CTGTAC Meeting # 47

Animal Models for Porcine Xenotransplantation Products Intended to Treat Type 1 Diabetes or Acute Liver Failure

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BRIEFING DOCUMENT

INTRODUCTION

This meeting is convened to provide the Food and Drug Administration (FDA) with insights and perspectives regarding the use of available animal models to support the administration of porcine xenotransplantation products in patients with acute liver failure (ALF) or Type 1 Diabetes (T1D). No specific products will be presented for regulatory review at this meeting. The source origin of these products will be restricted to porcine. Animal husbandry and product manufacturing issues will not be discussed in the meeting. At this meeting, invited experts who are developing animal models to evaluate xenotransplantation products to treat these two diseases will present their viewpoints on the major issues confronting the development of these models. Members of the committee will be requested to consider this information, as well as the information presented in this document, and to discuss and provide advice regarding FDA questions provided in this briefing package. Discussions of specific issues related to risks of xenogeneic infections are beyond the scope of this meeting. These issues are addressed in multiple guidances and guidelines and contribute to the basis of FDA assessment of risks to subjects in clinical trials [1-5]. Finally, discussions of genetically engineered animals are beyond the scope of this meeting and are addressed in FDA guidance [6].

BACKGROUND

FDA is responsible for assessing the safety and efficacy of new therapies, as well as ensuring that subjects enrolled in clinical trials of such products are not exposed to unreasonable risk. These responsibilities include regulation of clinical trials of xenotransplantation products. According to both the PHS guideline released in January 2001 entitled '*PHS Guideline on Infectious Disease Issues in Xenotransplantation,'* and the FDA guidance document released in April 2003 entitled, *'Guidance for Industry: Source Animal, Product, Preclinical, and Clinical Issues Concerning the Use of Xenotransplantation Products in Humans,'* xenotransplantation is defined as any procedure that involves the transplantation, implantation, or infusion into humans of: 1) live cells, tissues, or organs from a nonhuman animal source or 2) human body fluids, cells,

tissues, or organs that have had *ex vivo* contact with live nonhuman animal cells, tissues, or organs [1-5]. Xenotransplantation products may be able to offer novel treatments for patients suffering from a variety of diseases and injuries. Ongoing scientific research is directed towards improving the potential of new xenotransplantation products to provide clinical efficacy, while continuing to investigate issues related to safety.

The potential risks of xenotransplantation in the targeted patient population must be considered in the overall regulatory decision-making process for permitting the initiation of a first-in-human clinical trial. These risks include, but are not necessarily limited to: 1) the transmission of known pathogens, 2) the potential for introducing a new infectious disease into the general population, 3) the potential for adverse inflammatory and immunological responses of the host to the product or its secreted proteins, 4) the potential for rejection of the source animal cells/tissues/organs and any associated adverse effects, 5) the risks of using immunosuppressive agents in an attempt to prolong the transplanted graft, and 6) potential zoonotic risks to personal contacts and healthcare professionals. Although preclinical animal studies are conducted in order to characterize these risks, current investigational modalities cannot provide absolute assurance that all risks will be fully identified and evaluated. In developing clinical trial protocols, prospective sponsors should take the preclinical data into account when considering what the possible benefit to subjects from participating in the trial would be and/or how the resultant data from the first-in-human trial would contribute substantially to scientific knowledge and prudent product development. Demonstration of pharmacodynamic action of the investigational product will be helpful in these considerations. Given the potential risks due to the particular product, administration procedure, and concomitant medications and treatments, studies of animal models of the targeted disease are important to estimate the potential for positive pharmacodynamic action of the xenogeneic product, as well as the potential for harm. The goal of this meeting is to obtain expert advice regarding the availability and use of relevant animal models to evaluate and define the safety and clinical activity of porcine xenotransplantation products to treat patients with ALF or T1D.

U.S. GOVERNMENT ACTIVITIES RELATED TO XENOTRANSPLANTATION PRODUCTS

Xenotransplantation products are subject to FDA regulation under Section 351 of the Public Health Service (PHS) Act (42.U.S.C.262) and the Federal Food, Drug and Cosmetic Act (21 U.S.C. 321). In addition to various published guidances intended to assist investigators in this field [1-5], several public advisory committee meetings have been held to discuss scientific, medical, ethical, social, and regulatory issues on xenotransplantation products.

