

FDA Briefing Document

Cellular, Tissue, and Gene Therapies Advisory Committee Meeting

June 29 and 30, 2022

Office of Tissues and Advanced Therapies

DISCLAIMER STATEMENT

The attached package contains background information prepared by the Food and Drug Administration (FDA) for the panel members of the Advisory Committee. The FDA background package often contains assessments and/or conclusions and recommendations written by individual FDA reviewers. Such conclusions and recommendations do not necessarily represent the final position of the individual reviewers, nor do they necessarily represent the final position of the Review Division or Office. We have brought xenotransplantation to this Advisory Committee in order to gain the Committee’s insights and opinions, and the background package may not include all issues relevant to the final regulatory recommendation and instead is intended to focus on issues identified by the Agency for discussion by the Advisory Committee. The FDA will not issue a final determination on the issues at hand until input from the Advisory Committee process has been considered and all reviews have been finalized. The final determination may be affected by issues not discussed at the Advisory Committee meeting.

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Table 1 Examples of Genetic Modifications of Pigs for Xenotransplantation

Glossary

AC	Advisory Committee
BD	Briefing Document
BLA	Biologics License Application
BRMAC	Biologic Response Modifiers Advisory Committee
CBER	Center for Biologics Evaluation and Research
CDC	Center for Disease Control
CDRH	Center for Devices and Radiological Health
CDER	Center for Drug Evaluation and Research
CFR	Code of Federal Regulations
CVM	Center for Veterinary Medicine
FDA	U.S. Food and Drug Administration
DHHS	Department of Health and Human Services
HIV	Human Immunodeficiency Virus
IGA	Intentional Genomic Alterations
IDE	Investigational Device Exemption
IND	Investigational New Drug Application
MuLV	Murine Leukemia Virus
NAD	New Animal Drug
NIH	National Institutes of Health
NHP	Non-human Primate
OTAT	Office of Tissues and Advanced Therapies
PERV	Porcine Endogenous Retrovirus
PHS	Public Health Service
SIV	Simian Immunodeficiency Virus

1.0 Executive Summary/Draft Points for Consideration by the Advisory Committee

1.1 Purpose/Objective of the AC Meeting

This meeting is convened for the Food and Drug Administration (FDA), xenotransplantation product developers, and other stakeholders to gain insights and perspectives regarding development of xenotransplantation products to ensure the safety and efficacy of these products. This meeting will focus on human cells that have had *ex vivo* contact with animal cells and on the transplantation of porcine organs into human subjects.

Topics for Committee discussion include:

- Infectious disease risks associated with xenotransplantation products and porcine donor animals, and how to assess these risks.
- Strategies for meeting regulatory requirements for measuring identity, purity and potency of xenotransplantation products.
- Current strategies to control xenotransplant rejection by gene modification of donor animals and by systemic immune suppression of the human recipients.
- Characterization studies to ensure the function of the pig organ before and after transplantation.

Specific investigational products will not be discussed.

1.2 Context for Issues to Be Discussed at the AC

The 2016 FDA *Guidance for Industry: Source Animal, Product, Preclinical and Clinical Issues Concerning the Use of Xenotransplantation Products in Humans* defines xenotransplantation as any procedure that involves the transplantation, implantation, or infusion into a human recipient of either (a) live cells, tissues, or organs from a nonhuman animal source; or (b) human body fluids, cells, tissues, or organs that have had *ex vivo* contact with live nonhuman animal cells, tissues, or organs [1]. Products in which human cells have had *ex vivo* contact with live animal cells are under clinical study, and some have met standards for FDA approval.^{1,2} First-in-Human research clinical studies for whole organ xenotransplantation have not been initiated due to the lack of effective strategies to prevent rejection by the recipient. However,

¹ Epicel HDE approval documents. <https://www.fda.gov/vaccines-blood-biologics/approved-blood-products/epicel-cultured-epidermal-autografts>

² Stratagraft BLA approval, <https://www.fda.gov/vaccines-blood-biologics/stratagraft>

recent advances in immunosuppression and the creation of genetically modified animals have renewed interest in animal organ transplantation, primarily pig organs, into humans.

