Xenotransplantation 2016: 23: 3–13
doi: 10.1111/xen.12231

Original Article

First update of the International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes—Executive summary

Hering BJ, Cozzi E, Spizzo T, Cowan PJ, Rayat GR, Cooper DKC, Denner J. First update of the International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes—Executive summary. Xenotransplantation 2016: 23: 3–13. © 2016 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd.

Abstract: The International Xenotransplantation Association has updated its original "Consensus Statement on Conditions for Undertaking Clinical Trials of Porcine Islet Products in Type 1 Diabetes," which was published in Xenotransplantation in 2009. This update is timely and important in light of scientific progress and changes in the regulatory framework pertinent to islet xenotransplantation. Except for the chapter on "informed consent," which has remained relevant in its 2009 version, all other chapters included in the initial consensus statement have been revised for inclusion in this update. These chapters will not provide complete revisions of the original chapters; rather, they restate the key points made in 2009, emphasize new and under-appreciated topics not fully addressed in 2009, suggest relevant revisions, and communicate opinions that complement the consensus opinion. Chapter 1 provides an update on national regulatory frameworks addressing xenotransplantation. Chapter 2 a, previously Chapter 2, suggests several important revisions regarding the generation of suitable source pigs from the perspective of the prevention of xenozoonoses. The newly added Chapter 2b discusses conditions for the use of genetically modified source pigs in clinical islet xenotransplantation. Chapter 3 reviews porcine islet product manufacturing and release testing. Chapter 4 revisits the critically important topic of preclinical efficacy and safety data required to justify a clinical trial. The main achievements in the field of transmission of all porcine microorganisms, the rationale for more proportionate recipient monitoring, and response plans are reviewed in Chapter 5. Patient selection criteria and circumstances where trials of islet xenotransplantation would be both medically and ethically justified are examined in Chapter 6 in the context of recent advances in available and emerging alternative therapies for serious and potentially life-threatening complications of diabetes. It is hoped that this first update of the International Xenotransplantation Association porcine islet transplant consensus statement will assist the islet xenotransplant scientific community, sponsors, regulators, and other stakeholders actively involved in the clinical translation of islet xenotransplantation.

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Key words: porcine islets of Langerhans – type 1 diabetes – xenotransplantation

Abbreviations: cGMP, current Good Manufacturing Practices; CRISPR/Cas, clustered regularly interspaced short palindromic repeat/CRISPR-associated system; DPF, designated pathogen free; EMA, European Medicines Agency; FDA, Food and Drug Administration; GM, genetically modified; IE, islet equivalent; IXA, International Xenotransplantation Association; PERV, porcine endogenous retrovirus; NHP, non-human primate; TSE, transmissible spongiform encephalopathy; SAL, sterility assurance level; SACX, Secretary's Advisory Committee on Xenotransplantation; T1D, type 1 diabetes; TTS, The Transplantation Society; U.S., United States; WHA, World Health Assembly; WHO, World Health Organization.

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Received 16 February 2016;
Accepted 16 February 2016

Introduction

The International Xenotransplantation Association (IXA) published its original "Consensus Statement on Conditions for Undertaking Clinical Trials of Porcine Islet Products in Type 1 Diabetes" in Xenotransplantation in 2009 [1–8]. To remain relevant, it was intended to update this initial consensus statement in light of changes in the regulatory framework, progress in research, and comments and perspectives communicated by stakeholders active in the field.

To provide a forum for an in-depth discussion aimed at providing the underpinning of the first update of the initial consensus statement, IXA convened a full-day conference in San Francisco, CA, on August 1, 2014. This conference was open to all members of IXA and was attended by an international multidisciplinary panel of scientists active in islet xenotransplantation and related fields. To those members not able to attend in person, an Adobe Connect line was provided to allow active participation in the conference. The recordings of the conference were made available online to IXA members after the meeting.

This first update of the IXA consensus statement, published in this issue of xenotransplantation, is largely based on the discussion that took place at the above-referenced conference; also considered were the viewpoints communicated in scholarly review articles published on clinical translation of islet xenotransplantation since 2009 [9–13]. Included in this first update of the IXA consensus statement are this executive summary and seven chapters [14–20]. Except for the chapter on "informed consent," which has remained relevant in its 2009 version [8], all other chapters included in the initial consensus statement have been revised for inclusion in this update. These chapters are not to be viewed as complete revisions of the initial chapters; rather they restate the salient points made in 2009, highlight new and under-appreciated topics not fully addressed in 2009, suggest pertinent revisions, communicate opinions that complement the consensus opinion, and provide advice and information to those active and involved in clinical translation of islet xenotransplantation. Because many of the points made in the 2009 consensus statement remain valid, the reader is encouraged to study the chapters included in this issue in conjunction with the original chapters. The chapter on "Genetically Modified (GM) Source Pigs" has been added as a new chapter in view of the increasing significance of such source pigs in islet xenotransplantation. The following paragraphs summarize the significant points made in the six updated chapters and in the one newly added chapter and restate the key points made on informed consent in xenotransplantation trials as presented in the 2009 Executive Summary.

Chapter 1: Update on national regulatory frameworks pertinent to clinical islet xenotransplantation [14]

Considerable progress has been made in developing and implementing regulations in several countries to empower national health authorities to effectively regulate xenotransplantation trials and thereby ban unregulated procedures.

1. The comprehensive guidelines for conducting xenotransplantation clinical trials established in the United States (US) since 1993 by the Food and Drug Administration (FDA) collaboratively with other agencies within the US Federal Government and with other national and international governing bodies addresses three fundamental goals: (i) to provide a comprehensive approach for the regulation of xenotransplantation, (ii) to address potential public health safety issues associated with xenotransplantation, and (iii) to provide guidance to sponsors, manufacturers, and investigators regarding xenotransplantation product safety and clinical trial design monitoring [9]. In 2010, the FDA reviewed the existing regulatory framework within the United States that would be applied to the regulation of clinical trials utilizing xenogeneic porcine pancreatic islets to treat type 1 diabetes (T1D) and outlined the general review principles with respect to the infectious disease status of the donor pigs, manufacturing and final product testing of islets, preclinical testing in animal models, and finally the design of the clinical trial [9].
2. Recognizing the global concerns over the conduct of uncontrolled and unregulated xenotransplantation practices, the World Health Organization (WHO) urged its member states in its World Health Assembly Resolution WHA57.18 to "allow xenogeneic transplantation only when effective national regulatory control and surveillance mechanisms overseen by national health authorities are in place" [21]. Subsequently, the WHO convened WHO Global Consultations on Regulatory Requirements for Xenotransplantation Clinical Trials in Changsha, China, in 2008, and in Geneva, Switzerland, in 2011. WHA57.18 as well as the WHO Global Consultations emphasize the

importance of international collaboration to prevent unregulated xenotransplantation and to coordinate xenotransplantation vigilance, surveillance, and response to suspected infections. The recommendations for the roles and responsibilities of the WHO, member states, and investigators of proposed xenotransplantation clinical trials, as outlined in the Changsha Communiqué [22], were reviewed in the initial IXA consensus statement [2]. The more recent "Geneva Consultation" recommended to the WHO (i) to create a collaborative group of public/academic xeno-related infectious disease reference laboratories and appropriate health authorities' resources to support assay development, validation, standardization, and sample throughput; (ii) to encourage transparency in the development of national policies and procedures and in the conduct of any xenotransplantation trial to ensure harmonized practices and level of safety; and (iii) to convene regular global consultations between regulators and xenotransplantation subject matter experts on xenotransplantation activities [23]. In addition, the "Geneva Consultation" recommended to member states, investigators, proposers, or study sponsors to (i) seek global consistency in requirements for clinical trials by referring to best global standards and experts' advice especially in areas such as source donor animal, recipients, family members, and close contacts surveillance; risk/benefit analysis and trial infrastructure; (ii) to combat unfounded assertions on human xenotransplantation; and (iii) to assure access to independent (third-party) reference laboratories with identified expertise in xeno-specific infectious disease assays [23].

3. Several countries have embraced the suggestion of the WHO to harmonize xenotransplantation-related oversight and procedures on a more global scale [14]. Important changes of the regulatory framework pertinent to xenotransplantation have taken place or are in progress in several geographic areas that include Europe, Korea, Japan, and China. These changes encompass the most diverse facets of the clinical application of xenotransplantation and comprise ethical aspects, source animals, product specifications, study oversight, sample archiving, patient follow-up, and extent to insurance coverage in some legislations.

Chapter 2a: Source pigs—preventing xenozoonoses [15]

The original consensus statement set a reasonable bar at its time for the activities related to source pigs used in the preparation of clinical porcine islet products and still serves as an excellent platform from which to proceed, given interim progress in the field [3]. A summary of salient revisions to the original consensus statement is as follows:

1. Donor animal pathogen screening strategy should be geographically appropriate, product specific, adaptive, and dynamic.
2. As new rapid diagnostic technologies are developed and validated, they may enable the direct screening of islet products themselves.
3. Encapsulated islet products present different risk profiles than non-encapsulated islets primarily due to the lack of recipient immunosuppression. Some encapsulation methods enable *in vitro* islet culture of sufficient duration to perform viral screening on islet products prior to transplantation.
4. While porcine endogenous retrovirus (PERV)-C negative donor animals could be considered preferable, PERV animal selection criteria should be primarily based on low PERV expression levels and lack of infectivity.
5. Biosecure designated pathogen-free (DPF) animal facilities built to agricultural standards could be considered as appropriate source animal facilities if operated under standard operating procedures (SOPs) and current Good Manufacturing Practices (cGMPs).
6. The elimination of bovine products from the feed of donor animals throughout their lifetime should sufficiently mitigate the transmissible spongiform encephalopathy (TSE) risk.
7. The Sponsor's responsibility to archive donor samples should be for a limited duration and transferred to the appropriate regulatory government agency if additional duration is required.

Chapter 2b: Genetically modified source pigs [16]

Chapter 2 of the first IXA porcine islet transplant consensus statement focused on the conditions required for source pigs to fulfill DPF status [3]. However, the scope of the initial document did not extend to the use of GM pigs as donors. Because of the increasing significance of GM pigs in islet xenotransplantation [24–27], it was imperative to

include this dedicated new chapter in the updated consensus statement.

1. Genetic modification of the source pig offers the opportunity to improve the engraftment and survival of islet xenografts. The type of modification can be tailored to the transplant setting; for example, intraportal islet xenografts have been shown to benefit from the expression of anticoagulant and anti-inflammatory transgenes, whereas cytoprotective transgenes are probably more relevant for encapsulated islets.
2. The rapid development of pig genetic engineering, particularly with the introduction of genome editing techniques such as clustered regularly interspaced short palindromic repeat/CRISPR-associated system (CRISPR-Cas) [28–30], has accelerated the generation of new pig lines with multiple modifications. With preclinical testing in progress, it is an opportune time to consider any implications of genetic modification for the conditions for undertaking clinical trials.
3. Obviously, the stringent requirements to fulfill DPF status that are applied to wild-type pigs will apply equally to GM source pigs.
4. In addition, it is important from a safety perspective that the genetic modifications are characterized at the molecular level (e.g., integration site, absence of off-target mutations), the phenotypic level (e.g. durability and stability of transgene expression), and the functional level (e.g. protection of islets *in vitro* or *in vivo*, absence of detrimental effects on insulin secretion) [31].
5. The assessment of clinical trial protocols using GM pig islets will need to be done on a case-by-case basis, taking into account a range of factors including the particular genetic modification(s) and the site and method of delivery.

Chapter 3: Porcine islet product manufacturing and release testing criteria [17]

As in the first IXA porcine islet xenotransplant consensus statement [4], the pig islet product manufacturing quality and control requirements outlined here are based on the US regulatory framework where these products fall within the definition of somatic cell therapy [32,33] under the statutory authority of the US FDA. In addition, porcine islet products require pre-market approval as a biologic product under the Public Health Services Act. Pig islet products also meet the definition of a drug under the Federal Food, Drug, and Cosmetic Act and are subject to applicable provisions of that law [34]. As with other somatic cell therapies and human islet products [35–37], the following criteria must be met for pig islet products before proceeding to clinical trials:

1. To facilitate control of manufacturing as well as reproducibility and consistency of product lots, the same general principles of cGMP that apply to human pharmaceuticals also apply to xenotransplantation products [9]. Data must be provided to demonstrate that islet products can be consistently prepared that would meet basic lot release requirements.
2. Procuring pancreata from a closed herd of pigs in an operating room located within the source animal facility and following SOPs for organ procurement, preservation, and processing will assert considerable control over manufacturing. Similarly, if the final product is to be transported from the site of manufacturing to a distant clinical site, documentation is needed to show that under the proposed shipping conditions the islet products remain sterile, viable, and potent.
3. To facilitate product safety, (i) materials used in the manufacturing process, including the pig pancreas, must be free of adventitious agents; (ii) islets must be manufactured using aseptic processing; and (iii) the final product must undergo tests for sterility, mycoplasma (if cultured), and endotoxin. Safety specifications for pig islet product release include a negative Gram stain and an endotoxin content of < 5.0 EU/kg recipient body weight. Product post-release assessments must include sterility cultures on the final product. Because results for sterility are available only retrospectively, a plan of action must be in place for patient notification and treatment in case the sterility culture results are positive for contamination.
4. Product characterization information should be acquired from a sample of the final product to be used for transplantation and must address important aspects of lot release testing [35] such as identity/purity (cell composition), quantity (islet equivalents [IE], cell number), and potency (insulin secretory capacity, oxygen consumption rate corrected for DNA or transplant bioassay in immunoincompetent diabetic mice) of the product; it also provides critical information to demonstrate manufacturing control and product consistency across multiple islet preparations (lots).

5. Providing islet products containing an islet mass sufficient to restore euglycemia in trial participants (≥10 000 IE/kg) will require pooling of islets from multiple donor pancreata (≥2 to 4 from adult donors and ≥7 to 10 from neonatal donors). Demonstration of product consistency across products from individual pancreata would warrant release testing to be performed on a sample of the pooled product.
6. As product development and clinical trials advance, the increasingly more detailed specifications of potency assays on adult porcine islet products are expected to be predictive of post-transplant glycemic control. The immaturity of fetal and neonatal porcine islet tissue precludes the use of *in vitro* insulin secretion as a potency test as part of lot release testing unless demonstrated otherwise; another measure of potency appropriate to fetal and neonatal cells will need to be developed for product release testing and evaluation of aliquots of these products in mouse transplant bioassays should be performed to provide meaningful post-release information.
7. Several additional issues must be addressed when utilizing encapsulated xenogeneic islets for human transplantation [9]. All inert substances used in the encapsulation process should either be pharmacopeial grade, or meet rigorous pre-determined analytical specifications. All critical process steps should be validated to establish the consistency and reproducibility of the islet encapsulation process. Information on the base biomaterial such as the source, molecular weight and molecular weight distribution/polydispersity, relative compositions of the subunits (for copolymers such as alginate), purity, method of sterilization, and the sterility assurance level (SAL) should be provided [9,38]. Furthermore, information on the properties of the formed capsule, such as size, thickness, homogeneity, porosity, permeability, stability, and long-term durability, will need to be included [9]. Following encapsulation, a similar battery of tests to those listed in the previous section is necessary to confirm that this process has not adversely affected the viability, metabolic activity, or *in vitro* insulin secretory capacity of the islets [9,39]. Microscopic tests to determine capsule size, uniformity, and integrity are used to confirm that the encapsulated system has the physical properties required for free diffusion of lower MW components to and from the capsule while providing a sufficient barrier to immunological response. The assessment of the encapsulated islet product must also determine the number of islets within a capsule, the proportions of empty capsules and of unencapsulated cells, the bioreactivity and biocompatibility of the combined islet product and the device components. Specific defects may include the presence of an islet in the wall and a ruptured or distorted capsule. Assessment of the biological activity of the combined product is often a component of preclinical safety evaluations. It is recommended that studies should evaluate the duration and predictability of the device used in the combination product so that porcine islets contained in the device may be replaced at appropriate intervals to maintain life-supporting pharmacologic or metabolic activity.

Chapter 4: Preclinical efficacy and complication data required to justify a clinical trial [18]

The first IXA porcine islet xenotransplant consensus statement included IXA's opinion on what constituted "rigorous preclinical studies using the most relevant animal models" and was based on "non-human primate (NHP) testing" [5]. After careful consideration, it is believed there is no need to greatly modify the conclusions and recommendations of the original consensus document.

1. Preclinical studies should be sufficiently rigorous to provide optimism that a clinical trial is likely to be safe and has a realistic chance of success, but need not be so demanding that success might only be achieved by very prolonged experimentation, as this would not be in the interests of patients whose quality of life might benefit immensely from a successful islet xenotransplant.
2. When "free" islets are being transplanted and immunosuppressive therapy will be necessary, it is not unreasonable to expect the investigators to demonstrate in the pig-to-NHP model that insulin independence—or, at least, a greatly reduced insulin requirement—can be achieved and maintained for several weeks or months in a small number of experiments. A successful result should be achieved with a clinically tolerable immunosuppressive regimen. At the end of the period of follow-up, therefore, there should be evidence of functioning islets in the relative absence of complications from the immuno-

suppressive regimen, for example, infection and malignancy.

3. While hesitant to provide definitive guidelines on the exact number of experiments in NHPs that is believed to be necessary to justify advancing to a clinical trial, the majority opinion is that successful reversal of diabetes in 4 of 6 (or 5 of 8) consecutive experiments would be sufficient to indicate potential success of a clinical trial. However, there was a significant minority opinion that the number of experiments required should not be generalized, but rather determined by the investigators themselves with regard to their research objectives, possibly after discussion with the relevant regulatory authorities. A majority of those consulted indicated that a minimum follow-up of 6 months is essential, with, ideally, follow-up for 12 months in one or more cases, and that any graft failure that occurs during these periods of time should not be a result of graft rejection.
4. If the patient who will receive the pig islet xenograft is already receiving immunosuppression for a kidney allograft, there is little additional risk associated with the xenotransplant. However, to suggest a potential benefit to the patient, it should be demonstrated that the immunosuppressive regimen used to prevent kidney allograft rejection is also likely to be effective in preventing islet xenograft rejection.
5. If "encapsulated" islets are to be transplanted without immunosuppression, then arguments for insisting on studies in NHPs are reduced. Nevertheless, the majority of those consulted believe that studies in NHPs are essential if the efficacy of islet xenotransplantation is to be proven. If any form of pharmacologic immunosuppressive therapy is found to be necessary, for example, if the capsules do not provide complete immuno-isolation, then studies in NHPs to exclude significant complications from this therapy are considered mandatory. If studies in NHPs are deemed necessary, the same (or similar) criteria regarding the number of experiments in NHPs and the length of follow-up should be followed as outlined above for the transplantation of "free" porcine islets. However, a shorter length of follow-up, for example 3 months rather than 6 months, was suggested by some of those consulted to be adequate when encapsulated islets are being tested, particularly when exchangeable devices would allow replenishment of islets.
6. Although it is believed that investigators should err on the side of caution, some flexibility in these guidelines is necessary if clinical trials of pig islet transplantation are not going to be unduly delayed.

Chapter 5: Recipient monitoring and response plan for preventing disease transmission [19]

Xenotransplantation of porcine cells, tissues, and organs may be associated with the transmission of porcine microorganisms to the human recipient. The corresponding chapter of the initial IXA porcine islet consensus statement [6] focused on strategies to prevent transmission of PERVs. The updated chapter summarizes the main achievements in the field since 2009 and addresses potential transmission of all porcine microorganisms including monitoring of the recipient and provides suggested approaches to the monitoring and prevention of disease transmission [19].

1. Prior analyses assumed that most microorganisms other than the endogenous retroviruses could be eliminated from donor animals under appropriate conditions which have been called DPF source animal production. PERVs, integrated as proviruses in the genome of all pigs, cannot be eliminated in that manner and represent a unique risk.
2. Certain microorganisms are by nature difficult to eliminate even under DPF conditions; any such clinically relevant microorganisms should be included in pig screening programs.
3. With the use of porcine islets in clinical trials, special consideration has to be given to the presence of microorganisms in the porcine islet xenotransplantation products to be used and also to the potential use of encapsulation.
4. It is proposed that microorganisms absent in the donor animals by sensitive microbiological examination do not need to be monitored in the transplant recipient; this will reduce costs and screening requirements.
5. Valid detection assays for donor-derived microorganisms and those introduced during manufacturing must be established. Special consideration is needed to preempt potential unknown pathogens which may pose a risk to the recipient.
6. Although the clinical application of porcine islet products will require a comprehensive plan for the testing and archiving of donor and recipient tissues, the absence to date of reported *in vivo* transmission gives confidence that, with the appropriate safeguards

in place, well-planned pilot clinical trials could be safely undertaken.

Chapter 6: Patient selection for pilot clinical trials of islet xenotransplantation [20]

A central element of the design of any clinical trial, especially of xenotransplantation and also of cellular and gene therapy early-phase trials, is the definition of the study population. The aim is to select a trial population with a favorable benefit-risk ratio, while protecting the public from undue risks and also achieving the study's scientific objectives [40–43].

The 2003 U.S. FDA "Guidance For Industry on Source Animal, Product, Preclinical, and Clinical Issues Concerning the Use of Xenotransplantation Products in Humans" and the 2007 Health Research Council of New Zealand Gene Technology Advisory Committee "Guidelines for Preparation of Applications Involving Clinical Trials of Xenotransplantation in New Zealand" stipulate that, "because of the potentially serious public health risks of possible zoonotic infections, xenotransplantation should be limited to patients who (i) have serious or life-threatening diseases for whom adequately safe and effective alternative therapies are not available except when very high assurance of safety can be demonstrated, (ii) have potential for a clinically significant improvement with increased quality of life following the procedure, and (iii) are able to comply with public health measures as stated in the protocol, including long-term monitoring" [40,42]. The 2009 European Medicines Agency (EMA) Guideline on "xenogeneic cell-based medicinal products" similarly states that "the clinical development of xenogeneic cell-based products should involve initially patients with serious or life-threatening disease for whom adequately safe and effective alternative therapies are not available, or where there is a potential for a clinically relevant benefit" [41].

To identify, within this regulatory framework, suitable patient populations for early-phase clinical trials of xenogeneic islet cell products in diabetes, the following points should be considered:

1. Patients in whom type 1 diabetes (T1D) is complicated by impaired awareness of hypoglycemia and recurrent episodes of severe hypoglycemia are candidates for islet or pancreas transplantation if severe hypoglycemia persists after completion of a structured stepped care approach or a formalized medical optimization run-in period that provide access to hypoglycemia-specific education including behavioral therapies, insulin analogs, and diabetes technologies under the close supervision of a specialist hypoglycemia service.
2. Patients with T1D and end-stage renal failure who cannot meet clinically appropriate glycemic goals or continue to experience severe hypoglycemia after completion of a formalized medical optimization program under the guidance of an expert diabetes care team are candidates for islet or pancreas transplantation either simultaneously with or after a previous kidney transplant.
3. Similarly, patients with type 2 diabetes and problematic hypoglycemia or renal failure who meet these criteria are considered candidates for islet replacement.
4. Likewise, patients with pancreatectomy-induced diabetes in whom an islet autograft was not available or deemed inappropriate are candidates for islet or pancreas transplantation if extreme glycemic lability persists despite best medical therapy.
5. To justify participation of these transplant candidates in early-phase trials of porcine islet cell products, lack of timely access to islet or pancreas allotransplantation due to allosensitization, high islet dose requirements, or other factors, or alternatively, a more favorable benefit-risk determination associated with the xenoislet than the alloislet or allopancreas transplant must be demonstrated.
6. Additionally, in non-uremic xenoislet recipients, the risks associated with diabetes must be perceived to be more serious than the risks associated with the xenoislet product and the rejection prophylaxis, and in xenoislet recipients with renal failure, the xenoislet product and immunosuppression must not impact negatively on renal transplant outcomes.
7. The most appropriate patient group for islet xenotransplantation trials will be defined by the specific characteristics of each investigational xenoislet product and related technologies applied for preventing rejection. Selecting recipients who are more likely to experience prolonged benefits associated with the islet xenograft will help these patients comply with lifelong monitoring and other public health measures.

Chapter 7 of the first IXA porcine islet xenotransplant consensus statement: Informed consent and xenotransplantation clinical trials [8]

This chapter has not been updated as all the points made in the 2009 consensus statement on informed consent in xenotransplantation clinical trials have

remained relevant [8]. To include the discussion on this topic in the first update of the IXA porcine islet xenotransplant consensus statement, the key points on informed consent as provided in the 2009 Executive Summary [1] are repeated below.

In international and national codes and guidelines involving human subject research and in the laws of many nations, the informed consent of research subjects is obligatory. The moral foundations of informed consent include and also extend beyond respect for individual persons as autonomous agents in Western nations. Axioms regarding the value of human life and duties to protect innocent and vulnerable persons from harm, duress, and deceit underlie Western individualism and are broadly shared in many non-Western cultures. Accents on family and/or community consent in China and other nations are compatible with individual consent, as long as family and community consent supplement, rather than replace, individual consent.

Favorable harm-benefit determinations precede considerations of informed consent. When these harm-benefit assessments are favorable enough to warrant the onset of clinical trials, voluntary or freely given informed consent emerges as a pivotal moral precondition for these trials.

1. Xenotransplantation clinical trials involve a complex body of medical information, several procedures, numerous risks (associated with failure rates, immunosuppression, xenogeneic infections, and so forth), and the subject's obligation to abide by extensive national and international precautionary guidelines. In obtaining informed consent, the following criteria must be ensured: Informed consent should be enacted preferably through an informed consent team as an organized, sequential, thoughtfully paced, jargon-free process of communication.
2. The consenting process must cover a large number of topics, including treatment choices, participation information, study procedures, information about risks associated with immunosuppression, discomforts and other matters, xenogeneic infections of recipients (and possibly close contacts and the community) and, due to infectious risks, the following 10 post-protocol subject responsibilities: (i) regular post-clinical research checkups, (ii) informing researchers of future changes of address/contact numbers, (iii) timely reporting of all unexplained illnesses, (iv) following present and updated behavioral guidelines with respect to exchanges of body fluids with intimate contacts, (v) no future donations of blood, sperm or other body fluids or tissues, (vi) autopsy at time of death, (vii) education of family members and intimate contacts about their need to take precautions associated with infectious disease risks—that includes offered educational assistance from the research team, (viii) disclosure to future healthcare providers that subjects have received a xenotransplantation product, (ix) willingness to accept possible isolation and possible quarantine if necessary for public health, and (x) arrangements for assistance in meeting future responsibilities should the subject lose decision-making capacity.
3. Due to the unknown infectious risks, subjects must be informed that, while they may withdraw from the medical interventions of the protocol, they must abide by their post-protocol responsibilities as stated here.