The FDA/CBER Biological Response Modifiers Advisory Committee (BRMAC) Xenotransplantation Subcommittee [7]

The first BRMAC Xenotransplantation Subcommittee meeting was held in December 1997 in response to FDA's need to address regulatory issues associated with these products. This subcommittee consisted of 15 voting members, including the Chair, who was also a member of the BRMAC. The members had expertise in xenotransplantation, epidemiology, virology, microbiology, infectious diseases, molecular biology, veterinary medicine, immunology, transplantation surgery, public health, applicable law, bioethics, social sciences, patient advocacy, and/or animal welfare. Nonvoting participants also included experts from the Centers for Disease Control and Prevention (CDC), the National Institutes of Health (NIH) and FDA/CBER. This subcommittee was tasked with addressing risk assessment and management for xenotransplantation products and to highlight and discuss new scientific data generated in this rapidly evolving field. The last subcommittee meeting was held in 2000.

The Secretary's Advisory Committee on Xenotransplantation (SACX) [8]

The Secretary's Advisory Committee on Xenotransplantation (SACX) was chartered in 1999 to advise the

Secretary of the Department of Health and Human Services (DHHS) on all aspects of the scientific development and clinical application of xenotransplantation. The committee considered the scientific, medical, social, and ethical issues in the context of patient and public health concerns raised by xenotransplantation. These discussions extended to ongoing and proposed clinical trial protocols. Based on these public meetings, the SACX made recommendations to the Secretary of DHHS on policy and procedures associated with this pioneering area of research. The goal of the SACX was to facilitate DHHS efforts to develop an integrated approach to addressing emerging public health issues in xenotransplantation. The SACX consisted of 18 voting members that were appointed by the Secretary or a designee, with expertise as described for the FDA BRMAC Xenotransplantation Subcommittee. At least one SACX member was also a current member of the FDA BRMAC and at least one member was a current member of the CDC Healthcare Infection Control Practices Advisory Committee. Additional non-voting members from DHHS agencies including the Office of the Secretary, CDC, FDA, Health Resources and Services Administration, NIH and others as deemed appropriate by the Secretary or a designee, participated. The first SACX meeting was held in February 2001; the last meeting was held in February 2004.

In addition, FDA actively participates in international activities in the field of xenotransplantation because of the potential global impact of communicable disease outbreaks. Agency efforts in the global community include participating in the WHO efforts to develop international recommendations regarding xenotransplantation products.

CLINICAL CONSIDERATIONS

Xenotransplantation products offer the possibility of replacing, for varying periods of time, failed organ, glandular, or tissue function in conditions for which human-derived cells and tissues are in limited supply. Acute liver failure (ALF) and type 1 diabetes (T1D) represent particularly important examples of such diseases, because the need for cellular treatment options far exceeds the current or foreseeable supply of human-derived differentiated cells and there are no other currently-available or approved sources of such differentiated cells.

As reported in the published literature, investigational cellular products to treat ALF consist of hepatocytes or liver tissue placed in an extracorporeal blood circulation device, termed a Bioartificial Liver [BAL] assist system [9-11]. The intent is to use the system intermittently or continuously, under intensive care monitoring, until a liver is available for orthotopic transplantation or the patient spontaneously recovers. The acute clinical situation, which is life-threatening, generally reaches resolution (recovery, transplantation, or death) within weeks. To date, there are no approved human or xenogeneic cellular products or BAL systems containing human or xenogeneic cells or tissues for this indication.

For the treatment of Type 1 diabetes (T1D), the medical community has had considerable experience with allogeneic islet transplantation under the regulatory auspices of Investigational New Drug (IND) applications, as several hundred patients have received human islets via intraportal infusion, with immunosuppression. The major benefits, which have been demonstrated in a substantial proportion of subjects in clinical trials, are insulin independence, attenuation or disappearance of severe hypoglycemia, and improved metabolic control without increasing the frequency of hypoglycemia, even in recipients who still require some exogenous insulin. The principal limitations of this approach are the transient nature of clinical benefits (generally of the order of a few years), the need for continuous immunosuppression, and the limited supply of human islets. Current supplies of cadaveric islets could satisfy less than 1% of potential demand, even assuming very stringent clinical and metabolic criteria for treatment. Porcine-derived islets may offer the potential to provide cellular therapy for a large number of diabetic patients. Porcine and human beta cells have similar metabolic responses, and porcine insulin has been used therapeutically for decades. In addition, the use of islet encapsulation may reduce or eliminate the need for immunosuppression and may extend xenogeneic islet survival post-transplant. Accordingly, porcine islets, which are in plentiful supply, offer the possibility of treating large numbers of metabolically unstable diabetic patients.