1.3 Questions to be Discussed by the Advisory Committee

1. Archiving of source animal, product, and patient samples for up to 50 years is the current FDA expectation outlined in FDA-issued guidance titled, *“Source Animal, Product, Preclinical, and Clinical Issues Concerning the Use of Xenotransplantation Products in Humans”* (December 2016). Archived samples can aid in investigation of adverse events, and the archiving recommendations apply to xenotransplantation products, including those that have had *ex vivo* contact with animal cells, but are not themselves of animal origin. Please discuss whether the expectations for archiving of patient samples should be modified in terms of length of storage and/or sample sizes.
 - a. Please discuss technologies that could be used to analyze cell banks and final products and that might be sufficiently sensitive to allow for modification of archiving requirements.
 - b. Please discuss conditions that would alter the expectations for patient follow-up.
 - c. Please discuss conditions, if any, under which patient follow-up for disease transmission should not be required.
 - d. Please discuss conditions under which recipients of xenotransplantation products should be allowed to donate blood or tissues/organs.

2. Pigs can harbor endogenous viruses that may impact the health of transplanted tissues or organs or impart infectious disease risk to the recipient and their close contacts. Porcine circovirus 3 (PCV 3), porcine endogenous retrovirus (PERV) and Porcine Cytomegaliovirus (PCMV) have been identified as viruses that may impact organ function after transplantation or be transmitted to recipients of xenotransplantation products, their contacts, and the public. Please discuss the following:
 - a. Describe sensitive detection systems available for the detection of infectious agents in pigs used for xenotransplantation and which methods should be used orthogonally.
 - b. PCV 3 transmission from donor pigs to baboons has been reported in preclinical studies. Please discuss the potential for PCV 3 zoonotic infections in humans.
 - c. PCV 3-infected pigs have been reported to exhibit cardiac and multisystemic inflammation. Please discuss the impact of PCV 3 on transplanted organs.
 - d. Three subtypes of PERV (A, B, C) and PERV A/C recombinants have been found in various breeds of pigs. Please discuss which subtypes present the greatest risk and how PERV risk can be mitigated or eliminated.

- e. Please discuss any other known or emerging viruses that should be considered in the context of human xenotransplantation.
3. Pig cells or organs transplanted into humans are FDA-regulated articles and are subject to regulatory requirements such as identity, purity, and potency. Please discuss assays or testing strategies that might be appropriate to perform prior to transplantation to evaluate the safety and efficacy of these articles.
4. Transplantation of animal cells and organs into humans is associated with hyperacute rejection, vascular injury, cell-mediated rejection, and chronic rejection. Options for controlling rejection include genetic modification of donor pigs and modulation of the immune response in the recipient. Please discuss the most promising strategies to prevent rejection of pig organs. In your discussion, please consider the balance between the potential benefits of the desired genetic modifications and/or immune response modulation and the potential for detrimental transplant outcomes.
5. Transplantation of pig cells and organs is intended to provide replacement for non-functioning/damaged human cells and organs. Therefore, it is important to understand the characteristics of these cells or organs in the pig to ensure they have the characteristics needed to provide replacement therapy for the human recipient before transplantation. And, it is important to monitor these cells and organs to demonstrate they provide the expected functions after transplantation. Please discuss existing data to address the following issues related to pig cells and organs intended for transplantation into humans:
 - a. The ability of the target pig organ to support full organ function in humans.
 - b. The natural aging of the target organ in the pig relevant to expected organ function over time in humans.
6. Transplanted pig organs are likely to be exposed to a variety of drugs that were not routinely used in the donor animals. Such drugs could include products to treat the patient's underlying medical condition(s) (e.g., diabetes, hypertension), as well as drugs (e.g., immunosuppressants) intended to ensure the success of the transplant. The transplanted organ may alter the pharmacodynamic and pharmacokinetic profiles of these drugs, with consequences for the medical management of the organ recipient. In addition, these drugs could be toxic to the transplanted organ. Please discuss the importance, limitations, and feasibility of studies of such drugs in the pig model prior to transplanting the pig organ into humans.