The Secretary's Advisory Committee on Xenotransplantation (SACX) of the US Department of Health and Human Services has produced a draft document on informed consent containing a complete and understandable exemplary consent document for clinical research in xenotransplantation [28].

Conclusion

This "First update of the IXA consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes" has been prepared by an international multidisciplinary panel of investigators with a long-standing involvement in islet xenotransplantation to assist the islet xenotransplant scientific community, sponsors, regulators, and other stakeholders in the clinical translation of islet xenotransplantation.

In light of the substantial progress made since the preparation of the initial consensus statement in 2009, all chapters except for Chapter 7 have been extensively updated. The advancements in developing and implementing regulations in several countries to empower national health authorities to effectively regulate xenotransplantation trials and ban unregulated xenotransplantation practices have been reviewed in Chapter 1. Several important revisions regarding the generation of DPF source pigs have been suggested in the Chapter 2a (previously Chapter 2). The progress on GM source pigs [25–27] and genome editing technologies [28–30] necessitated the addition of Chapter 2b. Early-phase clinical trials of transplantation of micro-encapsulated neonatal

porcine islets have been completed under comprehensive regulation since 2009 [44], suggesting safety of transplantation of porcine islet xenotransplantation products when prepared from DPF source pigs in compliance with cGMP and transplanted into non-immunosuppressed recipients with T1D. Several updated chapters, in particular chapters 3, 4, and 6, have addressed the distinct circumstances of transplantation of encapsulated islet xenotransplantation products in the absence of immunosuppression. Chapter 4 provides a very thoughtful and balanced review of the critically important topic of preclinical efficacy and safety data required to justify a clinical trial and also includes minority opinions on the most relevant issues. The main achievements in the field of transmission of all porcine microorganisms, the rationale for more proportionate recipient monitoring, and response plans are reviewed in Chapter 5. Patient selection criteria and circumstances where trials of islet xenotransplantation would be both medically and ethically justified are examined in Chapter 6 in the context of recent advances in alternative and available therapies for serious and potentially life-threatening complications of diabetes.

Perhaps the most important remaining requirements to be met before clinical trials of porcine islet products in patients with diabetes can be initiated with more favorable and more definitive harm-benefit determinations are the development of a commercially viable porcine islet product and a clinically tolerable, effective, and available rejection prophylaxis [11,45]. The precise characteristics of the islet product deemed suitable for full clinical development and the precise immunosuppression, immunoisolation, or tolerance induction strategy selected for clinical development will determine the magnitude of the impact islet xenotransplantation can make in the care of patients with diabetes for which several other competing technologies including beta cell replacement technologies are under development [46–49].

By involving essentially all investigators who are very active in the field and by inviting participation of all interested members of our professional society, the IXA has again taken proper, proactive, and proportionate steps to outline a suitable framework for conducting clinical trials of porcine islet products in T1D without compromising unreasonably the safety of participants and the public. The IXA will continue to update this consensus statement as deemed appropriate in light of scientific advances, changes in the regulatory framework and comments submitted after publication. It is hoped that continued research, increasingly favorable safety and efficacy findings, and an improved understanding of the key factors affecting the harm-benefit determinations will build momentum to revisit with regulators the more challenging regulations and to engage funding agencies and industry to step up the commitment to developing porcine islet xenotransplantation products.

Acknowledgments

The Transplantation Society (TTS) and its section, the IXA, gratefully acknowledged an unrestricted educational grant provided by Otsuka Pharmaceutical Factory, Inc., to TTS. The grant was used to support the 2nd International Conference on Clinical Islet Xenotransplantation, which was convened by IXA in San Francisco, CA, on August 1, 2014, and which was entirely devoted to the preparation of the first update of the IXA consensus statement on islet xenotransplantation. OPF waived all rights to participation in the planning and the coordination of this conference and publications developed as a result of any related activities.

Conflict of interest

B.J.H. has served as a consultant to Dompé s.p.a. and Janssen Research and Development L.L.C. and is a Director of Diabetes-Free, Inc. E.C. has served as a consultant to Xenothera. T.S., P.J.C., G.R.R., D.K.C.C., and J.D. have no conflict of interest relevant to the content of this article.

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Xenotransplantation 2016: 23: 14–24
doi: 10.1111/xen.12222

Original Article

First update of the International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes – Chapter 1: update on national regulatory frameworks pertinent to clinical islet xenotransplantation

Cozzi E, Tönjes RR, Gianello P, Bühler LH, Rayat GR, Matsumoto S, Park C-G, Kwon I, Wang W, O'Connell P, Jessamine S, Elliott RB, Kobayashi T , Hering BJ. First update of the International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes – Chapter 1: update on national regulatory frameworks pertinent to clinical islet xenotransplantation. Xenotransplantation 2016: 23: 14–24. © 2016 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd.

Abstract: Islet xenotransplantation represents an attractive solution to overcome the shortage of human islets for use in type 1 diabetes. The wide-scale application of clinical islet xenotransplantation, however, requires that such a procedure takes place in a specifically and tightly regulated environment. With a view to promoting the safe application of clinical islet xenotransplantation, a few years ago the International Xenotransplantation Association (IXA) published a Consensus Statement that outlined the key ethical and regulatory requirements to be satisfied before the initiation of xenotransplantation studies in diabetic patients. This earlier IXA Statement also documented a disparate regulatory landscape among different geographical areas. This situation clearly fell short of the 2004 World Health Assembly Resolution WHA57.18 that urged Member States "to cooperate in the formulation of recommendations and guidelines to harmonize global practices" to ensure the highest ethical and regulatory standards on a global scale. In this new IXA report, IXA members who are active in xenotransplantation research in their respective geographic areas herewith briefly describe changes in the regulatory frameworks that have taken place in the intervening period in the various geographic areas or countries. The key reassuring take-home message of the present report is that many countries have embraced the encouragement of the WHO to harmonize the procedures in a more global scale. Indeed, important regulatory changes have taken place or are in progress in several geographic areas that include Europe, Korea, Japan, and China. Such significant regulatory changes encompass the most diverse facets of the clinical application of xenotransplantation and comprise ethical aspects, source animals and product specifications, study supervision, sample archiving, patient follow-up and even insurance coverage in some legisla-

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tions. All these measures are expected to provide a better care and protection of recipients of xenotransplants but also a higher safety profile to xenotransplantation procedures with an ultimate net gain in terms of international public health.

Key words: national regulatory frameworks – type 1 diabetes – xenotransplantation

Abbreviations: ATMP, Advanced Therapy Medicinal Products; AFMPS, National Agency for Drug Clinical Assays; CGMP, current Good Manufacturing Practices; CVM, Center for Veterinary Medicine; DPF, designated pathogen-free; EMA, European Medicines Agency; EU, European Union; FDA CBER, Food and Drug Administration Center for Biologics Evaluation Research; GLP, Good Laboratory Practices; HCT, Human Cellular and Tissue Therapies; IND, Investigational New Drug; ICH, International Council on Harmonizations; IXA, International Xenotransplantation Association; LCT, Living Cell Technologies Limited (LCT); MFDS, Ministry of Food and Drug Safety; MHLW, Ministry of Health, Labour and Welfare; NHFPC, National Health and Family Planning Commission of the People's Republic of China; PERV-C, porcine endogenous retrovirus-C; NHMRC, National Health and Medical Research Council; NZ, New Zealand; PHS, Public Health Services; SOPs, Standard Operating Procedures; TGA, Therapeutic Goods Administration; WHO, World Health Organization.

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Received 8 February 2016;
Accepted 8 February 2016

Introduction

Xenotransplantation represents an attractive solution to bridge the gap between the demand for human organs, tissues, and cells and the current availability of human donors for clinical needs. In particular, islet xenotransplantation is viewed as the most advanced form of xenotransplant. Indeed, pig islets represent a very attractive and potentially unlimited source of islet supply for the treatment of diabetic patients [1]. In this regard, considerable progress has been achieved in the last few years with several academic groups reporting long-term survival (more than one year in some cases) in relevant non-human primate models of type 1 diabetes [2,3]. Furthermore, initial clinical trials are already underway in diabetic patients [4].

The clinical application of porcine islet xenotransplantation, however, requires an ethically appropriate environment and the existence of a regulatory framework specifically designed to accommodate the peculiarities of xenotransplantation [5]. Furthermore, islet xenotransplantation should only take place in the presence of a convincingly favorable risk-benefit ratio. In particular, the theoretical risk of infection transmission to a xenograft recipient (or a close contact) mandates a cautious and tightly monitored clinical approach. Indeed, it is well-known that infections mediated by well-characterized [6] or even yet unidentified infectious agents [7] represent a risk intrinsic to transplantation medicine where recipients are more susceptible to infections as a consequence of the immunosuppression administered to enable graft acceptance.

In an earlier Consensus Statement, the International Xenotransplantation Association (IXA) has outlined the key ethical and regulatory requirements that need to be satisfied to enable the initiation of xenotransplantation studies in diabetic patients [5]. In this report, changes in the regulatory frameworks, which have occurred in the intervening period in the various geographic areas or countries, are herewith briefly described in specific sections that have been prepared by IXA representatives who are active in xenotransplantation research in their respective geographic areas.

Update on the regulatory framework pertinent to clinical islet xenotransplantation in the European Union

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In the European Union (EU), clinical trials using xenogeneic medicinal products are regulated by the European Medicines Agency (EMA), London, that takes care of centralized regulatory procedures for medicinal products. The EMA Guideline on xenogeneic cell-based medicinal products (EMEA/CHMP/CPWP/83508/2009) came into effect on January 1, 2010 [8]. The Guideline addresses the scientific requirements for xenogeneic cell-based medicinal products for human use. It is intended for products entering the marketing authorization (MA) procedure. In addition, the principles laid down in the Guideline should be taken into consideration by applicants who wish to enter into clinical trials. The document deals with the main criteria such as quality and manufacturing aspects, non-clinical testing, clinical development, pharmacovigilance and risk management plans, and particularly with requirements unique to xenogeneic specificities. The main scientific and technical issues identified concern the sourcing and testing of animals, quality control, adventitious agents safety as well as the non-clinical and clinical development of xenogeneic cell-based medicinal products. Relevant public health implications are discussed and measures to ensure a proper surveillance for infections, including zoonoses are highlighted. In addition, attention is given to principles of animal health and welfare in the processes of sourcing of xenogeneic materials for the medicinal products intended for human use.

For islets, in particular, the Guideline states that "unmodified islets" are considered as a drug and follow as such the guidelines for drug therapy. On the contrary, in the case of beta cells isolated from pig islets, the guideline should be read in conjunction with regulation 1394/2007/EC on advanced therapy medicinal products (ATMP) [9]. The multidisciplinary Committee for Advanced Therapies (CAT) at the EMA has been established in accordance with regulation 1394/2007/EC. In May 2013, EMA has published its scientific recommendation on classification of alginate-encapsulated porcine pancreatic beta cells as ATMP in accordance with 1394/2007/EC (Article 17). This particular mixture of cells was classified as somatic cell therapy products. The product is intended for the treatment of type 1 diabetes and might be considered to be effective in modifying abnormal glucose metabolism in such patients.

In Belgium, for the use of pig islets, the National Agency for Drug Clinical Assays (AFMPS) has recommended to precisely define the pig source (neonates/adult) and the characteristics of the SPF facility. It is only under such conditions that a pilot study in humans could be considered in Belgium and also EMA would be involved directly.

Update on the regulatory framework pertinent to clinical islet xenotransplantation in Switzerland

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The conduct of clinical transplantation in Switzerland is almost unique in the world, because it is regulated by law. The Federal Act on the Transplantation of Organs, Tissues and Cells became effective in October 2004 [10]. This was followed by specific ordinances for transplantation of human organs/tissues/cells in March 2007 [11], and for xenotransplantation of animal organs/tissues/cells in July 2007 [12].

The xenotransplantation ordinance addresses clinical trials and treatments with xenotransplant products with associated duty of care, special safety measures and conduct for the respective persons including contact persons, and insurance of liabilities. The definition of xenotransplantation is similar to that in guidance of the FDA and guidelines of the EMA, that is, organs/tissues/cells from animal origin, and human organs/tissues/cells/fluids that have been in contact with organs/tissues/cells of animal origin. In addition, the definition includes transplant products that have been manufactured from the products mentioned above: Transplant products are defined as "*products manufactured from human or animal organs, tissue or cells that can be standardised or whose manufacturing process can be standardised*" [1]. Following this definition, a porcine islet product is included in the transplant legislation, while a human islet product does not fall under this legislation. Regarding a porcine islet cell therapy product, the Swiss Competent Regulatory Authority (Swissmedic) does not acknowledge a product being an advanced therapy medicinal product (ATMP), but this is to some expect semantics because the associated requirements in compliance with a transplant product are similar if not the same. To illustrate this point, a porcine islet product is judged in a similar way as described in the European Regulation 1394/2007 on ATMPs [9]; the guidelines from the ICH [13] do apply, and regulatory filings should be structured according to the Common Technical Document [14].

According to the Verordnung, xenotransplantation is only allowed in Switzerland under certain strict conditions, which include the benefits and safety with respect to infectious risk, and which include the consideration that there is no alternative treatment with a similar benefit available.

Besides specific and detailed instructions for clinical trials, and specifics on (genetically modified) source animals, there are a number of items in the Swiss Ordinance that are more stringent than existing guidelines or guidance elsewhere in the world. For instance:

- the ordinance outlines specific requirements with regard to the information to be provided to the patient, which includes the request for lifelong monitoring and requirement for autopsy;
- the ordinance outlines specific requirements with regard to archiving of samples: for clinical trials, samples from the donor and patient should be stored indefinitely; for regular clinical use, samples should be stored for at least 20 years; and samples should be made available to cantonal authorities;
- An insurance coverage of CHF 20 Mio should be in place.

Thus, the development of products in compliance with Swiss regulations is similar to that in compliance with regulations and guidelines in other countries. Hence, following approval by Swissmedic, market entry will be possible in other countries after formal confirmation by competent regulatory authorities of such countries.

Update on the regulatory framework pertinent to clinical islet xenotransplantation in the USA

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The Center for Biologics Evaluation and Research (CBER) is the Center within U.S. Food and Drug Administration (FDA) that regulates biological products, including xenogeneic porcine pancreatic islet cells, for human use under applicable federal laws, including the Public Health Service Act and the Federal Food, Drug and Cosmetic Act. In accordance with these laws, the sponsors must obtain approval of an Investigational New Drug (IND) application from the FDA prior to the initiation of any clinical trial. From 2001 to 2003, with the objective of assisting sponsors, review teams, and the public, the FDA issued the following guidance documents relevant to clinical islet xenotransplantation:

- Public Health Services (PHS) Guideline on Infectious Disease Issues in Xenotransplantation 1/19/2001
- Draft Guidance for Industry: Precautionary Measures to Reduce the Possible Risk of Transmission of Zoonoses by Blood and Blood Products from Xenotransplantation Product Recipients and Their Intimate Contacts 2/1/2002
- Guidance for Industry: Source Animal, Product, Preclinical, and Clinical Issues Concerning the Use of Xenotransplantation Products in Humans 4/3/2003

These guidance documents can be found at http://www.fda.gov/biologicsbloodvaccines/xenotransplantation/default.htm.

The use of genetically engineered (GE) pigs as islet donors may have a number of significant advances over wild-type animals, including reduced immunogenicity of transplanted tissue. Within the FDA, regulatory oversight of GE animals for xenotransplantation is shared by the Center for Veterinary Medicine (CVM) and CBER. In January 2009, the Agency clarified its regulation of GE animals in its Guidance for Industry [15]. This guidance explains the process by which FDA is regulating GE animals and provides a set of recommendations to producers of GE animals to help them meet their obligations and responsibilities under the law. While the guidance is intended for industry, FDA believes it may also help the public gain a better understanding of this important and developing area.

In 2010, a review of the existing regulatory framework within the United States for the initiation of a clinical trial of xenogeneic porcine pancreatic islets for the treatment of type 1 diabetes was published by FDA staff [16]. In it, the authors summarized the review process by which the FDA will appraise xenotransplantation products, while also outlining the general review principles to be applied with respect to the infectious disease status of the donor pigs, manufacturing and final product testing of islets, pre-clinical testing in animal models, and finally the design of the clinical trial.

Update on the regulatory framework pertinent to clinical islet xenotransplantation in Canada

Gina R. Rayat, University of Alberta, Edmonton, Canada

In Canada, the national regulations pertaining to xenotransplantation clinical trials application have not changed since the release of the 2009 IXA Consensus Statement. In particular, xenotransplant products are considered therapeutic products (drugs or medical devices) and are subject to the requirements of the *Food and Drugs Act* [17, 18], as well as the *Food and Drug Regulations* [19,20] or the *Medical Devices Regulations* [21,22]. The Biologics and Genetics Therapies Directorate and the Therapeutics Products Directorate of Health

Canada are responsible for reviewing clinical trial applications and authorizing the sale of xenotransplant products in accordance with the *Food and Drug Regulations* or the *Medical Devices Regulations* [23,24]. Based on these regulations, sponsors of human clinical trials involving xenotransplants would be required to submit an application to Health Canada for approval before a clinical trial might proceed. Health Canada will review such an application and authorize clinical trials based on scientific evidence provided in the submission that the benefits are likely to outweigh the risks. In July 1999, Health Canada released for public comment a draft document of the proposed Canadian Standard for Xenotransplantation [25,26], which was prepared by a subcommittee of experts whose backgrounds involve regulatory, ethical, clinical, and scientific specializations. This document can be used to help researchers prepare submissions to Health Canada for clinical trial applications related to xenotransplantation. The document addresses the safety of xenotransplant products for human transplantation purposes and provides performance requirements aimed at preventing disease transmission and assuring optimum clinical performance of xenotransplants. The document also offers standards for animal production, care, and disposal as well as safety of recipients, personnel, and others who may be exposed or affected by the xenotransplant products. The Office of Regulatory Affairs in the Biologics and Genetic Therapies Directorate can be contacted for information related to Health Canada's Clinical Trial application process or the New Drug Submission process.

Update on the regulatory framework pertinent to clinical islet xenotransplantation in Japan

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In July 9, 2002, the "Public Health Guidelines on Infectious Disease Issues in Xenotransplantation (Iseikenhatsu No. 0709001)" were published by the Research and Development Division, Health Policy Bureau, Ministry of Health, Labour and Welfare (MHLW), which is the Japanese Regulatory Authority. The objective of this document is to prevent infection and expansion of emerging infectious diseases caused by xenotransplantation from the public health viewpoint. Therefore, Japan has a comprehensive guideline to prevent xenogeneic infections. However, two major issues are represented by the lack of update of these Guidelines since 2002 and the absence of any information regarding the steps to undertake for marketing approval of xenogeneic products.

Due to the possible clinical application of xenotransplantation in Japan, the MHLW, the Japan Society for Transplantation and Japanese Society for Xenotransplantation are currently working jointly to update the Guidelines. In addition, to enable marketing approval of xenogeneic products, two new laws have been approved in 2013 [27,28]. These are the Act on the Safety of Regenerative Medicine and the Amended Pharmaceutical Affairs Act, namely the Pharmaceutical and Medical Devices Law. The key points of these two Laws are summarized in Table 1.

Update on the regulatory framework pertinent to clinical islet xenotransplantation in Korea

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Currently, there is no official regulation on clinical islet xenotransplantation in the form of a law confirmed by the Korean government. The Ministry of Health and Welfare (MHW) has prepared a draft act (Act on Xenotransplantation) for xenotransplantation in 2012 with the help of the XRC (Xenotransplantation Research Center: a multiyear program for preclinical and clinical xenotransplantation research supported by Korean government since 2004). However, this has not yet been submitted to the National Assembly. Indeed, the government and the National Assembly appear to hesitate to ratify such an Act as they may not be sure of the benefits brought by xenotransplantation over the potential associated risks. However,

Table 1. Key points of the New Japanese Law for Regenerative Medicine Products

The Act on the Safety of Regenerative Medicine
1. This act will cover the Safety for the clinical research notified by authorities and furthermore the medical treatment not notified by authorities and not covered by health insurance.
2. There are three risk categories (high: Class 1, moderate: Class 2, and low: Class 3).
3. Xeno-cell therapy will be categorized as Class 1.
4. Collected data will be public open source by the MHLW.
5. The Act also requires hospitals and clinics to release to the Ministry regular reports regarding the status of implementation.
6. Cell culture and processing facilities are required to obtain a license.
The Pharmaceutical and Medical Devices Law
1. A new chapter for regenerative medicine products is made.
2. The demonstration of efficacy in an exploratory study will be considered to be sufficient for provisional approval that will grant the product a conditional term of seven years, during which further data collection of efficacy and safety will be obtained while the product is permitted to access the market.
3. After this phase, the stakeholder must submit an application dossier for the second authorization.
4. Should the benefit–risk assessment fail to provide a favorable ratio, the authorization will be revoked.

in the hope that xenotransplantation may enable the economic growth of the biomedical industry, the Korean government has been supporting xenotransplantation research since 2004.

The core of the draft act is 1) to suggest the basic ethical principles to be applied to all preclinical and clinical xenotransplantation research initiatives conducted in Korea; 2) to establish the necessary national institute supervising the xenotransplantation research; 3) to clarify the role of the governmental agencies involved in the xenotransplantation research; 4) to specify the details of the xenotransplantation clinical trial necessary to protect society from potential zoonotic infections and the human rights of the potential candidates and their close contacts.

The Department of Bioethics and Safety of the Ministry of Health and Welfare is responsible for stipulating the necessary regulations for xenotransplantation. The Ministry of Food and Drug Safety (MFDS) actually regulates the process of clinical trial authorization and approval of new medicines and medicinal products. Therefore, a clinical xenotransplantation trial should be approved and controlled by the MFDS. The MFDS has published the "Guide for the Scope of Xenografts and Source Animals" [29], the "Guide for the Quality Control and Infection Management of Xenografts" [30], and the "Guide for the Preclinical and Clinical Trials of Xenografts" [31] in 2006. These documents are actually effective for the manufacturer of xenografts and the responsible officials are represented by the MFDS, but have no strong legal binding power to the potential recipients, their close contacts, and other governmental agencies. This is why the MHW prepared the draft Act on Xenotransplantation. Besides the documents, many other acts and guidelines would be indirectly involved in xenotransplantation. The Pharmaceutical Affaires Act and the Medical Device Act are examples of these representative laws.

The fundamental points of the Korean regulatory system for xenotransplantation would be similar to those of the US system except for the regulatory structure from the governmental side. Nonetheless, in the development of its own guides for xenotransplantation, the Korean MFDS has relied on the guidelines of US FDA, EMA, and the related Japanese Agency.

Update on the regulatory framework pertinent to clinical islet xenotransplantation in China

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Consistent with the definition of WHO, xenotransplantation in China is also, animal to human, defined as living cells, tissues, or organs of animal origin and human body fluids, cells, tissues, or organs that have *ex vivo* contact with these living, xenogeneic materials, which have the potential to constitute an alternative to material of human origin and bridge the shortfall in human material for transplantation. Currently, only porcine islet xenotransplantation is approved for clinical trial in China. However, all affairs concerning xenotransplantation clinical trial should be thoroughly assessed by administrative authority on the basis of the Changsha Communiqué [32].

The conduct of clinical trials for porcine islet xenotransplantation in China is encouraged and subject to strict administration and surveillance of National Health and Family Planning Commission of the People's Republic of China (NHFPC, the former Ministry of Health) and the biosafety network which consists of Chinese Center of Disease Control and Prevention, National Institutes for Food and Drug Control and Wuhan Institute of Virology of the Chinese Academy of Science. Sponsors of human clinical trials involving xenotransplantation would be required to submit an application to NHFPC for approval before a clinical trial might proceed. Preliminary assessment of medical institution qualification would be carried out by an administrative authority appointed by NHFPC. For the qualified applicant, issues of the ethics, biosafety, and efficacy of porcine islet xenotransplantation clinical trial would be further assessed by a committee composed of experts in medicine, law, ethics, and other related fields. The requirement of the medical institution and personnel for transplantation has been stipulated by NHFPC [33]. Donor animals fulfilling designated pathogen-free (DPF) status by rigorous routines, standard operating procedures (SOPs) and compliance with Good Laboratory Practices (GLP) are required, and pathogens affecting herd health status or with the potential to cross-species barrier should be excluded. All the biosafety affairs not specific for but including xenotransplantation are under surveillance of biosafety network. When the safety and efficacy have been proved by the clinical trial, xenotransplant products are considered therapeutic products (new drugs or medical products) and are subject to the regulations of China Food and Drug Administration.

So far, encouraging progress has been achieved toward clinical trial of islet xenotransplantation in China, as a preclinical trial in monkeys has been

carried out in Changsha, China, with satisfactory results. Advocated by leading investigators, a National biosafety monitoring network was initially established in 2012 and consisted of national health authorities and of the Chinese academy of science. The network regulates and supervises all issues concerning biosafety affairs and public health related to islet xenotransplantation. Under the guide of the network, biosecure barrier facility for DPF animal has been set up in Changsha, and the list of pathogens has been established and being improved for donor animal screening and surveillance. DPF animals have been inbred and supplied for preclinical trials.