PRECLINICAL CONSIDERATIONS

Overall Concepts

According to Title 21 of the Code of Federal Regulations (CFR) Part 312.23 (a)(8), the sponsor of a clinical trial should provide *"…adequate information about the pharmacological and toxicological studies…on the basis of which the sponsor has concluded that it is reasonably safe to conduct the proposed clinical investigations. The kind, duration, and scope of animal and other tests required vary with the duration and nature of the proposed clinical investigations."* The design of preclinical studies is a critical determinant of their ability to provide appropriate and sufficient data to support clinical development of a product. For example, as previously stated in this document, the administration of xenotransplantation products in humans poses many risks, thus comprehensive preclinical studies can provide data to be considered in planning clinical trials to identify and characterize these concerns. Selection of the most appropriate animal model(s) to evaluate a xenotransplantation product intended for a specific clinical disease will serve to provide insight regarding dose/activity and dose/toxicity relationships. This information will provide the most reliable determination of the therapeutic potential of the product, as well as assess the likely duration of clinical effect. Duration of effect is important, given the inherent risks of xenotransplantation products. An understanding of the biological actions of the xenotransplantation product following administration in animals that model the intended clinical disease to the extent possible (pathophysiology, metabolically, immunologically) will potentially help to mitigate some of the risks to humans enrolled in clinical trials.

ANIMAL MODELS OF ACUTE LIVER FAILURE (ALF)

ALF presents as a multi-system syndrome, occurring as a consequence of acute or acute-on-chronic failure of hepatic function. Major elements of the syndrome include hepatic encephalopathy (HE), jaundice, coagulopathy, severe metabolic abnormalities, renal insufficiency and hemodynamic instability [12]. The pathophysiologic basis of HE is incompletely understood and considered to be multifactorial [13]. Elevations of blood levels of ammonia and other toxic substances, together with numerous ALF-associated metabolic and electrolyte derangements, may contribute to the syndrome. These associated disorders include hyponatremia; hypokalemia; hypophosphatemia; lactic acidosis; hypoglycemia; acute pancreatitis; and renal, respiratory, and circulatory failure.

The majority of extracorporeal BAL devices that are currently under development for clinical use in the ALF setting consist of liver tissue or hepatocytes obtained from porcine source animals. This approach is attractive because: 1) porcine tissue/hepatocytes are readily available, 2) important metabolic activities of porcine hepatocytes (i.e. clearance of ammonia and other metabolites, urea synthesis, p450 activity) are similar to those in humans, and 3) many of the porcine hepatic tissue/cell preparations can tolerate a wide range of handling and storage conditions and still have the potential to remain functional in a BAL device [9].

Numerous etiologies and complications of ALF in humans render a notable challenge in the development of an animal model that accurately reflects this condition. Many different procedures have been used to induce ALF in small and large animal species. Among these approaches are: 1) total or partial liver resection (hepatectomy model); 2) complete or transient hepatic devascularization by ligation or temporary clamping of the portal vein and hepatic artery (ischemia model); 3) administration of hepatotoxic drugs and chemicals (such as CCl_4) (hepatotoxic drug/chemical model); and 4) exposure to viral agents (infectious model). We refer the reader to Belanger and Butterworth 2005 for a comprehensive review of many of these models, and the table provided in this document is adapted from this reference [14].

The **infectious models** are probably the least favored approach due to the potential for human exposure to the hazardous infectious agents used to create the model [14]. Therefore we will not review them here.

The **hepatotoxic drug/chemical models** are generated via the use of agents such as acetaminophen, galactosamine, and thioacetamine (TAA). These are compromised due to the lack of inter-animal reproducibility, including the degree of toxicity relative to chemical dose, the clinical manifestations and laboratory abnormalities of ALF, and the time to death. In addition, extrahepatic toxicities occur commonly. For example, administration of acetaminophen in pigs and dogs has been reported to cause toxicity to the kidneys, heart, and lungs due to methemoblobinemia caused by the oxidation of hemoglobin by acetaminophen. TAA has been reported to cause neurotoxicity in animals, independent of liver toxicity, limiting the ability to assess the potential for a BAL to relieve hepatic encephalopathy in this model.