2.0 Introduction and Background

Ex vivo co-culture of human cells with animal cells has long been a method to improve the expansion, survival, and function of certain types of human cultured cells. Transplantation of animal organs has been identified as a possible remedy for the limited supply of organs for human transplantation. Both these scenarios are defined as xenotransplantation and carry risks associated with transmission of animal viruses to the recipients of the cells or organs, and to their close contacts. For the past 20 years, there have been attempts to transplant animal organs into humans. Early discussions led by the Public Health Service (PHS) and FDA promptly excluded the use of non-human primates as source animals, due to the similarity between Simian Immunodeficiency Virus (SIV) and Human Immunodeficiency Virus (HIV), and the potential for seroconversion in transplant recipients. However, until the advent of targeted, highly efficient gene editing, the issue of rejection due to pig antigens could not be surmounted.

2.1 Application of CBER Guidance[1] and PHS Guideline[2]

In formulation of its xenotransplantation guidance, FDA anticipated that its approach to regulation of xenotransplantation products would evolve with the increase in scientific knowledge in the area of xenotransplantation. FDA realizes that it may not be appropriate to apply every aspect of the xenotransplantation guidance to every xenotransplantation product. For example, some of the guidance recommendations for animal husbandry may not be needed for xenotransplantation products consisting of well-characterized, long-established animal cell culture lines or human cells co-cultured with such lines. For the advisory committee discussion, Epicel serves as an example of flexible application of xenotransplantation recommendations for a product that has contact with mouse fibroblasts from well-characterized donor mice.

3.0 Regulatory Framework for Xenotransplantation Products

Xenotransplantation products are regulated primarily as biologics under authority of section 351 of the PHS Act (42 U.S.C. 262) and the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 321 et seq.). The FDA Center for Biologics (CBER) has jurisdiction over biologic xenotransplantation products.

3.1 Source Animals with Intentional Genomic Alterations

When animals with intentional genomic alterations (IGAs) are used as a source of xenotransplantation products, the FDA Center for Veterinary Medicine (CVM) provides regulatory oversight over IGAs in animals under the New Animal Drug (NAD) provisions of the Federal Food Drug and Cosmetic Act (FD&C Act) (21 USC 321 et seq.) and its enabling regulations (21 CFR Part 511 and 514), as described in *Guidance for Industry: Regulation of Genetically Engineered Animals Containing Heritable Recombinant DNA Constructs* [3].

3.2 Clinical Studies

Xenotransplantation products regulated as biological products used in a clinical investigation require an Investigational New Drug (IND) application, and marketing requires a Biologics License Applications (BLA). The primary responsibility for designing and monitoring the conduct of clinical trials rests with the IND sponsor.

Expanded access INDs, often referred to as compassionate use, are intended to treat patients with serious or immediately life-threatening disease or conditions, and do not include clinical trials. The regulations for single-patient expanded access INDs are found in 21 CFR Chapter I, subchapter D Part 312.

3.4 Combination products

Some xenotransplantation products may be a combination of two regulated products, such as a biological product and a device. An example would be xenogeneic cells contained within a device used for extracorporeal hemoperfusion. Combination products³ are reviewed by the FDA Centers with designated expertise in the regulated articles that make up the combination product. FDA's Office of Combination Products provides recommendations or determinations for medical product classification and Center assignment.

4.0 *Ex Vivo* Exposure to Animal Cells

The definition of xenotransplantation includes human products that have had *ex vivo* contact with animal cells [1, 2].

Xenotransplantation poses unique zoonotic risks to individual recipients and potential risks to individuals coming into contact with the recipient, including, but not limited to, the healthcare team, close personal contacts, and the public. The potential public health risks of xenotransplantation products include:

1. Transmission of infectious agents that are pathogenic for humans but may not be pathogenic or even detectable in the source animal host.
2. Transmission of organisms that may not normally be pathogenic in humans but can become pathogenic in the immunosuppressed or immunocompromised individual; and,
3. Recombination or reassortment of infectious agents, particularly viruses, with nonpathogenic or endogenous human infectious agents, to form new pathogenic entities.