Update on the regulatory framework pertinent to clinical islet xenotransplantation in Australia

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In 2004, a Working Party of the National Health and Medical Research Council (NHMRC) published their recommendations for clinical trials of xenotransplantation in Australia. They recommended that clinical trials of non-vascularized cellular xenotransplants such as porcine islet grafts or transplantation of human cells, which were xenografts by virtue of their culture in direct contact with xenogeneic cells, should be permitted under strict regulatory oversight by the Therapeutic Goods Administration (TGA) provided that safety and efficacy could be demonstrated in preclinical models. This advice was not taken by the NHMRC Human Ethics Committee, who recommended a moratorium against clinical trials be put in place, concluding that the risks of transmission of animal viruses to humans and the wider community had not been resolved. However, ongoing research into xenotransplantation was encouraged. In 2008, a further NHMRC review of the field was undertaken where new data on the safety and the state of the art of xenotransplantation was re-evaluated and the findings published in 2009 [34]. After review of more recent published evidence, it concluded that the understanding of xenotransplantation technologies had progressed significantly and it was their opinion that the potential risks to individuals and the community were not sufficient to justify a continuing ban on clinical trials in Australia. Furthermore, maintaining such a ban effectively prevented development of the necessary regulatory and infrastructure frameworks, which were required to facilitate preclinical research. As a result, the NHMRC original recommendation was overturned in favor of control of risk through regulation. Issues identified as necessary for monitoring clinical trials included the following:

- Regulatory oversight by the TGA within the framework of the new Human Cellular and Tissue Therapies (HCT) Act
- Establishment of a National Surveillance System
- Development of a National Patient Register
- Appointment of a Xenotransplant Advisory Committee to provide advice to Human Research Ethics Committees
- Development of animal-to-human transplantation guidelines
- The possible addition of relevant supplementary material to the National Statement on Ethical Conduct in Human Research and the Australian code of practice for the care and use of animals for scientific purposes

Currently in Australia, the only xenotransplant product used in current clinical practice is human epithelial cells grown in culture on animal feeder cells and used as skin grafts for severe burns. Any future trials of xenotransplant products are to be regulated by the TGA under the new recently introduced regulatory framework for Human Cellular and Tissue Therapies [35]. Xenotransplantation is to be regulated as a Class 4 product (highest risk) of the HCT classification scheme. It is expected that they would fall under the Clinical Trial Exemption (CTX) Scheme, which requires TGA assessment and approval of the safety and quality of the trial product and proposed trial structure prior to commencement of the clinical trial.

Update on the regulatory framework pertinent to clinical islet xenotransplantation in New Zealand

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The New Zealand (NZ) legislation remains unchanged since 2009, and the details around the specific sections regarding xenotransplantation are provided in Part 7A of the Medicines Act 1981 (full copy available at: www.legislation.govt.nz). In particular, any study on new products requires specific Ministerial approval before it can proceed. The Act requires the Minister to be satisfied that: (a) the conduct of the procedure or class of procedure does not pose an unacceptable risk to the health or safety of the public; (b) any risks posed by the conduct of the procedure or class of procedure will be appropriately managed; (c) any ethical issues have been adequately addressed; (d) any

cultural issues have been adequately addressed and; (e) any spiritual issues have been adequately addressed; before granting consent to a clinical trial application. The Minister can call together an expert advisory panel if required, and the Act requires such a panel to review the application for compliance with a defined list of criteria and to call for public submissions on the application.

Two distinct clinical xenotransplantation trials are currently underway in NZ, one of which regards islet xenotransplantation in diabetic patients. In this study, promoted by Diatranz Otsuka Ltd, encapsulated porcine islet cells were transplanted in patients affected by type 1 diabetes. In the second study, promoted by Living Cell Technologies Limited (LCT), alginate-coated capsules containing neonatal porcine choroid plexus cells is used as potential source of nerve growth factors to promote new central nervous system growth in patients affected by Parkinson disease. In this context, it should be pointed out that this clinical study was temporarily halted following some concerns regarding data generated in earlier animal studies. However, following an intensive internal review process by the Data and Safety Monitoring Board of the Health Research Council of New Zealand, the study was resumed.

It is of interest that in both clinical studies, porcine cells are sourced from the unique herd of designated pathogen-free pigs bred from stock originally discovered in the remote sub-Antarctic Auckland Islands. In particular, these products contain pig cells from porcine endogenous retrovirus-C (PERV-C) transmission incompetent pigs.

Regulatory Issues related to Current Clinical Trials of Pig Islet Xenotransplantation in New Zealand

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Pilot studies of intraperitoneal transplantation of microencapsulated neonatal porcine islets were carried out in NZ before any national or international specific guidelines were available. Preclinical evidence of safety and efficacy allowed NZ regulatory authorities to approve these studies. The safety and partial efficacy of one of these patients has been reported [36].

Following the scare caused by the discovery of the in vitro infectivity toward human cells exhibited by some porcine retroviruses, a new herd of designated pathogen-free (DPF) pigs was discovered on a sub-Antarctic island and maintained to FDA guidelines in NZ. These pigs showed absence of any known pathogenic microorganism on extensive published Garkavenko et al. [37] in vitro studies. Preclinical evidence of limited efficacy of compound alginate microencapsulated islets from these pigs was seen in diabetic NOD mice, streptozotocin diabetic rats and monkeys, and alloxan diabetic rabbits.

A NZ law was enacted in 2003 that required all applications for xenotransplantation trials to gain prior approval of the Minister of Health on advice from the Ministry. A nationwide public consultation was carried out in 2004-2005 with a consensus result showing that such trials could be approved if they met certain stringent conditions for the source pig herd, compliance with current Good Manufacturing Practices (cGMP), and lifetime monitoring of transplanted patients together with archiving of all pig and human samples. A further public consultation was conducted in 2007 to seek consensus on approval before a specific trial was initiated. The opinion of an international expert consultant was also sought. These conditions having been met, the Minister approved the trial in 2008.

Sixteen patients were thus transplanted with no evidence of safety concerns (for up to 7-year follow-up to date) and preliminary evidence of efficacy for the secondary trial endpoint (i.e., unaware hypoglycemia) was provided. Using this approval as a default, regulatory approval was gained in Buenos Aires in 2009 to conduct similar trials involving 22 patients, transplanted twice. Promising results were attained.

From the evidence provided by these two trials, the balance of potential risk to potential benefit favors continuing with these endeavors, provided similar safety measures are employed.

Toward the definition of global regulatory framework pertinent to clinical islet xenotransplantation: the fundamental role of the WHO

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While the fundamental inspirational support provided by the above-mentioned US FDA documents has been instrumental in the development of the regulatory framework in many countries, it is apparent that the international regulatory landscape is fairly diverse. In particular, while some areas have a well-defined regulatory framework, other geographic areas are not as well equipped and appear to underestimate the risks potentially associated with the implementation of uncontrolled and unregulated xenotransplantation practices. In this context, it is highly relevant that the IXA has previously encouraged the successful initiation of clinical xenotransplantation trials in the

context of a well-coordinated international effort under the expert guidance of the WHO. Indeed, following the 2004 World Health Assembly Resolution WHA57.18, which urged member states to "allow xenotransplantation only when effective national regulatory control and surveillance mechanisms overseen by National Health Authorities are in place," the WHO has co-organized two global consultations on regulatory requirements for xenotransplantation clinical trials. The first of such events took place in Changsha, China, in 2008 and resulted in the publication of the Changsha Communiqué whose key messages have been reported in the 2008 IXA Consensus Statement [5,32]. More recently, the Second WHO International Consultation on Regulatory Requirements for Xenotransplantation Clinical Trials ("Geneva Consultation") convened at the WHO headquarters in Geneva, Switzerland, in 2011 with participants, health regulatory authority representatives and internationally recognized experts in xenotransplantation science, law, and ethics from every WHO region, representing 14 Member States [38]. The consultation's key objectives were to review the current status of xenotransplantation science and practice; to determine whether updates to the Changsha Communiqué were required; to discuss and refine draft guidance for infectious disease surveillance, prevention, and response to support various probable clinical xenotransplantation trial scenarios [39]. The recommendations issued from the Geneva Consultation are here summarized:

A. Recommendation to WHO

To facilitate global collaboration for laboratory investigations

WHO should facilitate the creation of a collaborative group of public/academic xeno-related infectious disease reference laboratories and appropriate Health Authorities' resources to support assay development, validation, standardization, and sample throughput. Such a network would include representation of CDC, FDA, Paul Ehrlich, NHMRC of Australia or NZ, Korea CDC, Chinese CDC, and other experts.

To encourage transparency in xenotransplantation-related activities

Indeed, it is regrettable to note that unregulated xenotransplantation continues to be advertised and performed in multiple jurisdictions in contravention of the fifty-seventh World Health Assembly Resolution WHA57.18 urging Member States "to allow xenogeneic transplantation only when effective national regulatory control and surveillance mechanisms overseen by national health authorities are in place".

To convene regular global consultations on xenotransplantation activities

WHO should foster regular (annual or biennial) interaction between regulators and xenotransplantation subject matter experts, as appropriate to the level of contemporary xenotransplantation activity. This global consultation would discuss planned or ongoing xenotransplantation clinical activities and provide a framework for exchanges identifying needs for advice and collaborations".

B. Recommendation to Member States, Investigators, Proposers, or Study Sponsors

- To seek global consistency in requirements for clinical trials by referring to best global standards and experts' advice especially in areas such as source donor animal; recipients, family members, and close contacts surveillance; risk/benefit analysis and trial infrastructure.

To combat unfounded assertions on human xenotransplantation

Stakeholders in xenotransplantation should only communicate on the basis of evidence. In particular, Member States should implement regulations that prohibit statements or advertisements for xenotransplantation trials or products that claim unproven benefits or that are (or may prove to be) false or misleading with respect to known or unknown risks.

- To refer to experienced independent laboratories

(a) Member States, Investigators, Proposers, and/or Sponsors of a clinical trial should assure access to identified expertise in xeno-specific disease assays.

(b) Member States should consider assuring access to an independent (third party) reference laboratory with identified expertise in xeno-specific infectious disease assays.

Conclusions

This update on the regulatory frameworks to undertake clinical trials of porcine islet products in type 1 diabetes documents that a refinement or integration of existing regulatory instruments to

accommodate xenotransplantation practices has occurred or is underway in many countries.

Indeed, important regulatory changes have taken place or are in progress in several geographic areas that include Europe, Korea, Japan, and China. Such significant changes in the regulatory frameworks encompass the most diverse facets related to the clinical application of xenotransplantation procedures and comprise ethical aspects, source animals and product specifications, study supervision, sample archiving, patient follow-up, and even insurance coverage in some legislations. All these measures have as the ultimate goal to provide a better care and protection to the patient but also a higher safety profile to xenotransplantation procedures, with an ultimate net gain in terms of international public health.

In addition, in the last few years, the WHO has proactively pursued its effort toward the harmonization of xenotransplantation procedures at a global level. Regrettably, however, several cases of unregulated xenotransplantation have been reported since the 2009 IXA Consensus Statement. Indeed, such unregulated procedures that do not comply with the requirements of the Changsha Communiqué represent a potential international threat and National Regulatory Authorities are strongly encouraged to identify and timely interrupt such unregulated practices.

In all cases, thanks to the indefatigable efforts put in place by the WHO and IXA, there is a wider international perception of the importance of developing an internationally harmonized ethical and regulatory framework for xenotransplantation. Indeed, all the steps underway in various geographic areas go in this direction and these will provide a solid basis for the safe development of this challenging medical discipline.

Acknowledgment

The authors are indebted to Dr Luc Noel for the central role he has played at the WHO to promote the development of an internationally harmonized ethical and regulatory framework for xenotransplantation.

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Xenotransplantation 2016: 23: 25–31
doi: 10.1111/xen.12223

Original Article

First update of the International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes—Chapter 2a: source pigs—preventing xenozoonoses

Spizzo T, Denner J, Gazda L, Martin M, Nathu D, Scobie L, Takeuchi Y. First update of the International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes—Chapter 2a: source pigs—preventing xenozoonoses. Xenotransplantation 2016: 23: 25–31. © 2016 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd.

Abstract: Chapter 2 of the original consensus statement published in 2009 by IXA represents an excellent basis for the production of safe donor pigs and pig-derived materials for porcine islet xenotransplantation. It was intended that the consensus statement was to be reviewed at interval to remain relevant. Indeed, many of the original salient points remain relevant today, especially when porcine islet xenotransplantation is performed in conjunction with immunosuppressants. However, progress in the field including demonstrated safe clinical porcine xenograft studies, increased understanding of risks including those posed by PERV, and advancement of diagnostic capabilities now allow for further consideration. Agents of known and unknown pathogenic significance continue to be identified and should be considered on a geographic, risk-based, dynamic, and product-specific basis, where appropriate using validated, advanced diagnostic techniques. PERV risk can be sufficiently reduced via multicomponent profiling including subtype expression levels in combination with infectivity assays. Barrier facilities built and operated against the AAALAC *Ag Guide* or suitable alternative criteria should be considered for source animal production as long as cGMPs and SOPs are followed. Bovine material-free feed for source animals should be considered appropriate instead of mammalian free materials to sufficiently reduce TSE risks. Finally, the sponsor retention period for archival samples of donor materials was deemed sufficient until the death of the recipient if conclusively determined to be of unrelated and non-infectious cause or for a reasonable period, that is, five to 10 yrs. In summary, the safe and economical production of suitable pigs and porcine islet xenograft materials, under appropriate guidance and regulatory control, is believed to be a viable means of addressing the unmet need for clinical islet replacement materials.

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Key words: closed herd – designated pathogen-free status – infectious disease – islet xenotransplantation – porcine endogenous retrovirus – porcine islet product – regulatory guidance – source pigs – type 1 diabetes – xenozoonoses

Abbreviations: AAALAC, Association for Assessment and Accreditation of Laboratory Animal Care International; AAVLD, American Association of Veterinary Laboratory Diagnosticians; BSE, bovine spongiform encephalopathy; cGMP, current good manufacturing practices; CLSI, Clinical and Laboratory Standards Institute; DPF, designated pathogen-free; EMEA, European Medicines Agency; ISO, International Organization for Standardization; MALDI-TOF MS, matrix-assisted laser desorption–ionization time-of-flight mass spectrometry; PERV, porcine endogenous retrovirus; SOP, standard operating procedure; TSE, transmissible spongiform encephalopathy; xPCR, polymerase chain reaction including, but not limited to, method variations nested, real-time, quantitative, reverse transcriptase, digital, and combinations thereof.

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Received 8 February 2016;
Accepted 8 February 2016

Introduction

A consensus statement that provided guidance for those contemplating clinical trials of porcine islet product xenotransplantation was published by the International Xenotransplantation Association (IXA) in 2009 [1]. It was intended that the consensus statement be reviewed at intervals to maintain relevance with the current state of xenotransplantation research. The first such review was conducted by the authors and presented at the 2nd International Conference on Clinical Islet Xenotransplantation (ICCIX), convened on August 1, 2014 by the IXA. The authors were not charged for rewriting the original consensus statement, but instead to (i) summarize salient points communicated in the 2009 consensus statement, (ii) update relevant progress, data, and understanding in the field since 2009, (iii) present new and underappreciated topics not addressed in the original statement, (iv) suggest pertinent revisions, and (v) offer other opinions and perspectives.

Salient points communicated in the 2009 IXA consensus statement

The original consensus statement—Chapter 2 Source pigs [2] summarized and presented the following salient points in the Executive Summary [1]:

To reduce the risks of transmission of xenogeneic infectious diseases to recipients of porcine islet products and of potential subsequent transmission to close contacts and the public, the following criteria related to source pigs must be met before undertaking a clinical xenotransplant trial:

1. To assure the absence of [designated] bacteria, fungi, protozoa and viruses in source pigs, source pig herds, and animal facilities, it is imperative that (i) well-defined routines of testing for designated pathogens be maintained and (ii) rigorous standard operating procedures (SOPs) and current good manufacturing practices (cGMP) of herd husbandry and veterinary care be followed, thereby fulfilling the DPF status.
2. DPF animals must be bred from a closed colony and housed in a well-controlled, pathogen-free environment with high standards of animal welfare
3. To monitor the DPF status, the herd must be extensively tested to ensure freedom from [designated] pathogens with appropriate biosecurity and surveillance in place to guarantee continued freedom from infectious disease.
4. The operation of the environment must be in compliance with cGMP and include a documented history of activities
5. As the DPF status of source animals cannot be obtained for [...] porcine endogenous retrovirus (PERV), a comprehensive plan for [...] monitoring of recipients after xenotransplantation must be followed to ensure timely identification, reporting, and management of possible xenotransplant-related infection episodes.

The authors believe these salient points remain relevant as indicated, especially when porcine islet xenotransplantation is performed in conjunction with immunosuppressants, considering progress in the field, and where not otherwise indicated hereafter. Of particular note, we have not restated the sixth salient point regarding the archival period of donor specimens as it is subsequently addressed and resulted in a revision based on the consensus of IXA.

Relevant progress, data, and understanding in the field since 2009

Prior to 2009, there was limited clinical safety data available for porcine islet products and as a result the degree of risk was largely unknown. To date, long-term preclinical use of porcine islet products has not demonstrated the transmission of porcine microorganisms including PERV [3,4]. All clinical results to date have also demonstrated no transmission of porcine microorganisms including PERV from porcine islet products [4–7], most recently in the clinical pig islet xenotransplantation trial conducted on fourteen patients in New Zealand [8], and long term (up to 408 months) in patients exposed to vital porcine skin xenografts [9]. Therefore, although porcine islet xenotransplantation still poses the theoretical risk for transmission of known and unknown infectious agents, the growing body of evidence indicates that these events will likely be rare and should they occur are unlikely to result in an infectious disease when utilizing appropriate source material and recipient safeguards (for further discussion on recipient monitoring refer to chapter 5).

The extensive lists of excluded agents defining designated pathogen-free health status in the original consensus statement still serve as an appropriate baseline for establishing DPF criteria. Additional agents of uncertain clinical significance continue to be identified around the world (e.g., enterovirus B, rotaviruses, porcine teschovirus, Paramyxoviridae sp., Tioman virus, parvovirus

PPARV-4 (bocavirus), kobuvirus, novel astroviruses, and Ljungan-like viruses) [4,5,10–12]. It bears note that these evolving lists represent a global summary of agents from which geographically appropriate, risk-, and product intended use-based profiles should be prepared, assessed based on the accumulated knowledge in the field, and agreed to with country- or region-specific regulatory agencies as was done in the case of the recent New Zealand clinical trial [8]. In this instance as part of a multilevel screening strategy of a donor herd, a comprehensive risk analysis was performed reducing 107 pig infectious organisms to 26 relevant pathogens deemed for the specific case of the New Zealand herd to necessitate demonstrated exclusion and reprinted as Table 1 to serve as an example. Once established, these DPF excluded agent lists need be dynamic and adaptive in response to new and emergent agents within the source geography. Select recent examples illustrating need for geographically based monitoring and adaptive responses include African swine fever (ASF) emergence in the Eastern E.U. in the summer of 2014 and porcine epidemic diarrhea (PED) emergence in USA in April 2013. These example diseases have never infected human beings [13,14] but are of significant concern to pig health and production in affected areas.

Table 1. Example testing schedule for the NZ donor pig herd [8]

Microorganism[a]	Frequency of testing	Method[b]	Present and ubiquitous in New Zealand	Absent within the herd
Bacteria				
Leptospira tarrasovi	Quarterly	MAT	Yes	Yes
Leptospira hardjo	Quarterly	MAT	Yes	Yes
Leptospira pomona	Quarterly	MAT	Yes	Yes
Mycoplasma hyopneumoniae	Annually	PCR	Yes	Yes
Campylobacter	Annually	Culture	Yes	Yes
Isospora	Annually	Culture	Yes	Yes
Cryptosporidium	Annually	Culture	Yes	Yes
Yersinia	Annually	Culture	Yes	Yes
E. coli K88	Annually	Culture	Yes	Yes
Salmonella	Annually	Culture	Yes	Yes
Viruses				
PCV2	Quarterly	ELISA & rt-PCR	Yes	Yes
PCV1	Annually	PCR	No	Yes
PLHV2	Quarterly	rt-PCR	Yes	Yes
PCMV	Quarterly	rt-PCR	Yes	Yes
RotaV A-C	Annually	RT-PCR	Yes	Yes
ReoV	Annually	RT-PCR	Yes	Yes
PTV	Annually	rtRT-PCR	Yes	Yes
PEVB	Annually	RT-PCR	Yes	Yes
PHEV	Annually	RT-PCR	Unknown	Yes
HEV	Quarterly	ELISA & RT-PCR	Yes	Yes
BVD	Quarterly	ELISA	Yes	Yes
AujD	Annually	ELISA	No	Yes
PPV	Quarterly	ELISA & rt-PCR	Yes	Yes
PRRSV	Annually	ELISA & PCR	No	Yes
EMCV	Annually	VNT & rtRT-PCR	Yes	Yes
Protozoa				
Toxoplasma	Quarterly	LAT	Yes	Yes

[a]PCV2, Porcine circovirus type 2; PCV1, porcine circovirus type 1; PLHV2, porcine lymphotropic herpesvirus type 2; PCMV, porcine cytomegalovirus; RotaV A-C, rotavirus A, rotavirus B, and rotavirus C; ReoV, reovirus (all types); PTV, porcine teschovirus; PEVB, porcine enterovirus B; PHEV, porcine hemagglutinating encephalomyelitis virus; HEV, hepatitis E virus; BVD, bovine virus diarrhea; AujD, Aujezsky's disease; PPV, porcine parvovirus; PRRSV, porcine reproductive and respiratory syndrome virus; EMCV, encephalomyocarditis virus.
[b]MAT, microscopic agglutination test; ELISA, enzyme-linked immunosorbent assay; LAT, latex agglutination test; PCR, conventional nested PCR; RT-PCR, reverse transcriptase PCR; rt-PCR, real-time PCR; rtRT-PCR, real-time reverse transcriptase PCR; VNT, virus neutralization test; N/A, not applicable.

New and underappreciated topics not addressed in the original statement including other opinions and perspectives

Diagnostic capabilities have evolved and improved since 2009. Technology platforms including microarray/gene chip, deep/high-throughput DNA and RNA sequencing, MALDI-TOF MS, and xPCR may facilitate rapid, precise, and accurate assessment of pathogen status of donor animals, in-process materials, and final islet product preparations as diagnostic capabilities improve and new methods are developed. Studies have shown that digital PCR (dPCR) offers greater sensitivity and reproducibility; however, the role of dPCR within the diagnostic laboratory has yet to be confirmed and methods need to be further optimized to match the sensitivity of real-time PCR [15,16]. Deep sequencing also has great potential but is currently not a feasible option for most laboratories due to the high cost associated when performing routinely, the bioinformatics burden, and the potential for sequence variations to produce false-negative results. Currently, the ability to assess the presence of unknown and some adventitious viruses relies on time-consuming and costly *in vitro* coculture/cytotoxicity assays which limits the ability to determine a complete safety profile solely on a labile islet product with a typical culture period of days to weeks and which have been shown to lack sensitivity in the case of certain viruses [17,18]. In the future, if alternative methodologies are developed [19] and validated (e.g., per CLSI, ISO, or AAVLD guidelines), they may enable the screening of semen for safe genetic introduction into DPF closed herds. The ability to determine complete pathogen profiles on transplant materials prior to transplantation may shift the safety release point further toward the final islet product. Suitably validated assays, depending on the specific agents, could even be used to demonstrate the absence of specific agents in islet products derived from positive donors.

Immunoisolated islet xenografts such as micro- and macro-encapsulated islet products in the

absence of immunosuppressants offer different risk profiles than islet xenografts protected from immune response by immunosuppressants but not immunoisolation [4]. Depending upon the physical characteristics of the immunoisolation material, infectious agents including viral particles could be sequestered. Furthermore, in the event an infectious agent escapes the immunoisolation material, a normal recipient immune response would be more likely to react to and indicate the presence of the agent rather than allow for the agent's adaptation, increased virulence, and replication which is the main public health concern regarding xenotransplantation. Finally, in certain instances, the encapsulation of islets can permit extended *in vitro* survival which provides the time necessary for thorough microbial screening including coculture assays for unknown viruses [20]. Arguments such as these should be taken into consideration with respect to intended use when preparing specific porcine islet product risk profiles.

The understanding of PERV risks continues to evolve. The diagnostic tools available to assess PERV genetic and expression profiles in donor animals, organs, raw and in-process materials, and final islet products have evolved facilitating further study and understanding of the potential, albeit unlikely, risks PERV infection poses. Methods have been developed to screen for the absence of intact genomic PERV-C *env* sequences (hereafter referred to as "PERV-C negative"), which due to sequence variations require multiple assays [21] as well as the PERV mRNA expression levels [5,8,22–24], allowing for the potential selection of donor animals and porcine islet products. Methods being developed in the field of epigenomics promise to illuminate the role of epigenetic transcriptional silencing of retrovirus [25] and PERV expression [26]. The appropriate role of the PERV infectivity assay which entails the long-term *in vitro* coculture of islet product or surrogate donor cells with susceptible human cells for the assessment of PERV infectivity potential remains uncertain. The PERV infectivity assay represents the "gold standard" assay for the release of human tropic infective PERV-A and PERV-B by coculturing—as an islet product surrogate—mitogen-stimulated donor PBMCs (representing highest possible infective particle release [23]) or non-stimulated donor PBMCs with susceptible 293 human embryonic kidney cells. Similar to adventitious virus coculture assays, the PERV infectivity assay system is problematic for islet product release except in cases where prolonged islet culture is feasible due to the prolonged amount of time required to complete the assay. Interestingly, it has been demonstrated that PERV expression levels in the pancreas are consistently lower than other analyzed organs [8,27]. Furthermore, in cases of low PERV-A, PERV-B, and, if present, PERV-C expression levels, it is thought that the release of infectious PERV particles is highly improbable, and as a result, the use of PERV infectivity assays to characterize individual donor animals, donor materials, and islet products may not be required. Therefore, the PERV infectivity assay is suitable as a component of PERV risk assessment (i.e., on founder lines, breeding pairs, sows, or other donor surrogates such as sentinel animals) to demonstrate non-infectivity along with characterized herd and donor PERV expression levels. The appropriate frequency and proportion of herd requiring infectivity assays on an ongoing basis will require agreement by regulatory authorities. With further study and consistent low PERV expression levels, the necessity of infectivity assays for porcine islet product safety release should be minimal and could readily be replaced by a suitable combination of more timely and simpler assays to determine the absence of spliced PERV env mRNA, PERV RNA, PERV Env protein, and intact PERV particles. Finally, regarding PERV risk assessment, it was the consensus of most ICCIX participants that PERV-C-negative animals and materials are not an absolute requirement for suitable islet xenograft materials but rather that low PERV subtype expression levels and demonstrated lack of infectivity are of primary import. This consensus is in alignment with the authors' feedback from the USA and NZ regulatory authorities.

A "Barrier facility" by definition is highly biosecure and is recommended by guidance [28] and echoed in the 2009 consensus statement to be built and operated in accordance with National Research Council's *Guide for the Care and Use of Laboratory Animals "The Guide"* [29] as a research laboratory animal facility and accredited by AAALAC. This represents a standard, albeit the "gold standard" of laboratory animal care, that is misaligned with the purpose of a DPF islet donor animal production facility—to humanely and efficiently produce agricultural DPF donor animals and resultant safe donor-derived materials including porcine islet products. Therefore, as long as biosecurity is maintained and SOPs and cGMPs are followed, DPF porcine islet product donor production facilities built and operated in accordance with the AAALAC agricultural standard the *Guide for the Care and Use of Agricultural Animals in Research and Teaching,* the *"Ag Guide"* [30] or alternate standard of care such as the UK Farm Animal Welfare Council's 5 Freedoms [31] could

arguably be considered more appropriate. This would enable DPF biosecure facilities built and operated in accord with agricultural standards to more efficiently scale up manufacturing of safe islet products.