Such direct extrahepatic toxicities can generally be avoided in the **hepatectomy and ischemia models**, but the latter rely heavily on the surgical technique and expertise of laboratory personnel to ensure that reproducibility of the model is achieved. Models that depend on a total hepatectomy or a complete devascularization are too severe, as death inevitably results, with no evidence of liver regeneration/spontaneous recovery. Animals that are subject to a **total hepatectomy** are more artificial in nature, as the patho-physiology of an injured liver to link to the clinical setting does not exist. For example, encephalopathy is detected only a few hours before death in this model, whereas it is observed early on in patients with ALF, and progressively worsens with time. Minimal hematologic and hemodynamic changes and brain edema have also been reported with this model, which does not reflect the severity of these symptoms when humans present to the hospital. Animals that are subjected to a **partial hepatectomy** do not usually progress to hepatic coma, and this model has been historically poor in recapitulating the clinical manifestations of ALF in patients.

Progressive encephalopathy and hepatic coma, accompanied by brain edema and intracranial hypertension, which are observed in patients with ALF, have been documented in animals exposed to **complete devascularization** (without hepatectomy). While these abnormalities are also manifested in animals exposed to **transient devascularization**, the ability to reproduce these laboratory and clinical changes with acceptable consistency is relatively poor.

The generation of an animal model of ALF through a **combined surgical and hepatoxic drug/chemical-**induced approach has also been reported. An example is a rat ALF model created via a partial hepatectomy (70% liver resection), followed by intravenous injection of endotoxin [15]. However, induction of an ALF model via this combined strategy is infrequently reported in the scientific literature.

Various animal models of ALF reported in the published literature

Model	Species Used					HE	Intracranial Hypertension	Reproducibility	Reversi Spontar Reco
	Pig	Dog	Rabbit	Rat	Mouse				
Acetaminophen	√	√	√			Yes	NA	+/-	Poten
Galactosamine		√	√	√		Yes	Yes	+/-	Poten
Thioacetamide			√		√	Yes	Yes	Yes	Poten
Viral hepatitis			√			NA	Yes	+/-	Poten
Total hepatectomy	√	√		√		Yes	Yes	Yes	No
Partial hepatectomy	√			√		No	NA	Yes	Yes
Complete hepatic devascularization	√	√	√	√		Yes	Yes	Yes	No
Transient hepatic devascularization	√					Yes	NA	+/-	Poten

HE = Hepatic encephalopathy; **NA** = data not available
Reversibility = possible liver regeneration

[Belanger M and Butterworth RF (2005). Acute liver failure: a critical appraisal of available animal models. Metab Brain Dis 20:409-23]

Selected Published Examples of the Use of BAL Devices in Animal Models of ALF

Flendrig and colleagues used a BAL device containing porcine hepatocytes in a rat ALF model created by an end-to-side portacaval shunt placed three days before hepatic artery and bile duct ligation [16]. The rats were treated with the device, starting 30 minutes after ligating the hepatic artery and bile duct until death. The study endpoints included HE assessment (neurological symptoms of normal to deep coma) and determination of arterial pressure, blood ammonia levels, lactate levels, and hepatic enzyme levels. Per the investigators, a treatment effect was demonstrated by reduction of blood ammonia and lactate levels, and by improvement of HE scores ($p<0.05$). All the non-treated control and empty device (lack of porcine hepatocytes)-treated control animals died between 3 to 8 hours after inducing liver ischemia, while the treated animals died between 8 to 14 hours after inducing liver ischemia.

Abouna and colleagues studied the use of a BAL device containing dog liver or calf liver in dogs (6/group) that had end-to-side portacaval shunts put in place the day prior to induction of ALF by occlusion of the hepatic artery for 2 hours [17]. At the onset of ALF, which was defined as the appearance of encephalopathy, hyperammonemia, hyperbilirubinemia, and elevated prothrombin time and hepatic enzymes (occurring about 10-12 hours after clamping the hepatic artery); the dogs were treated using the BAL device for 6-8 hours. The study endpoints included: serum bilirubin levels (excretion of toxic metabolites), hepatic enzyme levels, blood ammonia levels (detoxification), prothrombin time, various physiological parameters (i.e. portal vein pressure, hepatic artery pressure, body temperature, bile flow, blood pressure, pulse rates), overall survival, and gross and microscopic examination of the animal's liver. According to the authors, a treatment effect was demonstrated by a reduction in bilirubin levels and decreased hepatic necrosis in the dogs. The control animals that received medical support only, died between 32 to 38 hours post shunt placement, while the treated animals died between 60 hours to 7 days post shunt placement.