4.1 Archiving Samples

As described in the PHS Guideline and CBER Xeno guidance [2], [1], risks associated with zoonosis may be mitigated by implementation of multiple layers of safety, including source animal controls, appropriate testing of products, and clinical monitoring and follow-up. Source animal controls include animal husbandry practices, herd health screening and surveillance, and

³ 21 CFR 3.2(e)

donor animal segregation, testing and quarantine, and archiving of donor animal records and tissue samples for possible follow-up testing. The PHS Guideline and CBER Xeno Guidance stipulate archival of such records and samples for 50 years. Clinical monitoring and follow-up include archiving of clinical samples and passive screening programs.

4.1.1 Archives of Source Animal Medical Records and Specimens

Sections VII A.4 of the CBER Xeno Guidance and 3.7 of the PHS Guideline contain recommendations for archives of source animal medical records and specimens. Systematically archived source animal biologic samples and record keeping that allow rapid and accurate linking of xenotransplantation product recipients to the individual source animal records and archived biologic specimens are essential for public health investigation and containment of emergent xenogeneic infections.

Recommendations detailed in the PHS Guideline and CBER Xeno Guidance call for cryopreservation and archiving of samples of all final xenotransplantation products, including cells, tissues, or organs. For human cells, archives should include tissues or organs that have been in contact *ex vivo* with live nonhuman animal cells, tissues, or organs and samples of the final product and the nonhuman animal cells, tissues, or organs. As in the case of the animal source samples, the archive should include sufficient quantities and numbers of replicates of the:

- a. dedicated sample(s) for use by PHS as described in the PHS Guideline
- b. for use if needed for recipient diagnosis and care; and
- c. for use by the sponsor, as appropriate.

These samples should be stored for 50 years from the time of manufacture of the xenotransplantation product.

4.1.2 Archives of Patient Specimens

Sections X.H.1-2 of the CBER Guidance and 4.1.2 of the PHS guidance describe archiving of patient-derived biological specimens. The PHS guidance recommends that biological specimens obtained from the xenotransplantation product recipients and designated for public health investigations (as distinct from specimens collected for clinical evaluation or laboratory surveillance) should be archived for 50 years after the date of the xenotransplantation to allow retrospective investigation of xenogeneic infections. The type and quantity of specimens archived may vary with the clinical procedure and the age of the xenotransplantation product recipient.

Section X.F.3. of the CBER Guidance has additional recommendations for testing and scheduling of testing of recipients for infectious agents for patients who appear to be ill in X.F.3.a-b, and for routine screening for clinically inapparent infections and seroconversions using a passive screening approach in X.F.3.c. This passive screening program is in addition to the collection and archiving of biologic specimens designated for PHS use as described in the PHS Guideline.

4.2 Application of the PHS Guideline and CBER Xeno Guidance to Xenotransplantation in the setting of product exposure to well-characterized cell lines

In the setting of exposure to well -characterized mouse cell lines, FDA has modified application of some of the PHS Guideline and CBER Xeno Guidance regarding animal husbandry and the archiving of animal and recipient samples. Particularly, in January 2000, an FDA subcommittee of the Biologics Response Modifiers Advisory Committee (BRMAC) discussed three issues related to xenotransplantation products: 1) blood donor deferral; 2) examination of risks posed by different xenotransplantation products, and 3) porcine endogenous retrovirus. The subcommittee specifically considered Epicel[®], a sheet of epidermal keratinocytes manufactured from a small piece of autograft cultured in the presence of replication-inactivated murine feeder cells, which remain present in the final product at the time of product use.

Recommendations of the subcommittee and FDA for Genzyme (manufacturer) and patients treated with Epicel[®] were:

- Genzyme will obtain samples of the 3T3 mouse cells and the final patient product (Epicel[®]) will be archived.
- Genzyme will archive baseline (i.e., pre-Epicel[®] treatment) samples of the patient's blood.
- Epicel[®] recipients, but not their intimate contacts, should not donate whole blood, blood components, source plasma, source leukocytes, tissues, breast milk, ova, sperm, or other body parts for use in humans.
- The patient label and physician label will communicate to the patient, or through the treating physician, the xenogenic nature of Epicel[®].
- Epicel[®] will contain a peel-off label for the patient's medical chart history indicating that the patient was treated with a xenotransplantation product. The peel-off label states: This patient has been treated with Epicel[®] (cultured epidermal autografts), a product manufactured with murine cells.
- The patient label and physician label will communicate to the patient and through the treating physician that the patient should consider allowing an autopsy examination of their body upon death.
- Genzyme will construct a database to collect Epicel[®] patient information; this information will be provided to the National Xenotransplantation Database (NXD) when the NXD is completed.
- Genzyme will provide reports within 5 days to FDA regarding any clinical events that are suspicious of a xenogeneic cause.
- Epicel[®] recipients will be passively monitored, with active investigation of any suspicious clinical events.