The issue of transmissible prion-based disease was also addressed in 2009. Naturally occurring TSEs have not been identified in pigs to date [32–34]. FDA guidance to use feed free of rendered or recycled mammalian materials for at least two generations before islet donation has been utilized to mitigate potential TSE transmission risk by xenotransplantation [28] and was restated in the 2009 consensus statement. The original FDA guidance was broadly written to also address the potential use of non-porcine materials such as those derived from cattle, a species where the naturally occurring BSE has been linked with the human new-variant Creutzfeldt–Jakob (vCVD) disease [33,35]. BSE is the only TSE known to infect pigs, but both the interspecies oral transmission probability and efficiency are low [36–39]. Furthermore, the vertical (i.e., intergenerational) transmission of BSEs by non-bovine species of animals experimentally infected with BSE has not been demonstrated [40,41]. Therefore, the authors believe that a more appropriate standard, specifically in the case of porcine xenotransplantation products including islets, would be the elimination of rendered or recycled bovine materials from the diets of donor animals at minimum and their progenitors at maximum which is consistent with numerous governmental regulations banning recycled bovine materials in agricultural feed for animals reared commercially for consumption.

Suggested pertinent revisions

International guidances vary with respect to archival periods of source animal and recipient samples, ranging from a minimum of 30 yrs by EMEA [42] to indefinitely or at minimum 20 yrs after death by Swissmedic [43].

The 2009 consensus statement echoed the US FDA *Guidance for industry* by proposing an archival period of donor materials to enable the assessment of unknown pathogens for up to 50 yrs [28] and recommending that governmental agencies take ownership of and responsibility for establishing and maintaining such archives. These archives are specifically designed to safeguard against unknown xenozoonotic agents (not PERV) that might not be identified prior to transplantation, but the archival durations are arbitrary. IXA reached consensus reaffirming the importance of such archives and governmental roles and responsibilities proposing that clinical sponsors should be responsible for the archival of donor materials until the death of the recipient if conclusively determined to be of unrelated and non-infectious cause or for a reasonable period (i.e., 5 to 10 yrs) after which respective governmental agencies are encouraged to assume ownership and responsibility for archive if the responsible governmental agency deems further retention necessary.

Summary and conclusion

The original consensus statement sets a reasonable bar at its time for the activities related to source pigs used in the preparation of clinical porcine islet products and still serves as an excellent platform from which to proceed given interim progress in the field. A summary of salient revisions to the original consensus statement is as follows:

1. Donor animal pathogen screening strategy should be geographically appropriate, product specific, adaptive, and dynamic.
2. As new rapid diagnostic technologies are developed and validated, they may enable the direct screening of islet products themselves.
3. Encapsulated islet products present different risk profiles than non-encapsulated islets primarily due to the lack of recipient immunosuppression. Some encapsulation methods enable *in vitro* islet culture of sufficient duration to perform viral screening on islet products prior to transplantation.
4. While PERV-C-negative donor animals could be considered preferable, PERV animal selection criteria should be primarily based on low PERV expression levels and lack of infectivity.
5. Biosecure DPF animal facilities built to agricultural standards could be considered as appropriate source animal facilities if operated under SOPs and cGMPs.
6. The elimination of bovine products from the feed of donor animals throughout their lifetime should sufficiently mitigate TSE risk.
7. The sponsor's responsibility to archive donor samples should be for a limited duration and transferred to the appropriate regulatory government agency if additional duration is required.

Clinical safety data continue to accumulate; diagnostic capabilities improve; improved clinical protocols are under development to address safety and rejection issues; PERV-associated infectious risks continue to be understood; DPF facility

design and husbandry standards including modified feed restrictions evolve; and archival roles, responsibilities, and terms clarify. As a result, we believe the cost-effective production of safe porcine islet products from suitable source pigs will ultimately be a viable means to address the vast unmet need for clinical islet replacement. This chapter regarding source pigs should serve, until modified in a subsequent review, to guide investigators and source pig/islet product producers with their programs and regulatory discussions.

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Xenotransplantation 2016: 23: 32–37
doi: 10.1111/xen.12224

Original Article

First update of the International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes—Chapter 2b: genetically modified source pigs

Cowan PJ, Ayares D, Wolf E, Cooper DKC. First update of the International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes—Chapter 2b: genetically modified source pigs. Xenotransplantation 2016: 23: 32–37. © 2016 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd.

Abstract: Genetic modification of the source pig offers the opportunity to improve the engraftment and survival of islet xenografts. The type of modification can be tailored to the transplant setting; for example, intraportal islet xenografts have been shown to benefit from the expression of anticoagulant and anti-inflammatory transgenes, whereas cytoprotective transgenes are probably more relevant for encapsulated islets. The rapid development of pig genetic engineering, particularly with the introduction of genome editing techniques such as CRISPR-Cas, has accelerated the generation of new pig lines with multiple modifications. With pre-clinical testing in progress, it is an opportune time to consider any implications of genetic modification for the conditions for undertaking clinical trials. Obviously, the stringent requirements to fulfill designated pathogen-free status that are applied to wild-type pigs will apply equally to genetically modified (GM) source pigs. In addition, it is important from a safety perspective that the genetic modifications are characterized at the molecular level (e.g., integration site, absence of off-target mutations), the phenotypic level (e.g., durability and stability of transgene expression), and the functional level (e.g., protection of islets in vitro or in vivo, absence of detrimental effects on insulin secretion). The assessment of clinical trial protocols using GM pig islets will need to be performed on a case-by-case basis, taking into account a range of factors including the particular genetic modification(s) and the site and method of delivery.

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Key words: genetic modification – genome editing – islet xenotransplantation – multitransgenic – site-specific nuclease

Abbreviations: αGal, galactose-α1,3-galactose; Cas, CRISPR-associated system; CRISPR, clustered regularly interspaced short palindromic repeat; DSB, double strand break; GM, genetically modified; GTKO, α1,3-galactosyltransferase gene knockout; h, human; IBMIR, instant blood-mediated inflammatory reaction; Neu5Gc, N-glycolylneuraminic acid; RGN, RNA-guided endonuclease; TALEN, transcription activator-like effector nuclease; tracrRNA, target-independent trans-activating crRNA; TFPI, tissue factor pathway inhibitor; ZFN, zinc-finger nuclease.

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Received 8 February 2016;
Accepted 8 February 2016

Introduction

Source pigs are necessarily a key consideration in the formulation and revision of guidelines for clinical trials in islet xenotransplantation. Chapter 2 of the first IXA porcine islet consensus statement, published in *Xenotransplantation* in 2009 (and updated in this issue), focused on the conditions required for source pigs to fulfill designated pathogen-free status [1]. However, the scope of the initial document did not extend to the use of genetically modified (GM) pigs as "donors".

Our task in this section is to: (i) review the rationale for genetic manipulation of the donor pig and present evidence for the efficacy of existing modifications; (ii) summarize the rapidly advancing

technology for introducing multiple genetic modifications into the pig; (iii) address the question of genotypic and phenotypic stability, that is, the need for genetic "quality control"; (iv) describe assays to assess the effects of genetic engineering on islet function; and (v) discuss the potential safety implications of using GM donors.

Breeding and maintaining GM animals *per se* is subject to regulation; however, as regulatory requirements will likely vary from case to case and from country to country, a discussion of this issue is beyond the scope of this chapter.

Genetic modification of the donor pig to improve islet engraftment

The primate response to porcine islet xenografts depends on several factors, including the type of islets (adult, neonatal, fetal), the mode of delivery ("naked," microencapsulated, macroencapsulated), and the transplant site (intraportal, intraperitoneal, subcutaneous, intramuscular, bone marrow, etc.). This in turn will determine what type of genetic modification may be applicable.

In current clinical allotransplantation practice (with intraportal delivery of naked islets), engraftment can be compromised by the instant blood-mediated inflammatory reaction (IBMIR), which mediates early destruction of a significant islet mass through activation of complement and coagulation and infiltration by innate immune cells [2,3]. IBMIR is likely to be exacerbated in recipients of intraportal porcine islet xenografts by the action of pre-existing anti-pig antibodies [4,5].

An obvious genetic approach to improve engraftment is to modify the islet surface to eliminate xenoantigens and/or to express regulators of complement, coagulation, and platelet activation. Deletion of *GGTA1*, the gene encoding α1,3-galactosyltransferase, abolishes expression of the carbohydrate xenoantigen galactose-α1,3-galactose (αGal) and reduces the incidence of hyperacute rejection of solid organ xenografts in the non-human primate model [6]. This modification, termed GTKO, improved the engraftment and survival of neonatal pig islets transplanted intraportally in macaques, with evidence of reduced intrahepatic inflammation [7]. Interestingly, GTKO had no apparent impact on the early loss of adult pig islets transplanted into cynomolgus monkeys [8]. This lack of effect may be explained by the decline in αGal expression by pig islets as they mature [9]. Similarly, transgenic expression of the human complement regulator CD46 (hCD46) did not reduce early loss of adult pig islets, although it did promote long-term xenograft survival [8]. Nevertheless, the combination of GTKO with expression of a human complement-regulatory protein(s) is now generally regarded as the best platform on which to build further genetic modifications for both neonatal and adult islet xenotransplantation [10,11], just as it is for solid organ xenotransplantation [12]. A recent pig-to-baboon study showed that thrombosis induced by neonatal wild-type islets, while refractory to treatment with recombinant antithrombin, was dramatically reduced when GTKO/hCD55/hCD59 pigs were used as donors and the recipients were immunosuppressed [11]. Thus, the combination of GTKO with one or more complement inhibitor transgenes appears to improve xeno-islet transplant outcomes.

Two transgenes specifically designed to inhibit islet-mediated coagulation and thrombosis have been tested in the adult pig-to-cynomolgus monkey intraportal islet xenograft model [10]. Tissue factor pathway inhibitor (TFPI) regulates the extrinsic coagulation pathway, which appears to be pivotal to IBMIR, and CD39 inhibits platelet activation and inflammation. Transgenic overexpression of hCD39 in mouse islets has been shown to protect against IBMIR [13] and to protect islets from T cell-mediated injury in vivo [14]. When hTFPI and/or hCD39 were expressed through a beta cell-specific promoter on the GTKO/hCD46 background, there was evidence of a reduction in early islet xenograft destruction (e.g., reduced release of porcine C-peptide) and inflammation (reduced serum IL-6) [10]. However, the study was limited by the availability of the multitransgenic pigs and did not demonstrate a consistent improvement in long-term islet xenograft function.

It will be interesting to test whether expression of human thrombomodulin ± human endothelial protein C receptor on porcine islets further attenuates IBMIR. Thrombomodulin regulates the propagation phase of coagulation by converting thrombin's activity from procoagulant to anticoagulant; endothelial protein C receptor facilitates this process and has independent anti-inflammatory and cytoprotective properties. These transgenes have been shown to be beneficial in models of cardiac [15] and lung [16] xenotransplantation, respectively.

It is conceivable that further carbohydrate "remodeling" may improve the engraftment of intraportal islet xenografts. Pigs express N-glycolylneuraminic acid (Neu5Gc), which is recognized as the Hanganutziu-Deicher antigen by pre-existing antibodies in humans. To our knowledge, the presence of Neu5Gc on porcine islets has not been examined, nor whether its expression changes with maturity, analogous to αGal. The *CMAH* gene responsible for

Neu5Gc synthesis has been knocked out in pigs [17], although pre-clinical testing of the effect of this mutation would need to be performed in New World monkeys as they, like humans but unlike Old World primates, do not express Neu5Gc [18].

Thus far we have discussed only genetic modifications that affect the islet surface and are, therefore, more applicable to naked, but not encapsulated, islets. In contrast, intracellular cytoprotective molecules, such as heme oxygenase-1 and A20, may be relevant to both. Adenovirus-mediated expression of human heme oxygenase-1 has been shown to protect neonatal pig islets from the effects of inflammatory cytokines and oxidative stress in vitro [19], and transgenic pigs expressing human heme oxygenase-1 [19,20] or hA20 [21] have been generated. Transgene expression in islets from these pigs was either absent [19] or not reported [21], so in vivo testing of the potential benefit of these molecules to islet xenotransplantation has not yet been performed.

Genetic modification of the donor pig to prevent islet xenograft rejection

Minimizing early injury to islet xenografts, as described in the previous section, may reduce the antigenic stimulus to the adaptive immune system. Nevertheless, the T cell-mediated cellular immune response remains a significant barrier to successful islet xenotransplantation.

Two transgenic modifications designed to inhibit co-stimulation of T cells have been introduced into pigs: islet-specific secretion of LEA29Y (a high-affinity variant of human CTLA4-Ig) [22] or of pig CTLA4-Ig [10]. LEA29Y-expressing neonatal pig islets were protected from rejection for 28 days when transplanted into humanized mice [22], although they have not yet been tested in the non-human primate model. Expression of pig CTLA4-Ig provided no consistent long-term survival benefit versus GTKO/hCD46 adult pig islets when transplanted into cynomolgus monkeys [10]. Importantly, however, analysis of the latter pigs showed that islet-specific expression of up to 3 transgenes (plus ubiquitous expression of a fourth) had no overt detrimental effects on islet function [23].

New techniques for rapid multigenic modification

In recent years, several programmable nuclease-based technologies have been developed, providing new opportunities for genetic modification of pigs. Custom-designed nucleases produce site-specific DNA double strand breaks (DSBs), thereby triggering the endogenous DNA repair system. DSBs are repaired by two major mechanisms—non-homologous end joining or homology-directed repair [24]. Both repair pathways can be used to modify a specific target site (= gen(om)e editing). In the absence of a repair template, DSBs created by site-specific nucleases are repaired via error-prone non-homologous end joining through direct ligation of the break ends, often causing small insertions or deletions (indels). This can lead to a frameshift in the coding region, resulting in loss of function of the affected gene. More precise changes (specific point mutations or targeted insertions) can be introduced by homology-directed repair, achieved by including with the nuclease a donor template (DNA or single-strand oligodeoxynucleotides) containing homology arms corresponding to the target region.

Three major classes of customizable nucleases have been generated for genome editing in pigs—zinc-finger nucleases (ZFNs) [25], transcription activator-like effector nucleases (TALENs) [26], and, most recently, RNA-guided endonucleases (RGNs) [27]. One advantage of these nuclease-based systems over traditional homologous recombination methods is that they do not necessarily require somatic cell nuclear transfer, but can work via the less technically demanding method of cytoplasmic injection into zygotes. Both ZFNs and TALENs are artificial fusion proteins that contain a site-specific DNA-binding domain (zinc-finger motif or truncated transcription activator-like effector domain) and a non-specific DNA cleavage domain derived from the *Fok*I restriction enzyme. As *Fok*I must dimerize for DNA cleavage to occur, two appropriately targeted monomers are required to form an active nuclease, improving specificity.

RGNs derived from the bacterial CRISPR-Cas system have been used for genome editing in a variety of cells and organisms. CRISPR (clustered regularly interspaced short palindromic repeats) sequences and Cas (CRISPR-associated system) proteins are the two elements of an ancient prokaryotic adaptive restriction system. CRISPRs represent a repository of short, directly repeating nucleotide sequences that alternate with small unique DNA fragments acquired from invading bacteriophages or plasmids, thus forming a type of memory system [28]. These CRISPR regions are transcribed into target-specific crRNA and target-independent trans-activating crRNA (tracrRNA), which hybridize and form a complex with Cas nuclease that recognizes and cleaves foreign genetic material matching the CRISPR-derived RNA [29]. This complex system has been developed into an elegant genome editing tool by fusing the crRNA and the tracrRNA into a synthetic, small guide

RNA, that is, a hairpin RNA structure resembling the tracrRNA linked to a 20-bp sequence homologous to the target DNA.

RGNs have at least two advantages over ZFNs and TALENs: (i) the design and preparation are simple, as no complicated protein engineering is necessary and (ii) it is possible to modify several genes simultaneously using different small guide RNAs, allowing for efficient multiplex genome editing [30]. In 2014, knockout pigs were generated by zygote injection of CRISPR-Cas [27], demonstrating that RGNs are a promising tool for efficient and rapid genome editing in large animals. Recently, CRISPR-Cas has been used to generate pigs with mutant *GGTA1*, *CMAH* and putative *iGb3S* genes [31] and pigs lacking functional class I MHC alleles [32].

Genetic and functional quality control

Quality control for GM pigs and their islet products includes novel considerations in addition to those for wild-type pigs, including characterization of vector DNA, number of integrated copies, and transgene insertion site(s). Methods include quantitative polymerase chain reaction (qPCR), Southern blot hybridization, DNA fluorescence in situ hybridization (FISH), inverse PCR or thermal asymmetric interlaced (TAIL)-PCR, and next generation sequencing. The potential side effects of random integration can be avoided using recombination-mediated cassette exchange to insert expression vectors into "safe harbors" of the pig genome, such as the *ROSA26* locus [33]. If site-directed nucleases are used for genetic modification, the risk of off-target cleavage can be minimized by selecting optimal target sites using special bioinformatics tools, such as COSMID (CRISPR Off-target Sites with Mismatches, Insertions, and Deletions) [34].

Transgene RNA quantitation is classically performed by reverse transcriptase qPCR or northern blot hybridization of mRNA from isolated islets. At the protein level, the transgene product may be detected in pancreatic sections by immunohistochemistry, in isolated and dissociated islets by flow cytometry, and in islet protein extracts by Western blot or by other antibody-based methods such as enzyme-linked immunosorbent assay or radioimmunoassay.

The functional evaluation of the transgene product depends on its biological target. Examples are *ex vivo* clotting assays in human blood [13] to test genetic modifications directed toward overcoming IBMIR, or islet xenotransplantation into humanized diabetic mice to test genetic modifications addressing cellular immune rejection [22]. However, xenotransplantation of porcine islets into diabetic non-human primates remains the gold standard.

Although no major disturbances of glucose homeostasis and insulin secretion have been observed in multimodified donor pigs for islet xenotransplantation [23], the spectrum of physiological tests (fasting blood glucose levels, insulin, C-peptide, and glucagon responses to both glucose and arginine challenge) provides a solid basis for detecting disturbances of islet function which may result from even more complex genetic modifications.

Potential safety implications of GM donors

It is anticipated that islets from GM donor pigs will create a different safety and efficacy profile, and therefore a modified regulatory path, than islets from wild-type pigs. This different path will likely be triggered regardless of whether the genetic modification was due to gene knockout or insertion. However, each unique type of GM donor and its resulting GM islet derivative will need to be managed on a case-by-case basis. Assessment will differ based on different country-specific regulatory agencies and will depend on a variety of factors specific to the actual islet product. These factors include the genetic modification(s) present in the donor/islet, donor age (neonatal vs. adult), site of delivery (e.g., intraportal, subcutaneous, kidney capsule, peritoneum), method of delivery (e.g., encapsulated vs. naked islets), and number of islets transplanted. For example, the number of GM islets transplanted might be considerably lower than that required using wild-type islets due to the beneficial effects of transgenic modifications on islet survival and/or reduced cytotoxicity post-transplant.

Looking specifically at the nature of the genetic modification(s) in the donor islets and their impact on safety and related regulatory considerations, as described above for efficacy, it will be important from a safety perspective to have full molecular characterization of the DNA construct and the genomic integration site(s), phenotypic characterization of the donor animal (including health and viability of the pig), phenotypic characterization of transgene function and expression level, durability of the phenotype, and stability across multiple generations.

Containment and environmental considerations around the GM donor will also be important with respect to safety in regard to (i) keeping GM donors out of the food chain, (ii) housing and growth of a specific GM donor in a designated

pathogen-free facility if certain genetic modifications result in an immunocompromised pig, and (iii) excluding specific zoonotic pathogens (same as for wild-type donors/islets) according to regulatory guidelines. With these provisos, it is not anticipated that designated pathogen-free pig facilities would need to be significantly different for GM vs. wild-type donors, as they would be expected to similarly exclude the exogenous pathogens (viral or otherwise) described in detail in other sections of this consensus document.

In the USA, certain aspects of the donor pig (including containment, environment, and details of the genetic modification) are regulated by the Center for Veterinary Medicine (CVM), while the human aspects of islet xenotransplantation, including clinical trials (treatment of diabetes with GM islet products), would be regulated by another FDA agency, the Center for Biologics Evaluation and Research (CBER). Approval in each country would need to follow its own safety and efficacy guidelines.

While a number of specific genetic modifications with potential benefits for xeno-islet survival and function have been discussed here, it is premature to state which modification or combination of modifications will be essential for clinical application. If more than one genetic modification is required, the individual knockout or transgene addition needs to be well-characterized from a molecular and stability perspective, but also the combined phenotype and synergies of the transgene combination become the primary targets for safety and efficacy testing. Regulatory agencies are starting to embrace gene stacking modalities, and their requirements will likely evolve with these rapidly advancing technologies.

Whether one or multiple genetic modifications are required for bioactive gene products, there are likely safety benefits both for health of the donor pig and the islet recipient if the transgenes are under control of an islet-specific promoter, so that the therapeutic is delivered locally at the level of the islet cell rather than systemically (10, 22, 23). In addition, as part of the molecular characterization of the GM event(s), from a safety and regulatory perspective, there will be a need to demonstrate the absence of off-target events. Inactivation of essential genes or activation of deleterious genes (such as proviruses or oncogenes) has to be ruled out. The new gene editing systems described above, while highly efficient, can increase the potential for off-target events, and can generate very small indels that are difficult to track. Programs such as COSMID can reduce these CRISPR-based off-target events, and it seems likely that the development of genome analysis technology will advance as rapidly and as efficiently as the genome editing tools.

Conclusions

Recent data from the non-human primate pre-clinical model suggest that genetic modification of the donor pig will be important, perhaps essential, for the success of clinical porcine islet xenotransplantation. What can be achieved is no longer limited by the technology; it is now possible to precisely modify the pig genome in ways that would have been inconceivable a decade ago. The use of GM donors may entail safety and regulatory considerations above and beyond those for wild-type pigs, but it should be possible to manage these issues on a case-by-case basis.

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Xenotransplantation 2016: 23: 38–45
doi: 10.1111/xen.12225

Original Article

First update of the International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes – Chapter 3: Porcine islet product manufacturing and release testing criteria

Rayat GR, Gazda LS, Hawthorne WJ, Hering BJ, Hosking P, Matsumoto S, Rajotte RV. First update of the International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes – Chapter 3: Porcine islet product manufacturing and release testing criteria. Xenotransplantation 2016: 23: 38–45. © 2016 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd.

Abstract: In the 2009 IXA consensus, the requirements for the quality and control of manufacturing of porcine islet products were based on the U.S. regulatory framework where the porcine islet products fall within the definition of somatic cell therapy under the statutory authority of the U.S. Food and Drug Administration (FDA). In addition, porcine islet products require pre-market approval as a biologic product under the Public Health Services Act and they meet the definition of a drug under the Federal Food, Drug, and Cosmetic Act (FD&C Act). Thus, they are subject to applicable provisions of the law and as such, control of manufacturing as well as reproducibility and consistency of porcine islet products, safety of porcine islet products, and characterization of porcine islet products must be met before proceeding to clinical trials. In terms of control of manufacturing as well as reproducibility and consistency of porcine islet products, the manufacturing facility must be in compliance with current Good Manufacturing Practices (cGMP) guidelines appropriate for the initiation of Phase 1/2 clinical trials. Sponsors intending to conduct a Phase 1/2 trial of islet xenotransplantation products must be able to demonstrate the safety of the product through the establishment of particular quality assurance and quality control procedures. All materials (including animal source and pancreas) used in the manufacturing process of the porcine islet products must be free of adventitious agents. The final porcine islet product must undergo tests for the presence of these adventitious agents including sterility, mycoplasma (if they are cultured), and endotoxin. Assessments of the final product must include the safety specifications mentioned above even if the results are not available until after release as these data would be useful for patient diagnosis and treatment if necessary. In addition, a plan of action must be in place for patient notification and treatment in case the sterility culture results are positive. In terms of the characterization of porcine islet products and product release criteria, the information on the porcine islet products should be acquired from a

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Key words: good manufacturing practices – porcine islets of langerhans – type 1 diabetes – xenotransplantation

Abbreviations: cGMP, current Good Manufacturing Practices; FDA, Food and Drug Administration; IXA, International Xenotransplantation Association; FD&C Act, Federal Food, Drug, and Cosmetic Act; ICH, International Conference on Harmonisation; PERV, porcine endogenous retrovirus; PHS, Public Health Services; SOPs, Standard Operating Procedures.

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Received 8 February 2016;
Accepted 8 February 2016

sample of the final product to be used for transplantation and must include the morphology of the islets, specific identity, purity, viability, and potency of the product. In addition, information on the quantity of the islet products should also be provided in a standardized fashion and this should be in terms of islet equivalents and/or cell numbers. The current consensus was created to provide guidelines that manufacturing facilities may find helpful in the manufacture of and the release criteria for porcine islet products including encapsulated islets and combined islet products. Our intent with the above recommendations is to provide a framework for individual porcine islet manufacturing facilities to ensure a high level of safety for the initiation of Phase 1/2 clinical trials on porcine islet xenotransplantation.

Introduction

In the 2009 IXA consensus [1], the requirements for the quality and control of manufacturing of porcine islet products were based on the U.S. regulatory framework where the porcine islet products fall within the definition of somatic cell therapy under the statutory authority of the U.S. Food and Drug Administration (FDA) [2,3]. The FDA guidance was adopted from the International Conference on Harmonisation (ICH), which has published a number of documents that have relevance to the use of xenotransplantation products in humans [4–6]. In addition, the U.S. Public Health Services (PHS) published a draft of their guideline on infectious disease issues in xenotransplantation that the FDA guidance document restated. As such, porcine islet products require pre-market approval as a biologic product under the PHS Act [7]. Porcine islet products also meet the definition of a drug under the Federal Food, Drug, and Cosmetic Act and are subject to applicable provisions of the law [8]. As with other somatic cell therapies and human islet products, control of manufacturing as well as reproducibility and consistency of porcine islet products, safety of porcine islet products, and characterization of porcine islet products must be met before proceeding to clinical trials [9,10]. The current consensus will update and provide guidelines that manufacturing facilities may find helpful in the manufacture of and the release criteria for porcine islet products including encapsulated islets and combined islet products.