Fruhauf et al, conducted a study in a pig ALF model generated by a total hepatectomy [18]. One hour after the surgery the pigs (n=6/group) were treated continuously using a BAL device containing porcine hepatocytes until death. Note that the plasmapheresis cartouches were replaced every 8 hours. The study endpoints included albumin and ammonia levels and assessment of intracranial pressure (ICP). The investigators reported treatment effects demonstrated by increased albumin levels, decreased ammonia levels, a lower ICP, and extension of survival (24.8 ±4.3 hours) compared to untreated control animals (16.4 ±4.7 hours).

The studies using animal models of ALF described in the current scientific literature were largely conducted using a single cycle of exposure to the BAL device (i.e., a single treatment). However, due to the wide variation in individual device design, such as: 1) the xenogeneic components (hepatocytes vs. liver tissue), 2) the quantity/weight/volume of the xenogeneic components, 3) the rate of extracorporeal blood flow through the device, and 4) product viability (pre-and post-treatment), as well as the possibility of a host immune response to the source transplantation product, a single cycle of treatment may not be sufficient to achieve a clinically significant biological effect. Thus the duration of a single treatment and the ability to repeat the treatment, as would quite likely be required in the clinical setting, is an important consideration when developing animal models of ALF. In this regard, it is also useful to consider the theoretical assessments of Iwata and Ueda, in which the importance of hepatocyte mass and blood flow are discussed [19].

From the foregoing, the development of appropriate animal models for assessment of products designed to treat ALF has been fraught with numerous complexities and difficulties. The ability of existing animal models to reflect the immunologic, metabolic, and physiologic aspects of patients with ALF and the application of these models to inform first-in-human trial design are important considerations.

ANIMAL MODELS OF TYPE 1 DIABETES (T1D)

Rapid progress in several scientific areas may enable use of relevant animal models of diabetes in preclinical evaluations of xenotransplantation products prior to introduction of clinical trials in patients. Of particular importance are: 1) the increased understanding of the molecular and cellular basis of immune

suppressive/regulatory mechanisms and the subsequent development of animal models that are genetically or immunologically modified to tolerate xenotransplantation products; 2) advances in the ability to introduce genetic modifications in the pigs that will be the source animals; and 3) progress in techniques for the encapsulation and immunoisolation of xenogeneic islets.

Considerations for the use of an animal model(s) to generate safety and efficacy data using porcine islets include: 1) the method of induction of a diabetic state (i.e., spontaneous, chemical, surgical, immunological), 2) the final porcine islet 'formulation' (i.e., encapsulated vs. unencapsulated), 3) the transplant procedure, 4) the glucose metabolic set points of the recipient animal versus porcine islet set points, 5) the immunological profiles of the porcine source animal and of the recipient animal, 6) the duration of a clinically meaningful effect post-transplant and the need for re-transplantation to maintain this effect, and 7) identification of relevant parameters that indicate substantial clinical benefit (i.e. insulin requirements, fasting and glucose challenged animal source and recipient C-peptide levels, Hb_{A1c}, etc...). For each animal model that has been used in an attempt to satisfy these factors, advantages and limitations exist.

T1D occurs as a result of the complete or nearly complete destruction of the insulin-producing beta cells in the pancreas. Thus, for products designed to treat T1D, the induction of a diabetic state in recipient animals has generally been accomplished via one of three modalities that adversely affect beta cell function.
Spontaneously diabetic animals, commonly rodents (e.g. Non-Obese Diabetic [NOD] mice and Bio Breeder [BB] rats), permit the investigation of xenotransplant islet function in an autoimmune setting that more closely approximates the immunologic dysfunction associated with the human disease. In particular, as with humans, the MHC plays a role in the susceptibility of NOD mice and BB rats to diabetes. Transplanted islets in these two models are subjected to immune attack by T cells, B cells, NK cells, and macrophages, accompanied by the production of a variety of autoantibodies, such as anti-GAD and anti-insulin antibodies, similar to the human scenario. Insulitis occurs in NOD mice at around 4-5 weeks of age, with frank diabetes presenting at approximately 12-30 weeks of age. The BB rats exhibit weight loss, polyuria, polydipsia, hyperglycemia, and insulopenia at about 12 weeks of age, displaying a severe ketoacidosis that is fatal without exogenous insulin administration. However, differences between the human and animal autoimmune models do exist. For example, ketoacidosis in NOD mice is mild and animals can survive for weeks without exogenous insulin. There are also differences in genetic susceptibility markers that could potentially confound study outcome. Even so, spontaneous models of diabetes permit the study of a highly genetically controlled population from which valuable information can be interpreted from xenotransplantation studies [20].