All of the recommendations provided by the BRMAC subcommittee were adopted by FDA and all were implemented by Genzyme⁴. Although the National Xenotransplantation Database (NXD) has not been completed, data submitted in annual reports did not raise concerns regarding zoonic infectious agents in Epicel[®] or other human products that have had *ex vivo* contact with animal cells.

Both the PHS Guideline and the CBER Xeno Guidance describe some flexibility regarding archiving nucleic acid samples instead of cryopreserved leukocytes. Since the original PHS Guideline and CBER Xeno Guidance were published, significant advances in nucleic acid sequencing technology now allow greater sensitivity, the potential ability to quantify nucleic acid content of known infectious agents, the ability to include substantial sampling of potentially infectious material/product, and the potential to identify newly emerging infectious agents. For products that are included in the xenotransplantation category due to *ex vivo* exposure to living animal cells, FDA is asking this Advisory Committee to discuss the need for archiving of xenotransplantation product and patient samples.

4.4 Advances in Nucleic Acid Detection Systems

In recent years significant advances in nucleic acid detection technology have been developed. Such techniques allow greater sensitivity, potential ability to quantify nucleic acid content of known infectious agents, ability to include substantial sampling of potentially infectious material/product and clinical archival samples, and potential to identify newly emerging or previously unidentified infectious agents.

4.4.1 - Polymerase Chain Reaction (PCR) and Next Generation Sequencing (NGS) detection of virus

Evaluation of the safety of biological products often uses a combination of *in vitro* adventitious virus assays, *in vivo* tests, and specific polymerase chain reaction (PCR) analyses. PCR analyses are highly specific and can complement the *in vitro* and *in vivo* methods, but they are limited by the large number of potential virus contaminants that must be assayed [4]. PCR analysis is capable of single copy detection of viral RNAs [5] but is limited to detection of targets with known sequences. In a review by Onions in 2011 [4], the use of nucleic acid sequencing technology was becoming increasingly important: “As these deficiencies were becoming apparent, new massively parallel sequencing (MP-Seq) technologies emerged resulting in a renaissance in virus discovery, revealing new viruses of potential concern to the biotechnology industry [4, 5]. The same technology is also proving to be a key method in evaluating the safety of cell banks and in identifying viruses in raw materials and fermenter bulk harvests.” [6].

Next Generation Sequencing (NGS) sensitivity of detection depends on many factors, including sample quality, sample amount, and read depth, among others. NGS is sufficiently sensitive to detect rare mutational alleles in complex samples. Miura *et. al.* [6] demonstrated a relatively

⁴ FDA Executive Summary of BRMAC Subcommittee: <https://www.fda.gov/media/111543/download>

simple method to estimate the in-house limit of detection (LOD) for genetic mutations with low allele frequencies detected by whole-exome sequencing (WES). Allele frequencies of 20 mutations in the reference material that had been pre-validated by droplet digital PCR (ddPCR) were obtained from 5, 15, 30, or 40 G base pair (Gbp) sequencing data per run. The LOD of allele frequency in WES with the sequencing data of 15 Gbp or more was estimated to be between 5 and 10% using this approach.

NGS is capable of detecting at least 10 to 100 copies of certain viruses in clinical samples although RT-PCR may be more sensitive for others and reliably detect viruses that are present in low copy number [7].

FDA is asking the Advisory Committee to discuss strategies for sample collection and archiving of samples from patients who have received product that has had *ex vivo* exposure to well-characterized animal cells and patients who have received a product containing live animal cells. FDA is seeking the Committee's input on how increasing sensitivity of virus detection could address some of the public health concerns regarding xenotransplantation.