Criteria for the control of manufacturing, reproducibility and consistency of porcine islet products

To affirm control of manufacturing as well as reproducibility and consistency of porcine islet products, the manufacturing facility must be in compliance with current Good Manufacturing Practices (cGMP) guidelines appropriate for the initiation of Phase 1/2 clinical trials [11,12]. Sponsors intending to conduct a Phase 1/2 trial of islet xenotransplantation products must be able to demonstrate the safety of the product through the establishment of particular quality assurance and quality control procedures. All materials (including animal source and pancreas) used in the manufacturing process of the porcine islet products must be free of adventitious agents. Due to potential infectious disease risks associated with the use of xenotransplantation products, appropriate source animal qualifications should be developed [13]. These qualifications should include herd management and programs for prevention and screening for infectious agents. Although testing of the final xenotransplantation product for infectious agents is crucial, appropriate control of animal sources and husbandry provides important additional assurance for the safety of such products by controlling infections of both known and potentially even unknown agents [10]. The source animals should be derived only from closed herds with documented health screening programs as discussed in Chapter 2a (Source Pigs—Preventing Xenozoonoses). In addition, the welfare of the source animals should be considered. Procedures for animal husbandry, tissue harvesting, and termination of animals should be approved by an appropriate Institutional Animal Care and Use Committee, in accordance with the Animal Welfare Act (7 U.S.C. 2131, et seq.) [13]. If the funds are received from the PHS, manufacturing of porcine islet products must also comply with the PHS Policy on Humane Care and Use of Laboratory Animals according to section 495 of the Public Health Service Act (42 U.S.C. 289(d)) [13]. In addition, these animals should be shown to have pancreata that yield suitable numbers and quality of islets for undertaking the reliable and reproducible manufacture of the particular islets the facility is producing.

In particular, with respect to the manufacturing of adult pig islets, the manufacturing program

should ensure that only suitable donor pancreata with a maximal potential for yielding adequate numbers of islets and demonstrated islet function are used for the manufacture of islets for transplantation. There exists a tremendous variability among potential donor source herds and their pancreata, and not all pancreases are suitable for processing (in terms of cost/islet). It is important to start with good quality organs to maximize islet yield, which may allow sufficient islet numbers from fewer pig donors to restore normoglycemia in a single human patient and thus reducing the risk of potential xenozoonoses from the use of larger numbers of source pigs. Additionally, this aligns well with the principles of the 3Rs (Replacement, Reduction, and Refinement). Several manufacturing facilities have developed methods to maximize islet yield using good quality pancreases [14–16]. Warm ischemic injury must be avoided, and cold ischemic time should be minimized to maximize islet yield. Each facility must qualify the donor organs that they accept, and donor selection must adhere to stringent standards. For example, some facilities obtain biopsy from multiple pancreases to determine the best pancreas for digestion and exclude a donor pancreas that is of a poor quality due to the presence of abscess, suspected tumor mass, or other condition potentially unsafe for use in the manufacture process. Color of the pancreas, fat content, islet size, and islet demarcation have also been used as indications of the quality of the pancreas. Islet number (few or numerous) is evaluated informally as this does not always predict the final yield. It is important that information on the donor herd, individual pig donor (including parents and health history), and donor pancreas be recorded and archived.

An appropriate facility for the manufacture of porcine islets is also necessary to provide a high level of safety of the final islet product. The islet facility must be designed to prevent adventitious bacterial and viral contamination of the islets as well as unintended mixing of islet batches to assure the identity of the final product throughout processing and prior to transplantation into a patient. Procedures within the facility, including isolation methods, personnel traffic patterns, cleaning, and environmental monitoring, must be documented as standard operating procedures (SOPs), and the resultant data archived for ease of retrieval for verification and validation of the processes. Batch records must be established and should include complete donor histories (lineage and medical), raw material lot numbers, and in-process data including concentration and volume of enzyme used, digestion length, temperatures during isolation, digestion percentage and packed volumes and islet purity to name a few.

Reproducibility and consistency of porcine islets must be established prior to initiating a clinical trial. Although islets isolated from different pancreases will differ in total yield and insulin secretory capability, parameters for acceptance of isolated islets must be established by each manufacturing facility. For example, a range of islet equivalents per gram of digested tissue could be established as acceptance criteria to at least partially demonstrate a reproducible procedure. Similarly, insulin secretion could also be used as a measure of consistency among different islet isolations.

Criteria to ensure safety of the porcine islet products

To ensure product safety, materials used in the manufacturing process must be free of adventitious agents, and other agents identified in Chapter 2a—Source Pigs—Preventing Xenozoonoses. Porcine endogenous retrovirus (PERV) has been demonstrated to infect human cells and raised concerns that this virus might cause disease in a human recipient of porcine islets, and then spread to close contacts, or to the general population [13]. Recent studies have now shown that infection of human cells with PERV occurs only under unusual circumstances, and PERV appears to require permissive cell types to propagate [13]. The major concern being the presence of PERV provirus in host cells which has the potential for provirus integration resulting in insertional mutagenesis and chromosomal rearrangements [17]. Strategies to diagnose PERV in the recipient of an organ or cell xenograft have been developed, and these include serological and molecular assays, which have the capacity to detect productive infection and to help manage risk for subjects, close contacts, and the general population [13]. In addition, experts in the field now have a better understanding of other known infectious agents that may pose a risk to human recipients of porcine xenografts (Chapter 2a—Source Pigs—Preventing Xenozoonoses). Most known potential pathogens have been excluded from several pig colonies such as the specific pathogen-free pigs [18] and designated pathogen-free pigs that are currently being used for preclinical xenotransplantation research [19] and human clinical trials in New Zealand using Auckland Island pigs [20].

In terms of safety of the islet products, pancreas from designated pathogen-free or specific pathogen-free pig donors [21, and as discussed in Chapter 2a—Source Pigs—Preventing Xenozoonoses] should be used. In addition, procure-

ment of the pancreas must be performed in a manner that limits the risk of adventitious contamination. As such, pancreas must be acquired in an operating room or under equivalent conditions, which may reside either in the animal facility or at another location. If transport of the pigs to a manufacturing facility is required, equivalent biosecurity to that of the source animal facility must be maintained. SOPs for organ procurement, preservation, and processing must be followed by the manufacturing facility. As stated above, the manufacturing of islets must also be performed using aseptic processing and in facilities that are in compliance with cGMP guidelines appropriate for Phase 1/2 trials [11,12]. Materials used during in vitro manufacturing procedures, for example enzymes, antibiotics, chemicals, or solid supports such as beads, can affect the safety, purity, and potency of the final therapeutic product. These components should be clearly identified, and a qualification program should be established for each component to determine its acceptability for use during the manufacturing process. When using reagent grade material, the qualification program should include testing for safety, purity, and potency of the components where appropriate [9]. The reagents utilized for the manufacture of the islet product must also be clearly demonstrated to be free of potential pathogens such as bovine spongiform encephalopathy (BSE).

The final porcine islet product must undergo tests for the presence of adventitious agents including sterility, mycoplasma (if they are cultured), and endotoxin. Both direct and indirect mycoplasma screening should be performed as per USP <63> mycoplasma tests or EP 2.6.7 mycoplasmas. The porcine islet product must be negative for Gram stain and where possible should show negative growth from an aliquot taken of the final product as demonstrated by an automated microbiology growth and detection system designed to detect microbial growth. It should have an endotoxin content of <5.0 EU/kg recipient body weight, and negative results for other infectious agents identified in Chapter 2a—Source Pigs—Preventing Xenozoonoses. Fully validated viral assays are now available from several Good Laboratory Practices compliant contract laboratories for screening of porcine cell products using molecular and coculture assays. Assessments of the final product must include the safety specifications mentioned above even if the results are not available until after release as these data would be useful for patient diagnosis and treatment if necessary. In addition, a plan of action must be in place for patient notification and treatment in case the sterility culture results are positive. Aliquots of islets, serum, and various tissues collected at pancreas procurement should be archived for a minimum of 30 yrs and a maximum of 50 yrs [2,13,22] for future use in recipient diagnosis. As archived samples may not provide reliable results due to lack of correct storage, the quality of storage must be documented if archived samples are used [13]. A review of all donor, islet, and manufacturing records should be completed by the Head of Quality Assurance or team of scientists, virologists, microbiologists, veterinarians, clinicians, etc., as part of the release documentation. The clinician responsible for final transplantation of the product must be aware of and accept all release criteria prior to performing the transplantation of the individual batched product.

Characterization of porcine islet products and product release criteria

The information on the porcine islet products should be acquired from a sample of the final product to be used for transplantation and must include the morphology of the cell, specific identity, purity, viability, quantity, and potency of the product. The most important in the process of quality assurance of the islet product is the assessment of the quality of the islets. The sampling of the product to assess the islet morphology is a good indication of the overall product. Simple macroscopic assessment of the islets can indicate an intact islet of a transplantable size that has no sign of deterioration such as intact membrane structure, granularity around the nucleus, or cytoplasmic vacuolation. This simple macroscopic assessment can also easily indicate any acinar contamination as well as other forms of contamination. The identity of the islet products can also include endocrine cells (beta, alpha, delta, polypeptide, and epsilon), ductal cells, and contaminating acinar and immune cells. The identification of these cells will also provide information in terms of how pure the islet product is. These cells can be identified and quantified using dithizone-stained islets and visualization by conventional light microscope, flow cytometric analysis of stained single islet cells and/or staining of paraffin-embedded or cryopreserved islets or single islet cells. Dithizone staining of the islet products is limited in terms of the identification of specific endocrine and other types of cells in the product samples, and one may consider using antibodies to identify specific cell components of the islet products [23]. Some surface markers cannot be identified using available commercial antibodies on paraffin-embedded islet sections thus may require that the samples be

cryopreserved. A list of antibodies that could be used in the identification of cells in the islet products is included in Table 1. The viability of the islet products should also be provided and can be measured using live and dead assays. These assays utilize dyes such as the cell permeable esterase-substrate fluorescein diacetate (FDA) to demonstrate live cells that actively convert the non-fluorescent FDA into its green fluorescent product, an indication of viability. The cell-impermeant nucleic acid dye propidium iodide (PI) or ethidium bromide is used to stain the nuclei of membrane-compromised cells which fluoresce red/orange indicating cell death. These assays can also include acridine orange in combination with ethidium bromide to differentiate between viable, apoptotic, and necrotic cells. Acridine orange will stain both live and dead cells, while ethidium bromide will stain only cells that have lost membrane integrity. Live cells when stained with acridine orange will appear uniformly green, while apoptotic and necrotic cells will stain orange due to the incorporation of ethidium bromide [24]. The stained cells can be visualized using flow cytometry for single islet cells or fluorescent microscope for both intact islets and single islet cells.

The information on the quantity of the islet products should also be provided in a standardized fashion, and this should be in terms of islet equivalents and/or cell numbers. An example of the form one can use in assessing the quantity of islets in terms of islet equivalents is shown in Fig. 1. The potency or function of the porcine islet products must be demonstrated by *in vitro* insulin secretory capacity, insulin content relative to islet equivalents or corrected for DNA. *In vitro* insulin capacity of the islet products can be measured using static glucose stimulation assay where aliquots of the islet products are stimulated with low and high glucose concentrations, and the amount of insulin released after the glucose challenge is measured from the culture media using available commercial assay kits [23]. The immaturity of fetal and in part also of neonatal porcine islet tissue products precludes the use of *in vitro* insulin secretion as a potency test as part of lot release testing; another measure of potency appropriate to fetal and neonatal cells will need to be developed for product release testing, and evaluation of aliquots of these products in mouse transplant bioassays should be performed to provide meaningful post-release information. Additionally, there are also a number of cell specific ways to determine individual insulin content of the cells. This can be assessed by taking a small number of aliquots of the islet products and determine the insulin content using an insulin porcine radioimmunoassay kit (RIA). The specific DNA content of the islet products can also be determined from aliquots of the product using a quantitative DNA assay kit. These two results are then combined to form insulin/DNA ratio, which is calculated by dividing the insulin value (ng/IEQs) by the DNA content value (ng/IEQs). Additional cell specific functional assays that utilize a cell metabolic activity such as ATP content and oxygen consumption rate can also be used effectively as release criteria. These assays utilize small numbers of islets from the pooled product and can be performed quickly prior to release of the product for transplantation. In addition, they provide a good indication of the metabolic activity of the islet product and its potential functional capacity. Product characterization should also include the reversal of diabetes in immune-deficient mice relative to dose.

Table 1. List of antibodies for the characterization of porcine islet products

Antibodies	Catalogue number	Source
Exocrine and Endocrine Cells		
Rabbit anti-α-human amylase	A8273	Sigma
Polyclonal guinea pig anti-pig insulin	A0564	Dako
Monoclonal mouse anti-pig glucagon	G2654	Sigma
Polyclonal rabbit anti-human somatostatin	A0566	Dako
Rabbit anti-ppy/pancreatic hormone polypeptide	bs-8543R	BIOSS
Precursor and Endothelial Cells		
Monoclonal mouse anti-human cytokeratin 7	M7018	Dako
Mouse anti-proliferating cell nuclear antigen	M0879	Dako
Purified mouse anti-human Ki67	550609	BD Pharmingen
Monoclonal mouse anti-vimentin	M0725	Dako
Mouse anti-pig CD31	MCA1746	AbD Serotec
Carbohydrate and Immune cells		
Lectin from Bandeiraea simplicifolia (IB4)	L2895, L2140	Sigma
Mouse anti-pig CD29	561496	BD Pharmingen
Mouse anti-pig CD45	MCA 1222	AbD Serotec
Mouse anti-pig CD4a	561474	BD Biosciences
Mouse anti-pig CD8a	561475	BD Biosciences
Mouse monoclonal CD21 antibody (porcine)	NBP1-28248	Novus Biologicals
Mouse anti-pig macrophages	MCA 2317	AbD Serotec
Mouse anti-pig SLA I	MCA2261	AbD Serotec
Mouse anti-pig SLA II DR	MCA2314	AbD Serotec

Encapsulated islets and combined islet products

Encapsulated islets and combined islet products should follow the general framework for the clinical testing of xenotransplantation products before

Manual Islet Counting Worksheet

Product Number: _____ Date: _____ Initials: _____

Instructions:

- Perform islet counts using counter (manual or electronic).
- Record results in "Islets Counted" column.
- Multiply results by appropriate "IEQ Conversion Factor" based on the islet size.
- Sum the "IEQ's per Range" column.
- Multiply the result by the dilution factor (ml total volume/µl sample volume x 1000) to yield the IEQ.
- Estimate the percent purity and record in the appropriate box.
- Estimate the percent trapped islets and record in the appropriate box.

Islet Diameter Range (µm)	Islets Counted (n)	IEQ Conversion Factor (n x factor)	IEQ per Range
50–100		X 0.167	=
101–150		X 0.667	=
151–200		X 1.685	=
201–250		X 3.500	=
251–300		X 6.315	=
301–350		X 10.352	=
>350		X 15.833	=
Sum of IEQ's per Range (Σ last column)			=
Dilution Factor *(ml total volume/µl sample volume x 1000)*			=
Total IEQ (Σ last column x dilution factor)			=

Percent Islet Purity = % **Percent Trapped Islets =** %

Fig. 1. Sample of the form used to count islets after isolation. Percent islet purity is the percentage of islets compared to all tissue present in the islet preparation (islets, acinar, and ductal cells) [25]. Percent trapped is the percentage of islets that are embedded or trapped in acinar tissue (at least 25% of the border attached to acinar tissue) compared to all islets (free and trapped) [25]. Both percent islet purity and islet trapped are determined by visual inspection of a representative sample of the islet preparation [25]. For more details about the qualitative and quantitative assessment of islets using dithizone, please refer to reference [25].

use in clinical trials as outlined in Section VII of the U.S. FDA Guidance for Industry Source Animal, Product, Preclinical, and Clinical Issues Concerning the Use of Xenotransplantation Products in Humans [2]. In addition, these products warrant further preclinical characterization for bioreactivity and biocompatibility of the device components [2]. Porcine islets may be cultured for a number of days before encapsulation or added to a scaffold or pouch prior to implantation. The encapsulation process is designed to shield the isolated islets from the recipient's immune system and thus prevent local inflammatory responses and chronic rejection, while still allowing the islets to function by secreting insulin and controlling glucose metabolism in the body. An added benefit is that culture of the islets before and after encapsulation provides additional time to conduct standard pharmacopeial sterility tests for bacteriology, mycology, and viral screening.

Typical raw materials used for the encapsulation process include alginate and polycations, which determine the pore size, capsule strength, and robustness. Pores, which are approximately three nm in diameter, will allow diffusion of insulin of the capsule and retard the infusion of larger compounds such as IgG. All excipients used in the encapsulation process should either be pharmacopeial grade or meet rigorous pre-determined analytical specifications. All critical process steps should be validated to establish the consistency and reproducibility of the islet encapsulation process.

Following encapsulation, a similar battery of tests to those listed in the previous section are necessary to confirm that this process has not adversely affected the viability, metabolic activity, or *in vitro* insulin secretory capacity of the islets. In addition, microscopic tests to determine capsule size, uniformity, and integrity are used to confirm that the encapsulated system has the physical properties required for free diffusion of lower MW components to and from the capsule while providing a sufficient barrier to immunological response.

Specific defects may include the presence of an islet in the wall and a ruptured or distorted capsule.

Assessment of the biological activity of the combined product is often a component of preclinical safety evaluations. It is recommended that studies should evaluate the duration and predictability of the device used in the combination product so that porcine islets contained in the device may be replaced at appropriate intervals to maintain life-supporting pharmacologic or metabolic activity [2]. Animal studies of porcine islet product or combination products should be designed taking into consideration all aspects of the clinical trial and proposed patient population.

Discussion

The use of islet xenotransplantation products has the potential for transmission of infectious disease from pig donors to humans. Thus, during islet product manufacturing, it is important to consider the safety, not only of the recipients and their contacts, but also of the public. Our intent with the above recommendations is to provide a framework for individual porcine islet manufacturing facilities to ensure a high level of safety for the initiation of Phase 1/2 clinical trials. It is mandatory that appropriate safety procedures are demonstrated, best attained by the establishment of robust quality assurance practices. Full cGMP compliance requires numerous manufacturing controls, which must be implemented as clinical trials progress. Some of the assays for the release criteria we mentioned could be performed in a more practical but equally if not more reliable in terms of the results they produce. For example, the European Pharmacopoeia has recently accepted a PCR mycoplasma test for product release testing; however, the U.S. FDA to date has not accepted this assay. PCR test would reduce mycoplasma testing to about a day as compared to the 28 days required for the direct test. Moreover, some manufacturing facilities are using the PCR mycoplasma assay so that results are available before the product is released for use. In terms of the time required for archiving aliquots of final porcine islet products, serum, and various tissues collected at pancreas procurement, the group recommends the archiving time to be reduced to 10 yrs.

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Xenotransplantation 2016: 23: 46–52
doi: 10.1111/xen.12226

Original Article

First update of the International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes—Chapter 4: pre-clinical efficacy and complication data required to justify a clinical trial

Cooper DKC, Bottino R, Gianello P, Graham M, Hawthorne WJ, Kirk AD, Korsgren O, Park C-G, Weber C. First update of the International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes—Chapter 4: pre-clinical efficacy and complication data required to justify a clinical trial. Xenotransplantation 2016: 23: 46–52.

Abstract: In 2009, the International Xenotransplantation Association (IXA) published a consensus document that provided guidelines and "recommendations" (*not* regulations) for those contemplating clinical trials of porcine islet transplantation. These guidelines included the IXA's opinion on what constituted "rigorous pre-clinical studies using the most relevant animal models" and were based on "non-human primate testing." We now report our discussion following a careful review of the 2009 guidelines as they relate to pre-clinical testing. In summary, we do not believe there is a need to greatly modify the conclusions and recommendations of the original consensus document. Pre-clinical studies should be sufficiently rigorous to provide optimism that a clinical trial is likely to be safe and has a realistic chance of success, but need not be so demanding that success might only be achieved by very prolonged experimentation, as this would not be in the interests of patients whose quality of life might benefit immensely from a successful islet xenotransplant. We believe these guidelines will be of benefit to both investigators planning a clinical trial and to institutions and regulatory authorities considering a proposal for a clinical trial. In addition, we suggest consideration should be given to establishing an IXA Clinical Trial Advisory Committee that would be available to *advise* (but *not* regulate) researchers considering initiating a clinical trial of xenotransplantation.

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Key words: diabetes mellitus – islets – non-human primates – pig – xenotransplantation

Abbreviations: IXA, International Xenotransplantation Association; NHP, non-human primates; PERV, porcine endogenous retrovirus; WHO, World Health Organization.

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Received 8 February 2016;
Accepted 8 February 2016

Introduction

In 2008, the First World Health Organization (WHO) Consultation on Regulatory Requirements for Xenotransplantation Clinical Trials was held, resulting in the publication of the Changsha Communiqué [1,2].

"Principle 5" of this document states that "because of the community risk (from novel infections), in proposed clinical trials of xenotransplantation, there should be a high expectation of benefit to balance the risk. The level of expectation should be in proportion to the level of risk. The level of safety and efficacy should conform to recommendations from the international scientific community, when available, and requires rigorous pre-clinical studies using the most relevant animal models."

The "Key Recommendations" of the Communiqué include the statement that "investigators must provide clear justification for the (clinical) trial, including adequate pre-clinical data on safety and efficacy, usually from non-human primate testing."

In 2009, the International Xenotransplantation Association (IXA), as a relevant member of the "international scientific community," published a consensus document that provided guidelines and "recommendations" (*not* regulations) for those contemplating clinical trials of porcine islet transplantation [3]. These guidelines included the IXA's opinion on what constituted "rigorous pre-clinical studies using the most relevant animal models" and these were indeed based on "non-human primate testing." It was the IXA's intention that these recommendations should be reviewed at intervals to ensure they remained relevant to the xenotransplantation research community. We now report our discussion following a careful review of the 2009 guidelines as they relate to pre-clinical testing, which were included as Chapter 4 of the original document [4]. We believe these guidelines will be of benefit to both investigators planning a clinical trial and to institutions and regulatory authorities considering a proposal for a clinical trial.

It is not our intention to rewrite the entire statement published in 2009, but only to discuss specific points that we believe deserve review in light of experience gained since the original consensus statement was published. It should be noted that the number of contributing authors is considerably greater than was the case with the original document, and, therefore, this revision represents a wider range of opinions. Furthermore, these revisions have been made after considering the opinions of a large number of members of the IXA interested in the field of islet xenotransplantation. Our discussion relates only to the section "Determination of success of islet transplantation." Where we have not indicated that a revision may be necessary, the original recommendations remain relevant.

The need for a review of the document has been related to a number of factors that include (i) the continuing and increasing healthcare burden provided by type 1 diabetes, which requires an urgent solution, (ii) the steadily improving results of islet allotransplantation in patients with this condition, (iii) the continuing severe shortage of islets from deceased human donors (that limits the number of islet transplants that can be carried out each year) with no indication that this shortage is ever likely to be resolved, (iv) expert opinion that the potential risk of complications following the transfer of porcine endogenous retroviruses with the porcine graft is minimal (see Chapter 5), (v) renewed consideration of the fact that the recipient of encapsulated porcine islets *may* not require any exogenous immunosuppressive therapy (although this remains uncertain), thus reducing the risk of complications (e.g., infection, malignancy) from a clinical trial, and, furthermore, may involve exchangeable devices or devices in which the islets may be replenished at intervals, and (vi) greater insight into the immunologic and metabolic differences between species that assist in the appropriate interpretation of experimental results obtained in the pig-to-non-human primate model, and thus facilitate clinical application.

We have also taken into consideration opinions published recently [5,6]. We are fully aware of improvements that are taking place in exogenous insulin delivery and in management strategies for patients with "brittle" diabetes, as well as advances in stem cell technology, regenerative medicine, and other innovative technologies that might provide an alternative to xenotransplantation [6].

The pig-to-non-human primate model

Because they are not inbred species and their immune systems have close similarities to those of humans, NHPs are considered the optimal animal model for clinically relevant research into xenotransplantation, although the efficacy and adverse effects of certain biological and pharmacologic agents may differ between NHPs and humans. Most studies have been undertaken in monkeys [7], but baboons have also been utilized [8], and we believe both species are acceptable as experimental animals for pre-clinical studies of pig islet transplantation, although there are possibly more

background data relating to glucose metabolism in monkeys than in baboons (see Table 1).

There are, however, differences in glucose metabolism between pigs and monkeys that affect the results of pig islet xenotransplantation in this experimental model [9–14]. As glucose metabolism in the pig is closer to that in humans than in monkeys, clinical trials may be associated with better outcomes. Furthermore, NHPs do not develop autoimmune diabetes, but require the chemical induction of diabetes, for example, by streptozotocin, which might influence the success of islet xenotransplantation. For example, streptozotocin can be associated with lymphopenia [15], which could have an additive immunosuppressive effect, although hyperglycemia has the same effect, and therefore, streptozotocin may merely mimic the clinical condition [16]. However, streptozotocin can have other detrimental effects, for example, nephrotoxicity and hepatotoxicity, and its potential impact on engraftment and long-term function is not absolutely clear.

We do not believe these differences in glucose metabolism between monkeys and humans are sufficient to negate the value of the pig-to-monkey model as an indicator of the potential outcome of a clinical trial. The fact that the monkey maintains a lower blood glucose level and requires a higher level of C-peptide and insulin provides a greater hurdle for pig islet xenotransplantation than is likely to be faced in a clinical trial, and therefore, we suggest that success in monkeys is likely to be followed by a successful clinical trial.

The pig-to-NHP model, therefore, provides a valuable model for the assessment of the *efficacy* of any pig islet transplant, whether the islets are encapsulated or not, whether they are transplanted in association with other cells that may provide immune protection, for example, Sertoli or mesenchymal stromal cells, and/or whether immunosuppressive therapy is administered or not. They may also provide an indication of the *safety* of the islet transplant in that potential complications, for example, infection, can be monitored (although it may be more difficult to prevent infectious complications in laboratory-housed NHPs than hospitalized human patients). Assessment of safety, however, does *not* extend to monitoring of transfer of porcine endogenous retroviruses (PERV) (discussed in Chapter 5).

The original document was in part intended to dissuade clinical trials being undertaken irresponsibly, and so the pre-clinical hurdles were set high. We are concerned, however, that there is a risk that the original recommendations might deter investigators from undertaking clinical trials. In view of expert opinion that the potential risk of infection following pig islet xenotransplantation is significantly less than had previously been considered likely, we believe that some reduction in pre-clinical proven efficacy may be justified.