The second modality used to induce a diabetic state in large or small animal species is via **chemical induction**. The two most common agents employed are the toxic glucose analogues streptozotocin and alloxan. Streptozotocin, a nitrosurea derivative from *Streptomyces achromogenes*, induces a diabetic state when injected intraperitoneally in multiple small doses typically over five consecutive days (in order to avoid direct toxicity to the pancreas and other organs) [21]. Alloxan, an oxygenated pyrimidine derivative, is a toxic glucose analogue that accumulates in the beta cells via the GLUT2 glucose transporter and generates reactive oxygen species that ultimately trigger the death of the cells. As with streptozotocin, alloxan also inhibits glucose-induced insulin secretion [22]. When either diabetogenic agent is used in an animal species, adequate numbers of animals and utilization of appropriate controls are needed to ensure that baseline parameters (e.g. glucose, C-peptide, etc...) are established for a sufficient period of time both before and after a diabetogenic state has been reached. Based on the existing scientific literature, chemical induction is a primary means of generating a large animal diabetic model.

The third modality used to create an animal model of diabetes is via the **surgical** removal of the pancreas. Although pancreatectomies have been primarily conducted on large animal species, such as dogs and nonhuman primates (NHPs), generation of this model has also used mice and rats. Use of pancreatectomized animals can be compromised by complications of the surgery itself and by the complete removal of both endocrine and exocrine functions of the organ [23]. Pancreatic regeneration can also occur in the case of incomplete resection [24].

Small Animal Models of T1D

Small animal models of T1D can provide a wealth of information on disease initiation, progression, and potential therapeutic strategies. These models provide many advantages, including availability, cost, the potential for large sample sizes, and the existence of a wide array of diverse rodent strains that have been extensively studied. In addition, genetic manipulation of rodents, utilizing transgenic and knockout models, provides a relatively rapid tool for dissecting the molecular and cellular mechanisms of the disease itself, as well as a means to provide initial assessment of xenotransplant safety and potential benefit [25-26].

Porcine islet xenotransplantation studies in small animal models have helped to establish preliminary rationale and safety for novel immunosuppressive regimens, enabled rigorous characterization of molecular and cellular mechanisms of immune reactions and immunomodulation, and allowed for evaluation of the biocompatibility/biostability and potential effectiveness of encapsulation techniques [27-31]. However, translation of the data generated with these models to patients with T1D has limitations, such as: 1) significant immunological differences between rodents and humans, including MHC class II expression and the existence of preformed antibodies to pig antigens in humans; 2) metabolic differences (e.g., glucose set points, animal activity, and feeding cycles); and 3) dissimilarities in general physiology and other parameters due to large differences in physical size. These impediments provide a substantial challenge to the use of small animal models for the preclinical assessment of safety and pharmacodynamic function of islet products prior to testing in humans [25, 32].

Recent advances utilizing an array of transgenic and knockout mice may now provide more useful models to investigate pig-to-human xenotransplantation. For instance, studies of discordant xenotransplantation in pig-to-αGal-KO mice or the use of mice reconstituted with human immune cells (humanized mice) may provide powerful tools linking to a more clinically relevant scenario [33-34].

Large Animal Models of T1D

Large animal models, mainly dogs, pigs, and NHPs, provide genetic, metabolic, and physical profiles that more closely resemble the human situation [33]. Some of the major factors supporting the use of large animal models of T1D are immunological responses to the porcine islets and to any encapsulation components: metabolic set-points: product placement via the intended clinical procedure into the planned clinical anatomical site: and the ability to re-transplant the porcine islets (as will be the likely clinical requirement for an efficacious product). Based on the published literature, the diabetic state is primarily generated by chemical or surgical means. These animal models typically do not recapitulate the autoimmune condition that exists with the human disease, nor do they manifest the microvascular complications or hypoglycemic unawareness that often accompanies patients with established T1D. Nonetheless, these models can yield useful information to define the safety and possible benefits of xenogeneic islet transplantation, and thus potentially have the ability to provide some degree of assurance of clinical efficacy prior to the first-in-human trial.