5.0 Infectious Disease and Xenotransplantation

One of the primary risks associated with the use of xenotransplantation products is the transmission of known and unknown pathogens from the animals to humans, and the risks of zoonotic infection in patients, their personal contacts, health care professionals and the public. Zoonotic agents include viruses, bacteria, fungi, and parasitic organisms such as worms. The transmission into humans of pathogens previously restricted to animals has resulted in widespread human disease, but xenotransplantation presents a more serious risk, as these products are put in direct contact with human blood, or directly transplanted into the human body, often with accompanying immune suppression to prevent rejection.

5.1 Porcine Endogenous Retroviruses (PERV)

Retroviruses, which are enveloped RNA viruses, integrate into the genome of infected individuals, where they remain. This retroviral integration is a known risk for xenotransplantation products. Endogenous type C retroviruses are present in all reptiles, birds and mammals, including humans, but are not directly infectious for the species of origin and have a xenotropic host range (viruses replicating or reproducing only in cells or tissues of a species other than its normal host) [8]. Porcine Endogenous Retrovirus (PERV), a type C gamma retrovirus, is an agent of concern in xenotransplantation products derived from pigs. PERVs constitute an integral part of the porcine genome and are present in various proportions based on pig breed, tissue type and retrovirus subtype [9]. Purified PERV particles are antigenically related to mammalian leukemia viruses. PERV is closely related to murine leukemia virus (MuLV), feline leukemia virus (FeLV), and Koala retrovirus (KoRV), which are associated with leukemia and immunodeficiency in infected hosts [10-12].

Three replication-competent subtypes of PERV have been identified: PERV-A, PERV B, and PERV C. PERV A and B are polytropic, capable of infecting both porcine and human cells and therefore present a risk to xenotransplant recipients [13]. PERV C is an ecotropic virus that only infects porcine cells [14]. However, PERV A/C recombinants identified by Wilson *et al.* [15], were found to infect human cells. The PERV A/C recombination yields a retrovirus that contains PERV A sequence between the end of the *pol* and middle of SU region in the *env*, with the remaining sequence coming from PERV C. PERV A/C is 500-fold more infective than PERV A. Specific genetic determinants identified by Harrison *et al.* [16], suggest that high-titer recombinants may be produced *de novo* in xenograft recipients

Preclinical studies conducted to date have not found PERV transmission to recipients of xenotransplantation products [17, 18]. Animals used in these studies tested positive for PERV A, PERV B, and PERV C but not PERV A/C. Additional studies on PERV A/C recombinants may help to further understand the risks of PERV transmission to recipients of xenotransplantation products.

Various strategies to prevent the transmission of PERV to recipients of xenotransplantation products have been developed. One such strategy is focused on excluding the use of PERV C-positive pigs and using pigs that express low levels of PERV A and PERV B [20]. Another strategy to prevent the transmission of PERV is gene editing where all proviral copies in the genome are inactivated using CRISPR/Cas9 [21, 22] or zinc finger nucleases [23]. In addition, PERV-specific small interfering (si)RNA to reduce the expression of PERV may reduce the risk of PERV transmission [23].

If PERV transmission from donor pigs to human recipients of xenotransplantation products occurs, there is evidence that anti-retroviral drugs such as AZT (azidothymidine) can inhibit PERV replication *in vitro* [24]. However, to date there have been no reports of *in vivo* pig studies to better understand the potential for treatments should PERV infections arise in humans.

5.2 Porcine Circovirus (PCV)

Porcine circoviruses (PCV), found globally in pig populations, may be another potential source of zoonosis in the pig-to-human xenotransplantation setting. PCVs are non-enveloped spherical particles with a single-stranded circular small DNA genome. Three species have been identified thus far: PCV 1, PCV 2, and PCV 3. PCV 1, found as a contaminant in pig kidney cell lines [25] is apathogenic in pigs [26]. PCV 2 has been identified as the causative agent in post-weaning multi-systemic wasting syndrome (PMWS) [27]. PCV 3 was first identified in 2016 in U.S. herds suffering from porcine dermatitis and nephropathy syndrome (PDNS), reproductive failure, cardiac and multisystemic inflammation [28, 29]. PCV 3 was also found in healthy animals, suggesting that co-infection with other viruses, such as PCV 2 and porcine reproductive and respiratory syndrome virus (PRRSV), may be needed with PCV 3 to generate a pathogenic effect [30].