Moreover, we are cognizant that more rapid progress is likely to be achieved if clinical studies are undertaken (where there are far more sophisticated aids that assist in the care of the patient) and that the very considerable cost of experiments in NHPs undoubtedly limits the number of studies that can be undertaken by academic investigators, thus slowing the advances that can be made. Furthermore, we are fully aware of the restrictions being placed on studies in NHPs in several countries at the present time, greatly limiting or even preventing such studies by some groups experienced in the field of islet transplantation.

Table 1. Relevant normal parameters in healthy non-diabetic humans, monkeys, baboons, and pigs[a]

	Humans	Monkeys	Baboons	Pigs
Fasting blood glucose (mg/dl/mmol/l)	70–99/3.9–5.4	39–74/2.1–4.1	79–87/4.4–4.8	65–94/3.6–5.2
Fasting C-peptide (ηg/ml/nmol/l)	0.50–2.00/0.16–0.67	1.47–9.51/0.49–3.17	1.0–4.0/0.33–1.33	0.3–0.96/0.10–0.32
Fasting insulin (μU/ml/pmol/l)	4.8–19.2/33–136	1.92–28.06/13–194	3–37/21–257	1.0–5.3/7–106
Fold increase in C-peptide during ivGTT	2.6–3.4	1.3–3.6	NA	1.8–5.6
Glucose disappearance rate (K_G)	1.7–2.1	3.3–8.2	1.9–3.1	2.6–6.7
AIR_{Glu} (ηg/ml/pmol/l)	31–83/215–576	34.6–180/240–1250	69–121/279–840	2.8–56.1/19–389
ACR_{Glu} (μU/ml/nmol/l)	1.6–2.4/0.53–0.80	1.4–15.1/0.47–5.03	NA	0.46–2.20/0.15–0.73
ACR_{Arg} (ηg/ml/nmol/l)	0.7–1.2/0.23–0.40	0.42–3.05/0.14–1.01	NA	0.35–0.70/0.12–0.23
AIR_{Arg} (μU/ml/pmol/l)	31–83/215–576	4.9–45.7/34–317	58–102/403–708	7.4–16.9/51–117
HbA1c (%)	4.0–6.0	3.5–6.7	3.5–5.9	NA

[a]This table appeared as table 2 in the original IXA consensus document [2], but two modifications have been made to the units of measurement as these were incorrect in the original.

For sources of data, see Cooper [2].

ACR_{Arg}, acute C-peptide response to arginine; ACR_{Glu}, acute C-peptide response to glucose; AIR_{Arg}, acute insulin response to arginine; AIR_{Glu}, acute insulin response to glucose; HbA1c, glycated hemoglobin; K_G, log-linear (ln) decline in glucose level during the first 30 min of the ivGTT, where K_G = ln(glucose level at 5 min)−ln (glucose level at 30 min)/25 × 100; NA, not available.

Nevertheless, the pre-clinical requirements suggested by the IXA in 2009 remain as a basis for discussion [4]. In our opinion, no clinical trials should be initiated without animal studies that indicate a potential of benefit to the patient in the absence of a significant adverse effect. In patients who will be immunosuppressed following pig islet xenotransplantation, there will always remain a risk of complications associated with the immunosuppressive regimen. It would therefore be unethical to carry out a clinical trial unless there is evidence of potential benefit to the patient. Experience in "concordant" models, for example, rat-to-mouse, in which there are no (or low levels of) preformed anti-graft antibodies that might result in hyperacute rejection or early graft failure, is not acceptable as it is not relevant to the pig-to-human model.

Studies in NHPs are in some cases essential, and always recommended, to determine the potential risks (safety) and benefits (efficacy) of the transplant procedure. For example, if the early loss of islets from the instant blood-mediated inflammatory reaction [17–19] or other inflammatory response is almost total, then a clinical trial is unlikely to be in any way successful unless an alternative strategy can be identified. In such cases, in view of the (admittedly small) risk of adverse events, it is ethically questionable whether a patient should be subjected to islet xenotransplantation. However, (i) whether studies in NHPs are essential under all circumstances, (ii) the number of experiments in NHPs deemed necessary, and (iii) the length of the period of follow-up of each recipient NHP are topics on which there are differences of opinion and therefore worthy of discussion.

Encapsulated islet transplantation

If *encapsulated* islets are to be transplanted (or islets protected solely by Sertoli or mesenchymal stromal cells or other cell-based approaches), which involves *no* immunosuppressive therapy to the recipient [20,21], then arguments for insisting on studies in NHPs are reduced. It could be argued that studies in rodents demonstrating relatively long-term survival of the islets, with a beneficial effect on glucose metabolism, may be all that is required, but the majority of those consulted believe that studies in NHPs are essential if the *efficacy* of islet xenotransplantation is to be proven. However, there is a minority who feel that the associated risks to the patient are minimal and therefore studies in NHPs are unnecessary.

For example, if human (or possibly NHP) islet *allografts* have been successfully transplanted in the same site previously, and therefore, the *safety* of the procedure has been largely established, trials of pig islet transplantation in NHPs are possibly not essential from a *safety* perspective. Furthermore, if the pig islets are to be transplanted in a peripheral site, for example, subcutaneously, from which they can readily be removed (if necessary), then this arguably reduces the need to establish the *safety* of the procedure, although the regulatory authorities may well require evidence of this in a NHP model, a viewpoint with which the majority of us concur. However, if the islets are to be transplanted into an internal site, for example, the peritoneal cavity, where there is a potential for bowel obstruction or other serious complication, we believe it is essential to demonstrate the *safety* of the procedure in a NHP model before progressing to a clinical trial.

However, although the procedure may be deemed safe from rodent studies, potential clinically relevant infectious complications (excluding those relating to porcine endogenous retroviruses) are more likely to be identified in a NHP model than in rodents.

When a group has previous substantial clinical or NHP experience of the transplantation of porcine islets, then further studies in NHPs may well not be essential unless there have been major changes to the protocol.

If any form of pharmacologic immunosuppressive therapy is found to be necessary, for example, if the capsules do not provide complete immuno-isolation, then studies in NHPs to exclude significant complications from this therapy are considered mandatory.

To our knowledge, although the benefits (if any) of the transplantation of encapsulated porcine islets to a patient in a clinical trial have not yet been clearly reported, and may have been minimal or negligible, no adverse events have occurred as a result of these transplants [22–24].

If studies in NHPs are deemed necessary, the same (or similar) criteria regarding the number of experiments in NHPs and the length of follow-up should be followed as outlined below for the transplantation of "free" porcine islets. However, a shorter length of follow-up, for example, 3 months rather than 6 months, was suggested by some of those consulted to be adequate when encapsulated islets are being tested, particularly when exchangeable devices would allow replenishment of islets. It should be noted, however, that these shorter periods of follow-up are different from the original

guidance published by the US Food and Drug Administration [25].

Tolerance-inducing regimens

Even though the ultimate goal of a tolerance-inducing regimen is to enable long-term graft survival in the absence of chronic exogenous immunosuppressive therapy, these regimens generally require significant suppression of the immune response in the peri-transplant period. We would therefore recommend that studies in NHPs should be required to provide information on efficacy, morbidity, and safety before any clinical trial is considered.

"Free" islet transplantation

When *free* (i.e., *not* encapsulated) islets are being transplanted (and immunosuppressive therapy will be necessary), it is not unreasonable to expect the investigators to demonstrate in the pig-to-NHP model that insulin independence—or, at least, a greatly reduced insulin requirement—can be achieved and maintained for several weeks or months in a small number of experiments. Follow-up for several months would be particularly important when embryonic, fetal, or neonatal porcine islets have been transplanted where the production of insulin may be significantly delayed after transplantation [26,27]. A successful result should be achieved with a clinically tolerable immunosuppressive regimen. At the end of the period of follow-up, therefore, there should be evidence of functioning islets in the relative absence of complications from the immunosuppressive regimen, for example, infection and malignancy.

As we do not wish to inhibit discussion with regulatory authorities or restrict clinical trials unduly, we are hesitant to provide definitive guidelines on the exact number of experiments in NHPs that we believe is necessary to justify advancing to a clinical trial. However, if guidance in this respect is needed (by investigators, institutions, or regulatory authorities), the majority opinion is that successful reversal of diabetes in 4 of 6 (or 5 of 8) *consecutive* experiments would be sufficient to indicate potential success of a clinical trial. However, there was a significant minority opinion that the number of experiments required should not be generalized, but rather determined by the investigators themselves with regard to their research objectives, possibly after discussion with the relevant regulatory authorities. The minimum number of animals necessary to answer a specific research question should be influenced by the guidelines of national authorities involved in the oversight of experiments in animals, for example, the guidelines relating to refinement, reduction, and replacement of the U.S. Department of Agriculture (http://www.aphis.usda.gov/ac/Policy#12).

A majority of those consulted indicated that a minimum follow-up of 6 months is essential, with, ideally, follow-up for 12 months in one or more cases and that any graft failure that occurs during these periods of time should not be a result of graft rejection. Although graft failure may result from "exhaustion" of the pig islets, this may be associated with the demands made on the islets in the metabolically "hostile" environment of the monkey and may not be seen in the more benign environment of the human. However, when assessing the cause of graft failure, due attention needs to be paid to the immunosuppressive therapy that has been administered to the monkey, as the toxic effect on islets of calcineurin inhibitors may be a major factor in the "exhaustion" that may develop. However, in determining whether the transplant should be considered a failure or just a model-imposed limitation, due attention needs to be paid to the differences in drug metabolism between NHPs and humans [12].

However, as the goal of many trials would be to achieve improved glycemic control, with significantly reduced insulin administration, rather than insulin independence, a minority opinion was that 3 months follow-up would be adequate. Furthermore, if there is evidence that hypoglycemic episodes have been avoided for 3 months (although this might be difficult to document), this would be considered an adequate period of study.

Although the authors believe investigators should err on the side of caution, we agree that some flexibility in these guidelines is necessary if clinical trials of pig islet transplantation are not going to be unduly delayed.

If a good outcome has been obtained with an immunosuppressive agent that is not approved for clinical use by the regulatory authorities in the country concerned, for example, the Food and Drug Administration (FDA) in the USA, this would not negate the value of the pre-clinical study, but would necessitate discussion of the use of the agent with the relevant regulatory authority.

Pig islet transplantation in a patient already receiving immunosuppressive therapy

If the patient who will receive the pig islet xenograft is already receiving immunosuppressive therapy for a kidney allograft, or will be undergoing kidney allotransplantation at the same time as islet

xenotransplantation, we suggest there is a little additional *risk* associated with the xenotransplant, that is, that the *safety* of the transplant will not be unduly impacted. The major additional risk would be if the pig islets are a source of infectious microorganisms that are not present in the allograft, but we suggest this risk would be small (Chapter 5). Indeed, with the exception of PERVs, using current screening methods there should essentially be no risk. In these cases, possibly no evidence for pig islet graft survival in NHPs is necessary, although the majority opinion is that it would clearly be advantageous and is recommended.

However, once again the nature of the immunosuppressive therapy that will be administered to the patients needs to be taken into consideration. It should have been demonstrated in a NHP model (or possibly deduced from previous clinical experience with islet allotransplantation) that the immunosuppressive regimen to prevent kidney allograft rejection is also likely to be effective in preventing islet xenograft rejection – in the absence of toxicity that might compromise pig islet function and/or maturation. Calcineurin-sparing regimens would clearly be preferable, for example, those based on costimulation blockade. Furthermore, depending on whether the kidney allograft is already established or whether it is to be transplanted concomitantly with the pig islets, consideration would need to be given to whether the regimen is aimed at induction or maintenance of the kidney graft; a maintenance regimen sufficient to maintain a kidney allograft may prove insufficient to induce suppression of a new islet xenograft.

Prior clinical experience

Although not related to studies in NHPs, the clinical experience of the group planning to initiate a clinical trial is clearly important, and impacts the extent of pre-clinical studies that might be considered essential. For example, if the group has significant experience in the management of patients receiving the same immunosuppressive therapy proposed for the clinical trial, particularly if this is in association with islet allotransplantation, this may reduce the need for similar experience in a NHP xenotransplantation model. Previous studies in NHPs should also be taken into consideration.

Although no group currently has prior experience of the safety and efficacy of "free" islet xenotransplantation, when this initial experience has been obtained, subsequent studies in NHPs may not be essential unless there have been major changes to the protocol.

Conclusions

We suggest consideration should be given to establishing an IXA Clinical Trial Advisory Committee that would be available to *advise* (but *not* regulate) researchers considering initiating a clinical trial of xenotransplantation. The committee could also be a source of factual information and expert opinion for institutions and regulatory authorities considering a proposal for a clinical trial. This committee could possibly be based on the existing IXA Ethics Committee, with the addition of experts in the necessary fields, although the purpose and competence of the Clinical Trial Advisory Committee (related as it would be to assessment of the *safety* and *efficacy* of a trial) would be significantly different from that of the Ethics Committee, and therefore, a separate committee would be preferable.

In summary, after careful consideration, we do not believe there is a need to greatly modify the conclusions and recommendations of the original consensus document [4]. Pre-clinical studies should be sufficiently rigorous to provide optimism that a clinical trial is likely to be safe and has a realistic chance of success, but need not be so demanding that success might only be achieved by very prolonged experimentation. We believe this would not be in the interests of patients whose quality of life might benefit immensely from a successful islet xenotransplant. In view of the immense health problems associated with diabetes, research into porcine islet xenotransplantation should be given very high priority.

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Xenotransplantation 2016: 23: 53–59
doi: 10.1111/xen.12227

XENOTRANSPLANTATION

Original Article

First update of the International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes—Chapter 5: recipient monitoring and response plan for preventing disease transmission

Denner J, Tönjes RR, Takeuchi Y, Fishman J, Scobie L. First update of the International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes—Chapter 5: recipient monitoring and response plan for preventing disease transmission. Xenotransplantation 2016: 23: 53–59. © 2016 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd.

Abstract: Xenotransplantation of porcine cells, tissues, and organs may be associated with the transmission of porcine microorganisms to the human recipient. A previous, 2009, version of this consensus statement focused on strategies to prevent transmission of porcine endogenous retroviruses (PERVs). This version addresses potential transmission of all porcine microorganisms including monitoring of the recipient and provides suggested approaches to the monitoring and prevention of disease transmission. Prior analyses assumed that most microorganisms other than the endogenous retroviruses could be eliminated from donor animals under appropriate conditions which have been called "designated pathogen-free" (DPF) source animal production. PERVs integrated as proviruses in the genome of all pigs cannot be eliminated in that manner and represent a unique risk. Certain microorganisms are by nature difficult to eliminate even under DPF conditions; any such clinically relevant microorganisms should be included in pig screening programs. With the use of porcine islets in clinical trials, special consideration has to be given to the presence of microorganisms in the isolated islet tissue to be used and also to the potential use of encapsulation. It is proposed that microorganisms absent in the donor animals by sensitive microbiological examination do not need to be monitored in the transplant recipient; this will reduce costs and screening requirements. Valid detection assays for donor and manufacturing-derived microorganisms must be established. Special consideration is needed to preempt potential unknown pathogens which may pose a risk to the recipient. This statement summarizes the main achievements in the field since 2009 and focus on issues and solutions with microorganisms other than PERV.

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Key words: CRISPR/Cas – designated pathogen-free status – disease transmission – hepatitis E virus – infectious disease – islet xenotransplantation – porcine endogenous retrovirus – type 1 diabetes – xenozoonoses

Abbreviations: cGMP, current good manufacturing practices; PCMV, porcine cytomegalovirus; CRISPR/Cas, clustered regularly interspaced short palindromic repeat/CRISPR-associated system; CRO, Contract Research Organization; DPF, designated pathogen-Free; EMEA, European Medicines Agency; FDA, Food and Drug Administration; GLP, good laboratory practices; HCV, hepatitis C virus; HEV, hepatitis E virus; HIV, human immunodeficiency virus; LCMV, lymphocytic choriomeningitis virus; NGS, next-generation sequencing; NHP, non-human primate; PERV, Porcine Endogenous Retrovirus; TALEN, transcription activator-like effector nuclease; TRIM5α, tripartite motif-containing protein; WHO, World Health Organization; ZAP, zinc-finger anti-viral protein; ZFN, zinc-finger nucleases

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Received 8 February 2016;
Accepted 8 February 2016

Introduction

Xenotransplantation using pig materials may be associated with transmission of porcine microorganisms to the recipient. In general, most microorganisms could be eliminated by designated pathogen-free (DPF) production of the donor animals which includes Cesarean section, closed containment, special precautions concerning feed and waste, excellent training of the staff, and measures to prevent transmission of microorganisms from the staff to the herd. However, porcine endogenous retroviruses (PERVs) cannot be eliminated in this way as they are integrated in the genome of all pigs and may produce virus particles which are able to infect some human cells *in vitro* [1]. It is important to note that only certain transformed human tumor cell lines can be infected by PERV derived directly from pig cells. However, after adaptation on human cells associated with genetic modifications, PERV also infects human primary cells *in vitro* [1,2]. In the previous, 2009 version of this consensus statement [3], strategies to prevent PERV transmission were elaborated. A detailed analysis of risk posed by PERVs and the corresponding measures to prevent transmission was undertaken subsequently. In addition, other porcine microorganisms which could infect human recipients were studied and the risks posed by them were analyzed. Although they were thought to be eliminated easily by designated pathogen-free production, difficulties were observed in generating pigs free of designated pathogens such as hepatitis virus E and herpesviruses [4–7]. In addition, better detection methods were developed to identify pigs free of these microorganisms [8,9]. In general, if microorganisms are eliminated from the donor pig, there should be no need to continue to routinely monitor recipients for these specific microbes. With this approach, sterility of the preparation of the pig-derived transplant must be assured to avoid the transmission of infection to the recipient.

What is new since 2009: General aspects

Some new data have been developed since the previous version of the consensus statement published in 2009.

First, clinical studies transplanting pig islet cells have been performed and no transmission of PERV and other microorganisms has been observed [10–16]. Among these trials was the first New Zealand Government-approved clinical trial of alginate-encapsulated porcine islet cell transplants in fourteen patients suffering hypoglycemic unawareness. Each patient received between 5000 and 20 000 islet equivalents as a single dose from DPF Auckland Island strain donor pigs. In advance of the trial, pigs and islet preparations were tested for 26 microorganisms (15 viruses, 10 bacterial species, and one protozoan) using molecular and immunological assays. Recipients were found to be negative on testing for PERVs and other microorganisms at multiple time points up to 1 yr following transplantation [16]. In addition, it has been reported that patients receiving viable pig skin demonstrate strong IgG responses to pig antigens but lack evidence of PERV infection up to 35 yrs post-treatment. This is the longest time studied after xenotransplantation and shows that exposure to pig cells elicits a response, but more importantly, exposure evidently did not lead to infection [17].

Second, hepatitis E virus (HEV) and herpes viruses have been found in numerous animals even under SPF conditions using highly sensitive detection methods [6,18–25]. The risk posed by HEV is difficult to evaluate. Only genotype (gt) 3 is associated with zoonotic transmission, and severity of infection is dependent on a number of host factors [24]. There appears to be little clinical risk for healthy individuals; in some regions, up to 56% of the adult population has been exposed to the virus as shown by detection of HEV-specific antibodies [19,24]. There is great variation in the epidemiology of HEV, and the risk posed to transplant recipients remains to be fully clarified in clinical studies. HEV gt1 and gt2 represent the greatest risk in pregnant women. In hyperendemic gt1 and gt2 areas, pregnant women are at higher risk for severe disease and death, but this feature has not yet been reported for HEV gt3 infections. In pigs, only gt3 and gt4 were found. HEV is also of risk for patients with underlying chronic liver conditions and immunosuppressed individuals, either by the human immunodeficiency virus (HIV) or by pharmaceutical immunosuppression in the context of transplantation [24–32]. Transmission of HEV via xenotransplantation has not been demonstrated, and more studies are required to clarify any risks. It should be noted that the virus may be treated by the use of ribavirin based on studies of small numbers of immunosuppressed allotransplant recipients [33,34]. Using newly developed highly sensitive methods, HEV gt3 was also detected in Göttingen Minipigs produced under SPF conditions [8]. This may be explained by the finding that HEV can be transmitted from mothers to their piglets [8]. To improve the detection of porcine cytomegalovirus (PCMV) also new detection methods were developed und used for screening [9].

What is new since 2009: PERV update

Although PERVs can infect (non-productively) cells of non-human primates (NHPs) *in vitro* [35–38], transplantations of porcine tissues [39–42] and inoculations with highly concentrated PERV preparations under immunosuppression [43] into NHP *in vivo* demonstrated no PERV transmission or infection, respectively. However, later investigations demonstrated that the major receptor for PERV-A is mutated in NHP, and therefore, the infection is not efficient [44]. This means that NHPs do not represent a suitable model to be used for determination of the risk of transmission of PERV [45].

Sequencing of the pig genome [46,47] and analysis of the prevalence [48–50] and expression [50] of PERVs in different pig breeds have shown the heterogeneous nature of PERV distribution and differences between individual animals as well as breeds. With this in mind, simple screening for PERV loci cannot be applied routinely to all donor animals. However, this approach also provides an opportunity to select pigs with a lower expression of PERV-A and PERV-B if desired.

Improved methods allow better screening for PERV, both in the donor animals as well as in the human recipient (Table 1). Based on the fact that the human-tropic PERV-A, which is present in all pigs, can recombine with the ecotropic PERV-C, not present in all pigs, the selection of PERV-C-free animals may reduce the risk of PERV transmission to human recipients. Recombinant PERV-A/Cs are characterized by higher replication rates [51]. However, it is still unclear whether the exclusion of PERV-C-positive animals to avoid recombination between PERV-A and PERV-C is important. There are no data that indicate any PERV infection in human recipients receiving donor islets from PERV-C-positive animals [16].

Several restriction factors were characterized to be of particular importance for the replication of retroviruses: tripartite motif-containing protein 5 (TRIM5α), which disrupts the viral capsid after cell entry; TRIM28, which blocks viral transcription; ZAP (zinc-finger anti-viral protein), which directs degradation of viral RNAs; tetherin, which traps virions on the surface of infected cells, and APOBEC (apolipoprotein B mRNA-editing catalytic polypeptides), which are cytidine deaminases that disrupt viral DNA during synthesis [52,53]. Although PERV-A and PERV-A/C are insensitive to restriction by TRIM5α molecules [54], overexpression of either human or porcine tetherin in pig cells significantly reduced PERV production [55]. In addition, human and porcine APOBEC3s could inhibit PERV replication [56–58], thereby reducing the risk of potential infection of human cells by PERV in the course of pig-to-human xenotransplantation. Further studies of anti-viral restriction systems may help to develop therapeutic agents to regulate expression of these factors and to enhance anti-viral activities.

Table 1. Methods to be used to detect microorganisms in the donor pig and if necessary in the recipient

Method	What can be detected
Direct detection methods	
PCR, real-time PCR	DNA microorganisms
RT-PCR, real-time RT-PCR	RNA microorganisms, gene expression
Immunofluorescence, Immunohistochemistry, Western blot analysis	Protein expression
Electron microscopy	Microorganisms
Indirect detection methods	
ELISA, Western blot analysis	Detection of antibodies

To summarize, it is still unclear whether PERVs represent a risk in clinical xenotransplantation. No transmission of PERVs has been observed in multiple clinical trials enrolling more than 200 patients or up to 35 yrs post-xenotransplantation [1,10,11,16,17]. However, most of the patients in the clinical trials were not exposed for a prolonged period to the xenotransplants, and with some exceptions (associated with parallel kidney allotransplantation), no immunosuppression was applied. In addition, preclinical pig-to-non-human primate (NHP) transplants, or infection experiments in small animals or NHP with or without pharmaceutical immunosuppression have not demonstrated infection [1,37,39–43]. It is meanwhile clear that NHPs are not a suitable model to study the risk of PERV transmission as NHPs carry—in contrast to humans—a mutated receptor for PERV allowing infection only with reduced affinity [44,45]. Therefore, the question whether PERVs may be transmitted during xenotransplantation remains open. However, the availability of numerous sensitive and specific detection methods allows testing of the donor pigs and selection of suitable animals as well as screening of the xenotransplant recipients to detect a possible transmission very early. Indeed, selection of pigs free of PERV-C and with low expression of PERV-A and PERV-B is possible due to these excellent methods. Available antiretroviral agents have been shown to have activity against PERV *in vitro* [59–62]. Furthermore, genetic modification of donor pigs to exclude PERV loci, development of vaccines, and other preventive strategies may be available in the near future. The potential viability of clinical xenotransplantation has resulted in continued

investigation supported by the U.S. Public Health Service and the continued interest in the development of appropriate guidelines by the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMEA). The first clinical trials of pig islet cell transplantation received regulatory approval in New Zealand [16] and in Argentina (V. Morozov, S. Wynyard, R. Elliott, J. Denner, in preparation) without adverse events. Strategies to reduce the expression of PERV by siRNA or to knock-out PERV by zinc-finger nucleases (ZFN), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated system (Cas) (CRISPR/Cas) technologies are under development. The present data indicate that—when using donor animals well characterized concerning PERVs and sensitive detection methods—PERVs are unlikely to provide a public health security risk in clinical xenotransplantation.

Most importantly, recent findings demonstrate that 62 genomic copies of PERV could be inactivated in an immortalized pig cell line by gene editing using CRISPR/Cas9 [63]. This technology may be used in the future to derive porcine stem cells and embryos free of infectious endogenous retrovirus as well as to introduce desirable traits governing metabolic and immune functions. The impact of this advance remains to be explored [64]. Attempts to inactivate PERV sequences in pig cells by gene editing using another nuclease, ZNF, failed [65].

Open questions 2016

The main question is as follows: For which microorganisms should the recipient be monitored after xenotransplantation? The general consensus is that there is no need to monitor for pig-derived microorganisms absent in the donor pig. This assumes that available assays used in donor screening have the sensitivity required to avoid transmission of potential pathogens to immunosuppressed recipients. Assay validation might be examined in preclinical and clinical studies. This also requires the absence of infection during handling and transport. For animals free of known potential zoonotic pathogens, routine screening for PERV and, on the basis of clinical signs and symptoms, unknown pathogens, would be required. The methods to detect PERVs in the recipients are the same as used for pig screening (Table 1).

