wild-type pigs) Group II (n=5): ATG, anti-CD154, MMF, dextran sulfate (GKO/hCD46 transgenic)	<46 days >90 days	van der Windt et al.[17]
Neonatal	Rhesus (n=10)	Intraportal	Group I (n=5): anti-CD154, anti-LFA-1, CTLA4-Ig, MMF (wild-type pigs) Group II (n=5): anti-CD154, anti-LFA-1, CTLA4-Ig, MMF (GKO transgenic)	137 days 249 days	Thompson et al.[18]
Neonatal	Rhesus (n=9)	Intraportal	Group I (n=3): anti-IL-2R, anti-CD40, belatacept, sirolimus Group II (n=3): same as Group I with additional pre-tx dosing Group III (n=3): anti-IL-2R, belatacept, sirolimus	> 89 days > 203 days 71 days	Thompson et al.[19]
Neonatal	Rhesus (n=13)	Intraportal	Group I (n=3): anti-IL-2R, belatacept, anti-LFA-1, MMF Group II (n=5): anti-IL-2R, belatacept, anti-LFA-1, MMF, tacrolimus Group III (n=5): anti-IL-2R, belatacept, anti-LFA-1, LFA3-Ig, MMF	<5 days 99 days 114 days	Thompson et al.[20]

Table 2

Criteria for CIT enrollment

(From www.ClinicalTrials.gov, identifier number: NCT00434811)

Inclusion Criteria	Exclusion Criteria

Ages 18–65 years
Mentally stable and able to comply with study procedures
Clinical history compatible with type 1 diabetes with onset of disease at less than 40 years of age, insulin dependence for at least 5 years at study entry, and a sum of age and insulin dependent diabetes duration of at least 28
Absent stimulated C-peptide (less than 0.3 ng/ml) 60 and 90 minutes post-mixed-meal tolerance test
Involvement of intensive diabetes management, defined as:
 	
   a. Self-monitoring of glucose values no less than a mean of three times each day averaged over each week
 	
   b. Administration of three or more insulin injections each day or insulin pump therapy
 	
   c. Under the direction of an endocrinologist, diabetologist, or diabetes specialist with at least three clinical evaluations during the past 12 months prior to study enrollment
 	
At least one episode of severe hypoglycemia in the past 12 months, defined as an event with one of the following symptoms: memory loss; confusion; uncontrollable behavior; irrational behavior; unusual difficulty in awakening; suspected seizure; seizure; loss of consciousness; or visual symptoms, compatible with hypoglycemia in which the individual required assistance of another subject was unable to treat him/herself person and which was associated with either a blood glucose level less than 54 mg/dl or prompt recovery after oral carbohydrate, intravenous glucose, or glucagon administration in the 12 months prior to study enrollment
Reduced awareness of hypoglycemia. More information about this criterion, including specific definition of hypoglycemia unawareness, is in the protocol.
	Body mass index (BMI) greater than 30 kg/m2 or weight less than or equal to 50 kg
	Insulin requirement of more than 1.0 IU/kg/day or less than 15 U/day
	HbA1c greater than 10%
	Untreated proliferative diabetic retinopathy
	Systolic blood pressure higher than 160 mmHg or diastolic blood pressure higher than 100 mmHg
	Measured glomerular filtration rate using iohexol of less than 80 ml/min/1.73mm2. More information about this criterion is in the protocol.
	Presence or history of macroalbuminuria (greater than 300 mg/g creatinine)
	Presence or history of panel-reactive anti-HLA antibody levels greater than background by flow cytometry. More information about this criterion is in the protocol.
	Pregnant, breastfeeding, or unwilling to use effective contraception throughout the study and 4 months after study completion
	Presence or history of active infection, including hepatitis B, hepatitis C, HIV, or tuberculosis.
	Negative for Epstein-Barr virus by IgG determination
	Invasive aspergillus, histoplasmosis, or coccidioidomycosis infection in the past year
	History of malignancy except for completely resected squamous or basal cell carcinoma of the skin
	Known active alcohol or substance abuse
	Baseline Hgb below the lower limits of normal, lymphopenia, neutropenia, or thrombocytopenia
	History of Factor V deficiency
	Any coagulopathy or medical condition requiring long-term anticoagulant therapy after transplantation or individuals with an INR greater than 1.5
	Severe coexisting cardiac disease, characterized by any one of the following conditions:
	
	   a. Heart attack within the last 6 months
	
	   b. Evidence of ischemia on functional heart exam within the year prior to study entry
	
	   c. Left ventricular ejection fraction less than 30%
	
	Persistent elevation of liver function tests at the time of study entry
	Symptomatic cholecystolithiasis
	Acute or chronic pancreatitis
	Symptomatic peptic ulcer disease
	Severe unremitting diarrhea, vomiting, or other gastrointestinal disorders that could interfere with the ability to absorb oral medications
	Hyperlipidemia despite medical therapy, defined as fasting LDL cholesterol greater than 130 mg/dl (treated or untreated) and/or fasting triglycerides greater than 200 mg/dl
	Currently receiving treatment for a medical condition that requires chronic use of systemic steroids except for the use of 5 mg or less of prednisone daily, or an equivalent dose of hydrocortisone, for physiological replacement only
	Treatment with any antidiabetic medication other than insulin within the past 4 weeks
	Use of any study medications within the past 4 weeks
	Received a live attenuated vaccine(s) within the past 2 months

Inclusion Criteria	Exclusion Criteria
	Any medical condition that, in the opinion of the investigator, might interfere with safe participation in the trial
	Treatment with any immunosuppressive regimen at the time of enrollment.
	A previous islet transplant.
	A previous pancreas transplant, unless the graft failed within the first week due to thrombosis, followed by pancreatectomy and the transplant occurred more than 6 months prior to enrollment.