NHPs, like humans, possess xenoreactive natural antibodies including the well-studied Galα1-3Galβ1-4GlcNac (αGal) specific antibodies, which can lead to HyperAcute Rejection (HAR) and Acute Humoral Xenograft Rejection (AHXR) in vascularized tissue/organ xenotransplants. Islets typically express little αGal, but the use of NHPs could provide added confidence when assessing the success or failure of porcine islet preparation methods, immunosuppressive regimens, or encapsulation techniques. In addition, genetically engineered pigs that lack αGal or express complement inhibitory factors may further eliminate concerns over HAR and AHXR [32].

Additional immunological concerns that have been well characterized in NHPs include Instant Blood-Mediated Inflammatory Reactions (IBMIR) and the adaptive immune responses. IBMIR, responsible for early loss (within days) of intraportally transplanted porcine islets, involves activation of platelets, the coagulation process, complement cascades and the infiltration of neutrophils. This reaction has also been studied in athymic mice after intraportal injection of adult porcine islets [35]. IBMIR concerns can potentially be minimized through the use of immune modulating transgenic or knockout source pigs, immunosuppression, encapsulation,

and/or the transplantation of porcine islets into a different anatomical location. However, delayed adaptive immune responses that appear to be mediated primarily by T cells, B cells, and macrophages through mechanisms that are still not fully understood, remain major obstacles to successful long-term implantation in NHPs, as well as in other large and small animal models of diabetes. Even so, recent published studies in NHPs have reported porcine islet survival and function for up to 6 months post-transplantation utilizing a multi-agent immunosuppressant regimen or encapsulated islets [36].

In addition to the above-stated issues, other considerations when investigating the use of large animal models of T1D must also include cost, animal husbandry issues, availability of highly trained personnel, animal availability, and group sample size [37-38]. An understanding of the limitations and capabilities inherent in each animal model of T1D can provide insight into selection of the most appropriate species/model. When considering the preclinical data that will provide support regarding the safety of the xenogeneic islet cells and any associated agents/delivery devices that are used, it may be of benefit to consider a tiered approach using multiple disease models in order to address specific questions/unknowns. The limitations and advantages of existing animal models and what can be learned from these models to guide first-in-human trial design are important considerations.

Concluding Comment

As mentioned above, the use of animal models is essential to support the development and regulatory evaluation of xenotransplantation products for the treatment of ALF and T1D. Further as summarized above, the details of these complex models may place significant limitations on the conclusions that can be drawn from any specific study. Therefore the FDA is seeking scientific advice in this area to guide informed decisions regarding potential models in future applications that may be submitted for review.

ADVISORY COMMITTEE DISCUSSION QUESTIONS

FDA's decision to allow the initiation of a first-in-human clinical trial using a xenotransplantation product is based on careful examination of all available preclinical data, as well as the clinical indication and review of the proposed clinical trial protocol. This overall appraisal is greatly dependent on selection of appropriate animal models that can be employed to evaluate safety and demonstrate substantial pharmacodynamic activity of the xenogeneic product.

Animal Models of Acute Liver Failure (ALF)

1. Please discuss the limitations and capabilities of available animal models of ALF to assess the safety and clinical activity of bioartificial liver assist devices containing porcine cells or tissues as a bridge to spontaneous recovery or liver transplantation. Please consider the following in the discussion:

    a. The ability of the animals to model the clinical manifestations and laboratory abnormalities of ALF in humans.

    b. Treatment duration and the ability to repeat the treatment, as would likely be required by the clinical condition of ALF patients.

    c. Study endpoints – the changes in laboratory values and clinical responses in test animals that would be considered clinically meaningful and predictive of potential clinical benefit in patients

Animal Models of Type 1 Diabetes (T1D)

2. Porcine islet products are currently under development to treat Type 1 diabetics who are chronically metabolically unstable. Please discuss the limitations and capabilities of available animal models of Type 1 diabetes that can be used to assess the safety and clinical activity of porcine islet cell transplantation. Please consider the following in the discussion:

    a. The ability of the animals to model the immunological and metabolic manifestations of Type 1 diabetic patients.

    b. Treatment duration and the ability to re-transplant, as would likely be required by the chronic clinical condition of Type 1 diabetic patients.

    c. Study endpoints – the changes in laboratory values and clinical responses in animals that would be considered clinically meaningful in diabetic patients.

    d. The intended clinical immunosuppression regimen, as applicable.

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