Potential infection by unknown or emerging microorganisms is interesting and remains a research endeavor. With new methods, for example, next-generation sequencing (NGS) including RNA sequencing, many new viruses or other microorganisms may be detected which are, as yet, of unknown clinical significance. For example, several novel astroviruses, bocaviruses and Ljungan-like viruses were identified in stool samples from healthy pigs in China, using high-throughput sequencing [66]. In a similar approach kobuviruses, rotaviruses, astroviruses, enteroviruses, sapoviruses, picobirnaviruses, and a novel, previously unknown, virus, PigSCV, were detected in feces of German pigs [67]. A new porcine parvovirus was recently described in US pigs [68]. A long-term archiving of clinical specimens from donor swine and recipients was proposed; the optimal duration and modalities for such a repository remain to be described. The proficiency of the clinical laboratories charged with testing donor and recipient samples is essential to assure both researchers and the public regarding the stringency of clinical safeguards. This may require advanced, accredited (e.g., good laboratory practices (GLP)) laboratories available in major academic centers or Contract Research Organizations (CROs) and needs authorization by the competent regulatory authorities. Such laboratories must have the capacity also to test samples for human organisms that may infect transplanted porcine cells and tissues. Many recipients will have prior serological and clinical data available to indicate prior exposures to latent or persistent organisms such as the herpesviruses, hepatitis B or C viruses, HIV, or HEV. It is not known whether such pathogens will infect islets or encapsulated islets—such studies are required if the organism has the capacity to infect porcine cells *in vitro* or *in vivo*. The infectious challenge posed by encapsulated cells and tissues in non-immunosuppressed recipients may be less than that in immunosuppressed recipients of cellular or vascularized xenotransplants. Additional information may be obtained through use of standardized World Health Organization (WHO) questionnaires for recipients to indicate any changes in health status and the use of the "precautionary principle" [69]. That is to be prepared in advance for the identification, evaluation, and response to infectious syndromes. The monitoring of close contacts of the recipients should not be required unless data exist to demonstrate that the recipient is infected. It is not generally considered that transmissible spongiform encephalitis is a likely concern for islet xenotransplantation and is not a consideration of current WHO pathogen lists as there are no indications for prions in pigs. In contrast, prion transmission has been discussed in the context of islet allotransplantation [70].

Emerging viral concerns

As mentioned above, it appears that the potential for emerging viruses from donor or recipient would be of concern in the absence of other potential zoonotic pathogens [5]. In islet cell allotransplantation, a number of transmissions have been documented, the most common pathogens being cytomegalovirus (CMV) and enteroviruses; other viruses including HIV-1, HCV, lymphocytic choriomeningitis virus (LCMV), and rabies virus have been transmitted from organ donors to recipients [71–78]. To date, no emerging viral disease, as has been seen for human solid organ transplantation [75], has been documented in islet cell allo- or xenotransplantation [5]. In this context, the zoonotic potential of arenaviruses has been discussed [79]. Recognition of novel infections in immunosuppressed hosts can be difficult as the manifestations of infection including inflammation may be absent. Given that encapsulation of islets may reduce or negate the need for clinical immunosuppression of the recipient, the likelihood of infection may be reduced and any organ-derived infection may be more clearly recognized. As discussed above, routinely applied NGS or RNA sequencing could potentially identify novel/unknown pathogens to provide a microbiologic diagnosis.

With regard to PERV, as already reported in the consensus statement of 2009, different strategies have been developed to increase viral safety largely by preventing transmission of PERV. These strategies include vaccine development [80–84], RNA interference to knock down the PERV expression [85–87] and directed nuclease (e.g., ZFN, TALENs, CRISPR/Cas9)-based knockout of PERV [63–65,88,89]. However, due to lack of PERV transmission, the value of these techniques in a clinical setting has yet to be evaluated.

The clinical application of gene editing technology to the enhancement of xenotransplant safety is presently unknown. Other approaches to donor genetic modification (e.g., breeding) and to the reduction in infectious risk (e.g., monitoring, encapsulation) may also serve to enhance clinical safety and are under investigation.

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Xenotransplantation 2016: 23: 60–76
doi: 10.1111/xen.12228

XENOTRANSPLANTATION

Original Article

First update of the International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes—Chapter 6: patient selection for pilot clinical trials of islet xenotransplantation

Hering BJ, O'Connell PJ. First update of the International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes—Chapter 6: Patient selection for pilot clinical trials of islet xenotransplantation. Xenotransplantation 2016: 23: 60–76. © 2016 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd.

Abstract: Patients in whom type 1 diabetes is complicated by impaired awareness of hypoglycemia and recurrent episodes of severe hypoglycemia are candidates for islet or pancreas transplantation if severe hypoglycemia persists after completion of a structured stepped care approach or a formalized medical optimization run-in period that provides access to hypoglycemia-specific education including behavioral therapies, insulin analogs, and diabetes technologies under the close supervision of a specialist hypoglycemia service. Patients with type 1 diabetes and end-stage renal failure who cannot meet clinically appropriate glycemic goals or continue to experience severe hypoglycemia after completion of a formalized medical optimization program under the guidance of an expert diabetes care team are candidates for islet or pancreas transplantation either simultaneously with or after a previous kidney transplant. Similarly, patients with type 2 diabetes and problematic hypoglycemia or renal failure who meet these criteria are considered candidates for islet replacement. Likewise, patients with pancreatectomy-induced diabetes in whom an islet autograft was not available or deemed inappropriate are candidates for islet or pancreas transplantation if extreme glycemic lability persists despite best medical therapy. To justify participation of these transplant candidates in early-phase trials of porcine islet cell products, lack of timely access to islet or pancreas allotransplantation due to allosensitization, high islet dose requirements, or other factors, or alternatively, a more favorable benefit–risk determination associated with the xenoislet than the alloislet or allopancreas transplant must be demonstrated. Additionally, in non-uremic xenoislet recipients, the risks associated with diabetes must be perceived to be more serious than the risks associated with the xenoislet product and the rejection prophylaxis, and in xenoislet recipients with renal failure, the xenoislet product and immunosuppression must not impact negatively on renal transplant outcomes. The most appropriate patient group for islet xenotransplantation trials will be defined by the specific characteristics of each investigational xenoislet product and related

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Key words: porcine islets of Langerhans – problematic hypoglycemia – renal failure – type 1 diabetes – type 2 diabetes – xenotransplantation

Abbreviations: B4GALNT2, beta 1,4 N-acetylgalactosaminyltransferase; CGMS, continuous glucose monitoring systems; CMAH, cytidine monophosphate-N-acetylneuraminic acid hydroxylase; CSII, continuous subcutaneous insulin infusion; EMEA, European Medicines Agency; ESRF, end-stage renal failure; FDA CBER, Food and Drug Administration Center for Biologics Evaluation Research; GGTA1, alpha-galactosyltransferase 1; GTKO, α1,3-galactosyltransferase gene-knockout; HbA1c, glycated hemoglobin; HLA, human leukocyte antigen; IAH, impaired awareness of hypoglycemia; IAK, islet-after-kidney [transplantation]; IBMIR, instant blood-mediated inflammatory reaction; IXA, International Xenotransplantation Association; LGS, low-glucose suspend; mAb, monoclonal antibody; NICC, neonatal islet cell-like cluster; NHP, nonhuman primate; RCT, randomized clinical trial; SAP, sensor-augmented pump; SIK, simultaneous islet–kidney [transplantation]; SLA, swine leukocyte antigen; SHE, severe hypoglycemic episodes; T1D, type 1 diabetes; T2D, type 2 diabetes.

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Received 8 February 2016;
Accepted 8 February 2016

technologies applied for preventing rejection. Selecting recipients who are more likely to experience prolonged benefits associated with the islet xenograft will help these patients comply with lifelong monitoring and other public health measures.

Introduction

Encapsulated neonatal porcine islets have recently been tested in clinical trials in patients with type 1 diabetes (T1D) [1]. Further progress in developing safe and effective rejection prophylaxis protocols, when achieved, will generate interest in planning clinical trials of additional porcine islet products.

A central element of the design of any clinical trial, especially of xenotransplantation and also of cellular and gene therapy early-phase trials, is the definition of the study population. The aim of this review article was to select a trial population with a favorable benefit–risk ratio, while protecting the public from undue risks and also achieving the study's scientific objectives [2–5].

The 2003 U.S. Food and Drug Administration (FDA) 'Guidance For Industry on Source Animal, Product, Preclinical, and Clinical Issues Concerning the Use of Xenotransplantation Products in Humans' and the 2007 Health Research Council of New Zealand Gene Technology Advisory Committee 'Guidelines for Preparation of Applications Involving Clinical Trials of Xenotransplantation in New Zealand' stipulate that, 'because of the potentially serious public health risks of possible zoonotic infections, xenotransplantation should be limited to patients who (i) have serious or life-threatening diseases for whom adequately safe and effective alternative therapies are not available except when very high assurance of safety can be demonstrated, (ii) have potential for a clinically significant improvement with increased quality of life following the procedure, and (iii) who are able to comply with public health measures as stated in the protocol, including long-term monitoring' [2,4]. The 2009 European Medicines Agency (EMEA) Guideline on 'Xenogeneic Cell-Based Medicinal Products' similarly states that 'the clinical development of xenogeneic cell-based products should involve initially patients with serious or life-threatening disease for whom adequately safe and effective alternative therapies are not available, or where there is a potential for a clinically relevant benefit' [3].

To identify, within this regulatory framework, suitable patient populations for early-phase clinical trials of xenogeneic islet cell products in T1D, the questions to be addressed are as follows:

1. Which serious or life-threatening complications of T1D cannot be treated by adequately safe and effective alternative and available therapies?
2. Which patient with T1D has the potential for a clinically significant improvement with increased quality of life following the xenoislet transplant procedure?
3. Which patient with T1D will—more easily than others—be able to comply with long-term monitoring and other public health measures?

The answer to the first question is expected to change considerably over time as additional and more refined diabetes technologies will become available and as alternative, stem cell-derived islet cell sources will undergo clinical development. While these novel therapies hold great promise, it will take many more years before the safety and efficacy of alternative therapies for serious or life-threatening complications are tested and documented and before these therapies will become available to patients as approved therapies. The answer to the second question involves risk-benefit determinations that are largely determined by the specific characteristics of the xenoislet product under investigation and the associated technology for rejection prophylaxis. As with any early-phase clinical trial, there will be considerable uncertainty about expected risks and potential benefits. The clinical significance of these risks can depend on the study population that receives the product (e.g., sensitization in patients with and without chronic kidney disease) and the potential for benefit and the ability to detect the xenoislet product's activity (e.g., in restoring protection from severe hypoglycemia) might also depend on the choice of study population [5]. As early-phase pilot trials are likely to be small, single arm, and open label in which outcomes are compared to baseline status, the metabolic and clinical effects should be expected to be robust enough for detection in the selected study population, if meaningful information is to be obtained from the study [6]. As research is still underway, a challenge in answering the second question is the remaining uncertainty about the specific characteristics of xenoislet prod-

ucts (neonatal or adult, wild type or genetically modified) and related technologies for preventing rejection (immunoisolation, immunosuppression, tolerance) that will eventually proceed to clinical testing. The answer to the third question might, at first glance, predominantly depend on the age and life expectancy of the study population. Additionally, selecting patients in whom the potential for benefit is greater, in whom the durability of benefit is more likely, and/or in whom repeated administrations of the xenoislet product are more feasible might affect the compliance of study participants with long-term monitoring and other public health measures.

This chapter addresses these questions in more detail in the context of an updated discussion of the challenges related to the choice of suitable patients participating in early-phase clinical trials of islet cell xenotransplantation products in T1D. To do so, this chapter summarizes the salient points presented in the corresponding chapter on 'Patient Selection for Pilot Clinical Trials of Islet Xenotransplantation' in the 2009 Consensus Statement on Conditions for Undertaking Clinical Trials of Porcine Islet Products in Type 1 Diabetes by the International Xenotransplantation Association (IXA) [7], reviews-relevant progress made since 2009 in islet xenotransplantation and related fields, reviews topics that warrant further discussion and new topics that were not addressed in the original statement, and suggests pertinent revisions. The updated concepts and recommendations presented are based on progress reported in the literature and on expert opinions shared by participants in the 2nd International Conference on Clinical Islet Xenotransplantation, which was hosted by IXA in San Francisco, CA, on August 1, 2014 to provide a forum for reviewing and revising the original IXA Consensus Statement published in 2009 [8]. As concluded in the 2009 consensus statement [7], the selection of suitable study participants within the pertinent regulatory framework will largely be determined by safety and effectiveness of emerging alternative treatments for patients with serious or life-threatening complications of T1D, the particular characteristics of the yet-to-be-defined investigational xenoislet products and protocol-defined rejection prophylaxis strategies, the experience of the investigative team, and the resulting risk-benefit determinations.

Salient points regarding patient selection as communicated in the 2009 IXA consensus statement

Xenotransplantation carries unique risks and burdens for the patient [7,8]. These include the potential for unknown infective complications, the risks of immunosuppressive protocols tailored to control xenoimmunity, and the requirement for lifelong monitoring even in the absence of enduring graft function, some of which may also apply to close contacts and the general population. To balance these risks and burdens against anticipated benefits resulting from an islet xenotransplant, the following criteria must be met:

1. The patient must have been diagnosed with T1D for at least 5 yr.
2. The patient's unmet clinical needs must have remained serious, despite intensive efforts in collaboration with a diabetes care team to ameliorate the condition.
3. The risks of immunosuppression, if used, can be justified.
4. A partially or fully functional islet graft is expected to provide significant medical benefits for the patient.
5. The patient would not ordinarily be eligible for an allotransplant.
6. Preclinical studies suggest that the proposed clinical islet xenograft protocol results in significant medical benefits.
7. The requirements for lifelong surveillance should not be an unreasonable financial, psychologic, or social burden.

There are two clinical circumstances where trials of islet xenotransplantation would be both medically and ethically justified:

1. Hypoglycemia unawareness. This complication causes episodes of acutely life-threatening severe hypoglycemia, recurrent and at times persistent physical and psychosocial morbidity, and considerable mortality in affected patients. For these patients, the major difficulty is balancing the risk of immunosuppression with the potential benefits of improved glycemic control and protection from hypoglycemia. Islet xenotransplantation would be justified if, on a case-by-case basis, the risks associated with recurrent hypoglycemia were perceived to be more serious than the risks associated with the immunosuppression administered to prevent immunologic islet xenograft failure. Based on the degree of comorbidity and mortality, it has been widely accepted that long-term immunosuppression is justified to protect human islet allografts from rejection and, if possible, from recurrent autoimmunity.
2. Islet–kidney and islet-after-kidney transplantation. These patients are already undergoing

long-term monitoring and receiving conventional clinical immunosuppression. Islet xenotransplantation may present potential advantages, provided that the unique additional immunosuppressive requirements for an islet xenograft are not too onerous. However, this group of patients has substantial co-morbidities, and special consideration must to be given to ensure that the procedure does not impact negatively on renal allograft outcomes.

The level of associated risks, and the decisions regarding who will be eligible, will depend on the particular xenotransplant protocol proposed and the clinical experience of the team of investigators.

These key patient eligibility criteria and concepts proposed in 2009 continue to be valid. For an in-depth discussion of the medical and ethical justification for considering patients with T1D complicated by hypoglycemia unawareness or end-stage renal disease for xenoislet pilot trials, please see the corresponding chapter in the 2009 IXA consensus statement [7]. The following sections will discuss selected topics of importance in the context of new insights and perspectives related to patient selection of early-phase clinical trials of islet xenotransplantation products in T1D.

Recent progress (data or understanding) in the field (which could possibly impact patient selection)

Firstly, it is important to review whether adequately safe and effective alternative therapies have become available since 2009 for the treatment of serious and life-threatening complications of T1D that would question or even obviate the need for the clinical development of xenoislet products. Considerable progress has been made since 2009 in the development of new treatments of T1D and its complications. Particularly relevant in the context of alternative therapies is the progress in the development of interventions that restore awareness of hypoglycemia and prevent severe hypoglycemic episodes (SHE) in adults with T1D complicated by impaired awareness of hypoglycemia (IAH) [9,10] as well as progress in transplant modalities that restore glycemic control in patients with T1D and progressive microvascular complications such as diabetic nephropathy [11–13].

Compared with standard education, hypoglycemia-specific education (HyPOS) improved awareness of hypoglycemia and reduced the incidence of SHE in patients with T1D [14]. Education in flexible insulin therapy, also referred to as Dose Adjustment for Normal Eating (DAFNE), can significantly reduce the incidence of SH and restore awareness in almost half of those who report unawareness [15]. With a structured psychoeducational program, the DAFNE-Hypoglycemia Awareness Restoration Training (HART), which emphasizes behavioral changes identified through qualitative interviewing and delivers key aspects of blood glucose awareness training (BGAT) using cognitive behavioral therapy and motivational interviewing techniques, 17 of 24 patients with SHE despite previous treatments experienced complete resolution of SHE, and significant reductions in the incidence of SHE were found in the remaining seven patients [16]. In the HypoCOMPaSS trial, educational intervention and intensive and frequent contact with the diabetes care team led to significant reductions in SHE (from 8.9 to 0.8 episodes per patient annually) and to improvement in awareness scores in patients with long-standing T1D complicated by IAH and previous recurrent SHE [17].

Rapid-acting insulin analogs (aspart, glulisine, lispro) with faster onset and shorter duration than regular insulin and also newer basal insulin analogs (degludec and U300 glargine) can reduce the incidence of nocturnal hypoglycemia and SHE [18–22]. In the single study of insulin analogs performed in patients with T1D and recurrent severe hypoglycemia, the HypoAna trial, treatment with insulin detemir and aspart reduced the incidence of SHE by 29% compared with human insulin [22].

Progress has also been made in the development and evaluation of diabetes technologies. A meta-analysis comparing severe hypoglycemia and glycemic control in T1D during continuous subcutaneous insulin infusion (CSII) and multiple daily insulin injections (MDI) found a 4-fold reduction in the incidence of SHE and a 0.6% improvement in HbA1c level with CSII [23]. A before-and-after study in 20 patients with T1D, IAH, and recurrent non-severe and severe hypoglycemia demonstrated the effectiveness of CSII in reducing the incidence of non-severe and severe hypoglycemic episodes and in restoring awareness of hypoglycemia with no deterioration in glycemic control [24]. Real-time continuous glucose monitoring systems (CGMS) significantly reduced the incidence of SHE status in two small studies in patients with T1D and problematic hypoglycemia; in one of these studies, HbA1c levels but not the awareness status improved whereas HbA1c levels remained unchanged and restoration of awareness was not directly assessed in the other study [25,26]. Sensor-augmented insulin pumps (SAPs), which are CSII devices with an integrated CGMS, facilitated a significant improvement in glycated hemoglobin levels when compared with MDI therapy in a randomized

controlled trial (RCT) in patients with inadequately controlled T1D; patients with two or more SHE in the year prior to enrollment were excluded from participation [27]. The use of SAPs with low-glucose suspend (LGS) feature, which automatically interrupts basal insulin delivery for up to 2 h in response to sensor-detected hypoglycemia, reduced the duration of nocturnal hypoglycemia in those at greatest risk in a small before-and-after study in T1D [28]. The same technology, SAP with LGS, when compared in a randomized clinical trial with SAP without LGS, reduced nocturnal hypoglycemia without increasing glycated hemoglobin values; patients were excluded if they had more than one SHE in the previous 6 months [29]. When compared against conventional CSII in a RCT in patients with T1D and IAH aged 4 to 50 yr, SAP with LGS reduced the combined rate of severe and moderate hypoglycemia with no change in glycated hemoglobin in either group [30]. In this study, SH was defined as hypoglycemia resulting in seizures or coma and moderate hypoglycemia as hypoglycemia requiring assistance for treatment.

As discussed above and as recently reviewed [9,10], substantial progress has been made in recent years with the development of refined and novel educational, pharmacological, and technological interventions for the treatment of T1D complicated by IAH and recurrent SHE. However, these new therapies are also associated with limitations. While most of the discussed therapies reduce the incidence of SH, restoration of hypoglycemia unawareness is incomplete as evidenced by elevated Clarke or Gold scores post-intervention and protection from subsequent SHE has not been demonstrated without accepting an elevated HbA1c target of approximately 8.0% [15–17,26, 30–32]. Some of the most advanced diabetes technologies such as SAP plus LGS fail to provide superior protection from SHE compared with conventional CSII when the data of the rare RCT in patients with problematic hypoglycemia are carefully analyzed. The loss of statistical significance in the study reported by Ly et al. [30] for the primary outcome measure, the combined incidence of severe and moderate hypoglycemia, after exclusion of two children with the highest baseline rates of moderate hypoglycemia raises the possibility that the findings were due to chance imbalance rather than represent a true result. Also, when the data in this study were restricted to participants aged ≥18 yr, there was an equivalent reduction in SH incidence in SAP plus LGS and conventional CSII [10]. It is conceivable that next-generation diabetes technologies such as insulin pumps with predictive low-glucose management technology [33] and closed-loop pumps with glucose-responsive insulin or insulin and glucagon delivery [34] will provide superior results, but these emerging technologies remain yet to be tested in patients with long-standing T1D complicated by IAH and recurrent SHE. The most significant limitation of currently available educational, pharmacological, and technological interventions is the failure in preventing SHE in about one-third of patients with T1D and problematic hypoglycemia [35]. Of 36 patients with T1D and problematic hypoglycemia referred to a specialist hypoglycemia service where they were in frequent contact with an experienced team and where they had access to all these interventions, despite accepting an elevated target HbA1c of 8.0%, only 17 (47.2%) patients experienced resolution of their SHE, another 9 (25%) achieved clinically relevant improvement, and 10 (27.8%) required pancreas or islet transplantation [35]. These results indicate that currently available non-transplant medical therapies are very effective in resolving problematic hypoglycemia in a substantial proportion of patients with T1D and should therefore always be tried as first-line therapy before resorting to transplant therapies. These results also indicate that close to one-third of patients with T1D, IAH, and recurrent SHE remain completely unaware of hypoglycemia and continue to have problematic hypoglycemia despite utilization of all the current measures outlined above and despite close contact with a specialist hypoglycemia service [35].

Progress made since 2009 in the field of transplantation of human allogeneic islets has further supported the rationale for islet replacement as an effective treatment of T1D complicated by IAH and recurrent SHE. As previously demonstrated in pancreas transplant recipients [36–40], intraportal islet transplantation can normalize HbA1c, abolish time spent while hypoglycemic (<70 mg/dl), recover partial glucagon secretion, improve epinephrine secretion, restore autonomic symptom perception, and normalize endogenous glucose production in response to insulin-induced hypoglycemia in patients with long-standing T1D and IAH [41]. Even partial islet graft function improves hypoglycemia counterregulation by increasing endogenous glucose production [42], explaining in part that minimal islet graft function is sufficient to abrogate hypoglycemia (<54 mg/dl) [43].

Because of the clinical significance of restoring protection from severe hypoglycemia and near-normoglycemia in patients with T1D complicated by IAH and SHE, the FDA Center for Biologics Evaluation and Research (CBER) suggested in its 2008 guidance on allogeneic pancreatic islet cell

products a composite primary endpoint consisting of near-normal HbA1c (e.g., HbA1c ≤6.5%) and elimination of hypoglycemia for licensure trials of transplantation of human allogeneic islet cell products [44,45]. According to this guidance, this endpoint is best reserved for subjects who have significant hypoglycemia at baseline despite intensive therapy by a diabetes team. Both elements of this composite endpoint must be present simultaneously in the same subjects who may require some exogenous insulin or may be completely insulin-independent [45]. The proposed primary endpoint should be measured at least 12 months after the final islet infusion to allow the assessment of the durability of islet cell transplantation, and determination of appropriate reductions in HbA1c from baseline should be discussed with FDA prior to initiation of the pivotal trial [44]. Both the Phase 3 trial of transplantation of allogeneic islets in T1D complicated by severe hypoglycemia conducted by the NIH Clinical Islet Transplantation (CIT) Consortium [46] and the multicenter Australian trial [47] used the proportion of subjects with HbA1c of 7.0% at day 365 and absence of SHE from day 28 to day 365 inclusive after the first islet transplant as the primary endpoint. The CIT trial enrolled 48 subjects; the results are expected to be reported in 2016. Of the 17 recipients with T1D and IAH enrolled in the Australian trial, 14 (82%) achieved the primary endpoint. Similar metabolic goals were attained in the multicenter trial conducted by the Integrated UK Islet Transplant Program [48]. The Office of Biostatistics and Epidemiology at FDA/CBER analyzed data from the Collaborative Islet Transplant Registry (CITR) on 347 patients with T1D who had received infusions of allogeneic islets between 1999 and 2008 [45]. More than 90% of islet allograft recipients reported to CITR had experienced SHE prior to their first islet infusion [49]. Of the 347 recipients analyzed by the FDA/CBER, 59% were free of SHE and maintained HbA1c level of ≤6.5% at 1-yr post-transplant. The Kaplan–Meier survival analyses showed that 69, 54, and 44% of these 1-yr responders maintained this composite endpoint at 2, 3, and 4 yr, respectively. Ninety-one percent of all recipients were free of SHE at 1 yr, and KM survival estimates showed that 91, 85, and 80% of these subjects maintained this clinical benefit at 2, 3, and 4 yr, respectively. A CITR analysis reported in 2012 showed that, regardless of sustained graft survival, >90% of all type 1 diabetic islet allograft recipients in their database of whom >90% had IAH and who had received their first islet transplant between 2003 and 2006 had remained free of SHE through 5 yr of follow-up [49].

Increasing evidence indicates sustained benefits of islet transplants for T1D [50] in studies with longer follow-up. In a retrospective multicenter study of patients with T1D, islet alone and islet-after-kidney transplantation were associated with sustained HbA1c levels of <7.0% and freedom from SHE for 5 yr in 60% of immunosuppressed recipients, including those with recurrent severe pre-transplant hypoglycemia [51]. More potent induction immunosuppression including T-cell depleting antibodies and TNF-α inhibitors was associated with 5-yr insulin independence rates of 50% in non-uremic human islet allotransplant alone recipients with T1D; these outcomes were previously only attainable with more invasive vascularized pancreas transplants [52]. A prospective 13-yr follow-up study in patients with T1D and chronic kidney disease receiving either a pancreas or islet transplant simultaneously with or after kidney transplantation showed higher insulin independence rates and higher rates of operative complications in pancreas compared with islet transplant recipients [13]. The rate of severe hypoglycemia was reduced by >90% in both pancreas and islet transplant recipients. HbA1c levels declined to normal (5.9 ± 1.1%) and near-normal levels (6.5 ± 1.1%) after pancreas and islet transplantation, respectively, and remained stable at these levels during the 13-yr follow-up. The decline of calculated glomerular filtration rate at year 13 after simultaneous kidney–pancreas and after simultaneous kidney–islet transplantation was comparable [13]. In a prospective, crossover, cohort study in patients with T1D with a median follow-up of 4–5.5 yr, islet transplantation was associated with significantly less progression of diabetic retinopathy and nephropathy than intensive insulin therapy [53].

These preliminary findings showing near-normoglycemia for prolonged periods after islet transplantation are relevant in view of the association between glycemic control and excess mortality in T1D [54] and the difficulty of patients with T1D to achieve and maintain target HbA1c levels despite the availability of new insulin analogs and advanced diabetes technologies. A recent review of the overall state of metabolic control and current use of advanced diabetes technologies in the US by the T1D Exchange Registry showed that only a small proportion of children and adults had achieved their age-adjusted American Diabetes Association HbA1c goals [55]. Furthermore, despite the almost universal implementation of renoprotective treatment, patients with T1D and macroalbuminuria remain at high and undiminished risk for end-stage renal disease, suggesting

that more effective therapies are desperately needed [11,56].

Taken together, since the publication of the first IXA Consensus Statement in 2009, new evidence documents the effectiveness of structured psychoeducational programs and refined diabetes technologies in reducing the incidence of SHE in patients with T1D and IAH [10]. However, one-fourth to one-third of patients with T1D and IAH continue to experience SHE even with the use of these interventions [35]. Because human islet transplantation can completely abrogate SHE and restore near-normal glycemic control for several years [57–60], this intervention is now approved and reimbursed for this subgroup of patients in several countries including the UK, Switzerland, Australia, and by some Provinces in Canada. Human islet transplantation, while increasingly well established as a vital treatment option for T1D, is associated with potentially serious side effects of immunosuppression and also severely limited in its applicability by the shortage of suitable human donor pancreases. Only a fraction of the pancreases retrieved from deceased human organ donors yields human islet products of sufficient quantity and potency for sustained metabolic benefits after single-donor transplantation in a cost-efficient manner [61]. It is conceivable that approximately 100 000 patients with T1D and IAH in the US alone could benefit from islet replacement therapy, assuming a prevalence of T1D of 1.0 million [62], development of IAH in up to 30–40% of patients [15,63], and resolution of SHE with medical interventions in up to 75% of patients with T1D and IAH [35]. The number of patients with T1D and chronic kidney disease and other microvascular complications who could benefit from islet replacement therapy for the purpose of slowing progression of complications in native or transplanted kidneys remains high and is estimated to be at least 100 000 in the US, including many thousand patients on renal transplant waiting lists and approximately 5000 new patients with T1D developing end-stage renal disease every year [11,56]. Transplants of human islets prepared from deceased organ donors cannot meet that demand. Because of unprecedented advancements made in recent years, much more scalable, human embryonic stem cell-derived islet beta cells represent a highly promising, alternative cell source for beta cell replacement therapy in diabetes [64–67]. Although early-stage pilot clinical trials in T1D have already been initiated in 2014 [68], clinical development may proceed slowly in view of safety concerns and the need for innovating more suitable implantation technologies and is expected to take many more years to be completed. Of the many questions to be addressed, like for other cell sources as well, the most suitable patient population for transplantation of stem cell-derived beta cells remains to be identified. Whether the lag time between plasma and interstitial glucose concentrations at the subcutaneous implantation site and the diffusion kinetics imposed by the retrievable immunoisolation device will limit application of such cell-device combination products in patients with SHE and glycemic lability is not well understood and cannot be easily addressed in preclinical transplant models. Similar limitations apply in part to the evaluation of porcine islet cell sources in nonhuman primates (NHP). Thus, in summary, despite substantial progress in the development of psychoeducational programs, insulin analogs, diabetes technologies, and stem cell-derived cell sources, a considerable subgroup of patients with T1D remains to be challenged by significant unmet clinical needs for which the continued development of xenogeneic porcine islet cell therapy products appears to be warranted.

Secondly, it is pertinent to review the preclinical and clinical progress made in the field of islet xenotransplantation since 2009 to determine which patient with T1D has the potential for a clinically significant improvement with increased quality of life following the xenoislet transplant procedure. Such a favorable benefit-over-harm determination has long been viewed as a fundamental prerequisite for initiating clinical research and is rooted in the moral duties of beneficence (the duty to benefit others) and non-maleficence (the duty not to harm others) [69,70]. Because clinical research on xenotransplantation patients is at risk of adverse events due to the xenograft product, transplant procedure, and immunosuppressive therapy, and patients and possibly society at large are exposed to unknown potential infectious risks [71], one of the guiding principles of the Ethics Committee of IXA [72] and the Changsha Communiqué [73] is that there should be a relatively high expectation of benefit, based on rigorous preclinical studies using the most relevant animal models, before such risks can be considered acceptable in clinical trials [8].

As there is no animal model of hypoglycemia unawareness, high expectation of benefit associated with porcine islet transplantation in patients with T1D, IAH, and recurrent SHE cannot be directly supported by preclinical studies that demonstrate restoration of hypoglycemia unawareness in islet xenograft recipients. This limitation has been recognized by the Cellular, Tissue and Gene Therapies Advisory Committee of

FDA/CBER at its meeting on 'Animal Models for Porcine Islet Xenotransplantation Products Intended to Treat Type 1 Diabetes or Acute Liver Failure' in 2009 [74]. However, the committee considered measurement of insulin dependence and insulin dose, glucose levels, C-peptide levels, HbA1c, mixed meal tests, etc., good surrogates to address secondary pathologies such as hypoglycemia unawareness and microvascular disease [74]. Thus, these surrogates can support the determination of expected benefits both in diabetic patients with IAH and in patients with ESRF.

Several preclinical studies in the pig-to-NHP model reported since 2009 have extended previous observations [75–78] and provided additional evidence of prolonged islet xenograft survival associated with near-physiologic control of fasted and post-prandial glycemia in immunosuppressed recipients [79–85]. In one of the immunosuppressed monkeys, normoglycemia was maintained for more than 600 days post-transplant, the longest reported islet xenograft survival to date [85]. A closer look at the reported cohorts indicates that functional pig-to-NHP islet xenograft survival exceeding 180 days, the efficacy benchmark to be met in ≥5 of 8 NHP before initiating clinical trials according to the original IXA consensus statement [8,86], has to date been achieved, with one notable exception, only in a small proportion of transplanted NHP (i.e., 1–2 of 3–7 in several studied cohorts referenced above). Investigators from the Xenotransplantation Research Center at Seoul National University College of Medicine met this endpoint in 4 of 5 transplanted monkeys [85], thereby achieving a significant advance and strongly suggesting that this important milestone can be met. However, as most other protocols that allowed long-term survival of wild-type or genetically engineered porcine islet grafts at least on an occasional basis [75–77,83], their immunosuppressive protocol included anti-CD154 antibodies. The considerable thromboembolic risks associated with these antibodies precluded their clinical development [87] and the clinical translation of the Seoul protocol.

Antagonistic anti-CD40 monoclonal antibodies (mAb), not associated with thromboembolic complications, could possibly substitute for anti-CD154 mAb in regimens for prevention of islet xenograft rejection although they neither mediate Fc-dependent depletion of activated T cells [88] nor 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 [89]. Nevertheless, one such antibody, clone Chi220, has been explored in a pig-to-NHP islet xenotransplant model with promising results [79]. Several additional antagonistic anti-CD40 mAbs, that is, 3A8 [90], 2C10R4 [91], and ASKP1240 (4D11) [92], have proven effective in prolonging islet allograft survival in NHP. Although it would require substantial resources, it would be critically important for the clinical translation of islet xenotransplantation to determine the efficacy and safety of selected antagonistic anti-CD40 antibodies in combination with other immunotherapeutics in preventing rejection of islets from wild-type and genetically engineered source pigs. Anti-CD40 mAb-based regimens have allowed substantial prolongation of survival of xenogeneic hearts from genetically engineered pigs in baboons [93]. Because lack of consistent success in achieving long-term islet xenograft survival in a cohort of recipients can be ascribed, at least in part, to failure of intraportally transplanted islets from wild-type donors to engraft in the presence of the instant blood-mediated inflammatory reaction (IBMIR) [94], the efficacy of anti-CD40 mAb in facilitating long-term survival of neonatal porcine islets known to express galactose-α 1,3-galactose (αGal) [95] should preferably be tested using islets exhibiting genetic modifications known to mitigate IBMIR. Successful engraftment of porcine islets in rhesus macaques, as measured by attainment of insulin independence, was increased after intraportal transplantation of αGal-deficient neonatal islet cell clusters (NICC) from galactosyl transferase knockout (GTKO) porcine donors [96] compared with transplantation of wild-type NICC [80]. Profound reduction of IBMIR and prevention of intravascular clotting was also demonstrated in baboons after intraportal infusion of NICC from αGal-deficient porcine donors transgenic for the human complement regulators CD55 and CD59 compared with wild-type donors [97]. These findings are very relevant as control of IBMIR could substantially increase the proportion of recipients with long-term islet xenograft function. Control of IBMIR could also noticeably reduce the donor–recipient ratio and thereby reduce the risks and costs of porcine islet transplants. How critical the use of GTKO porcine donors is for mitigating IBMIR in adult pig-to-NHP islet xenotransplantation is less well understood.

Immunosuppression-free survival of porcine islet xenografts in NHP has been achieved with a novel macroencapsulation technology [98]. Islets in alginate were transplanted subcutaneously as an islet monolayer on an acellular collagen matrix in a macrodevice; these grafts maintained FBG levels <150 mg/dl for 20 to 28 weeks in 5

streptozotocin-diabetic, non-immunosuppressed cynomolgus monkeys, whereas 2 of the 4 control monkeys that received microencapsulated adult porcine islets under the kidney capsule maintained FBG levels <150 mg/dl for up to 2 weeks. Co-transplantation of mesenchymal stem cells with islets in such macrodevices increased oxygenation and neoangiogenesis without substantially improving or prolonging islet xenograft function [99]. Long-term functional survival of human islet allografts [100] and rat-to-pig islet xenografts [101] in the absence of immunosuppression has also been achieved by subcutaneous transplantation of an oxygenated devise containing islets immobilized in alginate and immunoprotected by a thin hydrophilized teflon membrane impregnated with alginate.

An open-label, safety, and dose-finding Phase 1/2a study of microencapsulated neonatal porcine islets was performed under a comprehensive regulatory framework in New Zealand following 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 [1,102,103]. This trial demonstrated the microbiological safety of the tested encapsulated porcine islet product, which was prepared in compliance with current Good Manufacturing Practices from designated pathogen-free porcine donors and transplanted intraperitoneally at doses of 5000–20 000 IE/kg in 14 non-immunosuppressed patients with unstable T1D [104]. Analysis of the efficacy data did not show a dose effect of porcine islets, and porcine C-peptide was not detected in the serum of any of the transplanted patients [1]. Nonetheless, transplantation of the microencapsulated neonatal porcine islets was associated with a reduced frequency of unaware hypoglycemic episodes, lower HbA1c levels, and up to 30% lower daily insulin requirements in some of the patients [1]. This encapsulated porcine islet product was subsequently studied at doses of 5000 and 10 000 IE/kg in a Phase 2a efficacy trial in 8 subjects with T1D and IAH in Argentina with authorization by the Minister of Health and approval by the local bioethical committee [103]. The safety of the porcine islet product was confirmed in this trial; compared with pretransplant, most participants were found to have lower insulin requirements, fewer unaware hypoglycemic events, and reduced HbA1c levels post-transplant. These promising but very preliminary findings await confirmation in a controlled trial.

The preceding review of the preclinical and clinical status of islet xenotransplantation will help select the most suitable patient populations for the emerging islet cell xenotransplantation products and associated rejection prophylaxis technologies. While considerable uncertainty remains about the specific characteristics of xenoislet products and technologies for preventing their rejection that will advance to clinical trials, the accomplishments made in recent years favor the continued development of three concepts [105].

The first of these concepts involves the intraportal transplantation of wild-type or genetically engineered adult porcine islets in immunosuppressed recipients. As discussed above, this concept is supported by several preclinical studies showing long-term normoglycemia in insulin-independent NHP immunosuppressed with anti-CD154-based protocols. Clinical translation of this concept requires the development of i) a controlled, consistent, and scalable islet manufacturing process to manufacture therapeutic porcine islet patient doses of approximately 1.5 million islet equivalents from ≤3 (to 5) porcine donor pancreases; ii) interventions that mitigate IBMIR for the purpose of maintaining the transplanted islet dose within reasonable limits and for the purpose of facilitating stable and long-term function of a high number of engrafted islets; and iii) effective, safe, anti-CD154-sparing and clinically applicable and available immunosuppression for prevention of islet xenograft rejection. Antagonistic anti-CD40 mAbs hold the potential to substitute for anti-CD154 mAbs in xenotransplantation [93], but the development of an effective, safe, and clinically applicable anti-CD40-based regimen will require commitment to their continued evaluation in the pig-to-NHP islet xenotransplant model in combination with other immunotherapeutics. Once these remaining requirements have been met, the benefit–risk determination of the resulting xenotransplantation protocol is expected to be sufficiently favorable to warrant its evaluation in patients in whom T1D is complicated by IAH and recurrent SHE. To warrant clinical trials of such a protocol in patients with T1D and ESRF undergoing simultaneous transplantation of an allogeneic kidney and a xenogeneic islet product and in patients with an established kidney allograft will additionally require prior documentation of the safety and efficacy of anti-CD40-based, preferably calcineurin inhibitor- and steroid-free immunosuppression in preventing rejection of renal allografts in clinical trials. Two antagonistic anti-CD40 antibodies, ASKP1240 and CCFZ533X2201, have entered clinical evaluation in de novo kidney transplant

recipients [106,107]. While it seems possible that a clinically applicable, anti-CD40-based regimen can be developed for use in porcine islet xenotransplantation in T1D, it is yet another question whether by the time such regimens have been developed and corresponding antibodies have become available for clinical research in islet xenotransplantation, the proposed islet xenotransplantation intervention will be the best of all treatment options then available to diabetic patients with IAH and/or ESRF [108].

The second emerging clinical concept incorporates the intraportal transplantation of NICC from genetically engineered donors in immunosuppressed recipients and is as such very similar compared with the first concept in its requirements for clinical translation and very similar with respect to the patient populations that appear appropriate for participating in pilot clinical trials testing this concept. The only difference is the demonstrated advantage of using GTKO porcine donors for the purpose of mitigating IBMIR to intraportally transplanted NICC [80,97], which is less well established for adult porcine islets. It is unknown whether, in the NICC-to-human and in the adult pig-to-human settings, donors with multigenic modifications such GGTA1 and CMAH double- and GGTA1, CMAH, and B4GalNT2 triple-knockout donors will provide additional advantages [109–112].

The third clinical concept entails the transplantation of neonatal or adult porcine islets behind an immunoisolation barrier in non-immunosuppressed recipients. Should investigators and sponsors of such a proposed clinical trial be in a position to provide very high assurance of safety of their hybrid xenogeneic cell and immunoisolation device product, evaluation of the technology must then not be limited to patients who have serious or life-threatening diseases for whom adequately safe and effective alternative therapies are not available [2,4]. Because restoration of protection from SHE has been demonstrated in human islet allograft recipients with marginal graft function [43] and in view of the preliminary mixed efficacy findings in pilot trials of microencapsulated neonatal porcine islets suggesting a reduced incidence of unaware hypoglycemic episodes despite overall marginal graft function [1,103], the potential of benefit and the ability to detect the microencapsulated xenoislet product's activity are expected to be higher in patients with T1D and IAH than in patients with T1D and microvascular complications. The risk of sensitization to porcine antigens, including swine leukocyte antigens (SLA), is considerable in non-immunosuppressed recipients of microencapsulated porcine islets, assuming that the stability and integrity of infused microcapsules containing islets is incomplete over time. As a number of neonatal donors will be required for a single therapeutic islet patient dose and as our understanding of sensitization to SLA and cross-reactivity of anti-HLA antibodies with SLA alleles is incomplete, consideration should be given to excluding patients with severe hypoglycemia refractory to medical management and patients with or at high risk of developing ESRF from participation in early-phase trials of xenotransplantation protocols that are likely associated with a high risk of sensitization. Therefore, patients with T1D that neither suffer from IAH nor have microvascular complications could be suitable candidates for safety trials of microencapsulation technologies as long as high assurance of safety can otherwise be demonstrated (e.g., no requirement for investigational immunosuppression). These considerations might not apply to pilot clinical trials evaluating islet macroencapsulation devices [98,101] in non-immunosuppressed recipients if investigators can demonstrate in preclinical studies prolonged restoration of normoglycemia and insulin independence with a low risk of sensitization. As discussed above in the context of possible limitations of transplantation of stem cell-derived islet products in retrievable macrodevices, the implications of the lag time between plasma and interstitial glucose concentrations at the subcutaneous implantation site and the diffusion kinetics altered by the immunoisolation membrane and device for the selection of T1D patients with recurrent SHE and glycemic lability should be considered.

Thirdly and finally, in the context of the existing regulatory framework referred to above and in Chapter 1, it is appropriate to ascertain briefly which patient with T1D will—more easily than others—be able to comply with long-term monitoring and other public health measures. As discussed in the original consensus statement [7], it is inevitable that a proportion of islet xenografts will fail and lead to sensitization, thereby complicating retransplantation, reducing the prospects of having enduring benefits associated with participation in the xenotransplantation trial, and possibly reducing the participants' adherence to lifelong monitoring. As in human islet allotransplant trials, candidates should undergo psychological evaluation to determine their capability to cope with islet xenograft loss and to comply with long-term monitoring. Avoiding porcine antigens to which the recipient is sensitized in islet xenografts prepared for re-transplantation will be mandatory. Selecting recipients at an advanced age or with a

reduced life expectancy will certainly lower the duration of monitoring commitments participants are asked to make. Refining donor–recipient matching in pig-to-human xenotransplantation could in the future facilitate selection of recipients in whom prolonged xenograft survival is more likely. Whether long-term monitoring of the islet xenotransplant recipient can be incorporated in the management of the patient's diabetes and renal disease, which are chronic diseases requiring life-long monitoring as well, will depend on the scope of xenotransplantation-specific monitoring defined in the trial protocol and on the degree of overlap with routine monitoring. Renal transplant recipients receive long-term monitoring that includes monitoring of immunosuppression, infective risks, and protective immunity; thus, the additional assessments required to comply with participation in a xenotransplantation trial are expected to be more limited for this subgroup of patients compared with nonuremic patients with T1D and IAH.

New and underappreciated topics not addressed in the original statement

This section will suggest for consideration four additional patient populations, not discussed in the original consensus statement, for whom an islet allotransplant could be medically justified but who are unlikely to receive such a transplant for various reasons and who could be appropriate candidates for participating in early-phase trials of porcine islet cell xenotransplantation products. Firstly, islet xenografts could substantially increase access of patients to islet replacement therapy who meet medical criteria for an islet allograft as discussed above but who would not have ready access to a transplant because of high immunization to human leukocyte antigens (HLA). Allosensitization does not increase the risk of xenoreactivity to tissues and organs from GT-KO porcine donors in patients on transplantation waiting lists [113,114]. While cross-reactivity of anti-HLA antibodies with SLA alleles may limit the use of porcine xenografts in some highly sensitized patients, most patients with anti-HLA class I antibodies should be able to find pig donors lacking SLA antigens that cross react with their antibodies [115]. Secondly, insulin-treated patients with type 2 diabetes (T2D) and IAH are another possible patient population for early-phase trials of islet xenotransplantation. The prevalence of IAH in this patient population is 9.8%, and in those with IAH the incidence of SHE is 17-fold higher than in those with normal hypoglycemia awareness [116]. Xenogeneic islets are not at risk of recurrent autoimmunity as in recipients with T1D. Thirdly, with substantial progress made in renal xenotransplantation [117,118], simultaneous kidney–islet xenotransplantation in patients with ESRF and T1D or T2D could be considered. Simultaneous pancreas and kidney transplantation is increasingly being utilized for the treatment of T2D and ESRF [119,120]. Finally, encapsulated porcine islet xenotransplantation products could be evaluated in non-immunosuppressed patients with pancreatectomy-induced diabetes, who underwent total pancreatectomy for the treatment of chronic pancreatitis or pancreatic cancer but did not receive an islet autograft or islet or pancreas allograft and are challenged by extreme glycemic lability [121]. Post-pancreatectomy diabetes can present significant diabetes management challenges [122–127] and would allow evaluation of the efficacy of xenoislets and immunoisolation without the risk of recurrent immunity.

Suggested revisions

Firstly, in view of the considerable progress made in the treatment of diabetes since 2009, one fitting revision of the original IXA consensus statement appears to be the mandatory incorporation of a formalized medical optimization run-in period in the selection of appropriate participants in early-phase trials of islet xenotransplantation. The purpose of such a run-in period is to evaluate whether candidate participants can meet their treatment targets when they have access to evidence-informed interventions in a structured, stepped approach under the supervision of an expert diabetes care team. Only those candidates with microvascular complications such as ESRF who cannot meet clinically appropriate glycemic goals and candidates with IAH in whom SHE persist after completion of the formalized medical optimization run-in period should be deemed to have failed optimized medical therapy and considered satisfying medical criteria for undergoing islet (or pancreas) transplantation. Alternatively to a formalized run-in period that is part of a clinical pilot trial and lasts preferably at least 6 months, trial participants could be recruited from specialist diabetes complications or hypoglycemia services where patients are given similarly access to a structured and stepped care approach to meeting treatment targets. Recently proposed, evidence-informed treatment algorithms for patients with T1D and problematic hypoglycemia involve structured diabetes education in flexible insulin therapy, which may incorporate psychotherapeutic and behavioral

therapies, and progress to diabetes technology, incorporating sensors and insulin pumps, or very frequent contact with care team, in those with persisting need [9,10]. Patients in whom SHE are deemed refractory to best educational and technological interventions should be evaluated for transplant interventions. Individual patient circumstances should direct suitability and acceptability to ensure prudent use of technology and transplant resources [9].

Secondly, the original IXA consensus statement is revised to suggest that sponsors/investigators include in their application the requirement for justifying the rationale for islet xenotransplantation over islet or pancreas allotransplantation in those patients who, based on outcomes of a formalized medical optimization run-in period or a structured steeped care approach as outlined above, meet medical criteria for islet replacement. As dictated by the patient population and specifics of the study protocol, the discussion of the rationale for favoring xenoislet replacement should show that the selected patients, who meet medical criteria because of refractory SHE or persistently above-target HbA1C levels, will not have timely access to islet or pancreas allotransplantation as a result of allosensitization to the majority of possible human donors, high islet dose requirements that are unlikely to be met by human islets, or other factors. Alternatively, the discussion of the rationale could point out why the risk-benefit determination associated with a xenoislet transplant is expected to be more favorable than the determination associated with an alloislet (e.g., superior xenoislet product quality) or allopancreas (e.g., patient's cardiac comorbidity) transplant.

Thirdly, the original IXA consensus statement is revised to suggest that sponsors/investigators address in their application why the expected benefits and risks of the proposed study protocol are more favorable in the proposed than in any other study population. For example, as discussed elsewhere in this chapter, the potential for benefit and the ability to detect the xenoislet product's activity might be highest for microencapsulated neonatal islets in patients with T1D and IAH. However, the risks of sensitization after transplantation of such a product are expected to be high in any non-immunosuppressed recipients of islets from multiple neonatal donors, and the implications of possible sensitization are expected to be particularly significant in patients with T1D at risk of ESRF and possibly also very relevant in T1D patients with IAH and recurrent and refractory SHE.

Conclusion

The purpose of this review was to assist investigators, sponsors, and other stakeholders active in the development of porcine islet cell xenotransplantation products to navigate the questions related to the selection of appropriate participants in early-phase xenoislet trials. No clear consensus can be anticipated as long as research on medical modalities, xenoislet and other cell replacement technologies for diabetes continues and uncertainty about their potential for benefits and risks remains.

The improved educational programs involving behavioral therapies, insulin analogs, CGMS, and diabetes technologies such as SAP with predictive low-glucose management technology [33] will help insulin-treated diabetic patients meet treatment goals. For those patients in whom diabetes is complicated by IAH and recurrent SHE, extreme glycemic lability, and progressive microvascular lesions and in whom SHE persist or glycemic goals cannot be maintained despite access to refined interventions under the close guidance of an expert diabetes care team, transplant interventions should be considered and continue to be optimized. Patients who present with these persistently unmet clinical needs may have T1D, T2D, or pancreatogenic diabetes resulting from total pancreatectomy. To warrant enrollment of these transplant candidates in pilot clinical trials of porcine islet cell products, lack of timely access to alloislet replacement due to allosensitization, high islet dose requirements, or other factors, or alternatively, a more favorable benefit–risk determination associated with the xenoislet than the alloislet or allopancreas transplant must be demonstrated. Additionally, in non-uremic xenoislet recipients, the risks associated with diabetes must be perceived to be more serious than the risks associated with the xenoislet product and the rejection prophylaxis, and in xenoislet recipients with renal failure, the xenoislet product and immunosuppression must not impact negatively on renal transplant outcomes.

Rapidly evolving, alternative cell transplant technologies such as stem cell-derived islet beta cell replacement will soon also need to be considered. Xenoislet transplant technologies are also rapidly evolving and the specific characteristics of each investigational xenoislet product and related technologies applied for preventing rejection will determine the patient group in whom the xenoislet product's activity, either beneficial or adverse, can be best detected and for whom participation in an early-phase xenoislet trial is the best option. As the xenoislet products advance through research and development and the full potential of xenoislet

transplantation is increasingly realized through genetic modifications of porcine donors [128], antigen-specific immunotherapy [129], donor thymus co-transplantation [130], and other technologies not available to allotransplantation, the selection of appropriate recipients will need to be entirely revisited.

Acknowledgments

The authors' work was supported in part by grants from the National Institutes of Health, the National Health and Medical Research Council of Australia, and the Juvenile Diabetes research Foundation. The authors wish to thank Brian Flanagan and Christopher Burlak for their critical review of the manuscript.

Conflict of interest

B.J.H. has served as a consultant to Dompé s.p.a. and Janssen Research and Development L.L.C. and is a Director of Diabetes-Free, Inc. P.O.C has served as a consultant to Otsuka Pharmaceutical Factory. No other potential conflict of interests relevant to the content of this article were reported.

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