Evidence of PCV 3 in the xenotransplantation setting was reported in a genetically modified pig population that was knocked out for α 1,3-galactosyltransferase (GT-KO), and expressed human membrane cofactor protein (CD46) and human thrombomodulin (hTM) [31]. Baboons received an orthotopic heart transplant from these pigs. In four cases where PCV 3-positive pig hearts were transplanted, all four of the baboons tested positive for PCV 3 in all organs. Higher viral loads were found in animals with longer survival times, indicating the replication of the virus [31]. In experiments where PCV 3-positive pig PBMCs stimulated with a T cell mitogen were co-cultured with human 293 cells for various time points, no transmission was observed. Further studies may be needed to investigate the potential for PCV 3 to infect human cells.

5.3 Porcine Cytomegalovirus (PCMV)

PCMV is also of concern in the xenotransplantation setting. PCMV is related to the human cytomegalovirus (HCMV), also called human herpesvirus 5 (HHV-5). HCMV causes fatal infections in human organ transplant recipients. PCMV transmission in orthotopic pig heart xenotransplantation was associated with a reduced survival time of recipient baboons [32]. Baboon transplant recipients had increased serum levels of IL-6 and TNF α , and high levels of tPA-PAI complexes in the serum. This finding suggests a loss of the pro-fibrinolytic properties of endothelial cells. Of greater concern is reactivation of Herpesvirus in immunosuppressed human recipients. Herpesvirus remains latent, typically in nerve endings, until the immune system is stressed.

Yamada *et al.*, (2014) conducted a retrospective study investigating the possible relationship between the presence of PCMV and early pig kidney graft loss. Average survival was substantially better in recipients of PCMV-negative kidneys compared to recipients of PCMV-positive kidneys [33].

In another investigation, PCMV distribution was studied in three baboons that received orthotopic pig heart transplants. A Western blot assay based on four PCMV protein sequences and real-time PCR were used to detect PCMV transmission. Two of the recipient baboons tested positive for PCMV despite the absence of PCMV in donor pig blood tested by RT-PCR. However, PCMV was detected in different organs of the donor pigs, and in sibling animals. Immunohistochemistry using an antiserum specific for PCMV detected virus protein expression in all organs of the recipient baboon, most likely representing virally-infected pig cells that became dispersed throughout the recipient baboons [34]. These studies suggest that donor pigs testing positive for PCMV should be excluded as donors of human xenotransplantation products. These studies also underscore the importance of infectious disease surveillance within the herds and implementation of criteria to govern exclusion of diseased animals from source herds.

5.4 Porcine Lymphotropic Herpes Virus (PLHV)

Porcine lymphotropic herpesviruses (PLHV 1, PLHV 2, PLHV 3) are gamma herpesviruses that are widespread in pigs. PLHV is closely related to the Epstein-Barr virus (EBV) and Kaposi

sarcoma virus, which cause serious disease in humans. PLHVs related to bovine and ovine herpesviruses are apathogenic in their natural host but cause disease in other species. Although no relationship between PLHV and diseases in swine has been observed, PLHV 1 causes post-transplantation lymphoproliferative disease (PTLD) after experimental transplantations in mini-pigs [35]. In humans, PTLD is a serious complication of human solid organ transplant and is often linked to EBV. Although PLHV 1 was present in genetically modified donor pigs, PLHV 1 was not transmitted to baboon recipients in preclinical studies.

6.0 Donor Animals

Risks associated with emerging pathogens and zoonosis may be controlled through multiple layers of safety. Screening programs for common zoonotic microorganisms, including bacteria, viruses, and fungi using sensitive methods of detection is the first line of preventing the spread of infectious disease within the animal herd and to human recipients and their close contacts. To prevent zoonotic infections, animals testing positive for certain viruses (e.g., PERV, PCV 3, and PLHV) can be excluded as donors of xenotransplantation products.

To reduce the risks of infectious disease in pig herds, the following can be employed:

- Adequate herd containment, protection, and stability
- Judicious use of antimicrobials, vaccines, diagnostics
- Diet and drinking water considerations
- Procedures to identify and handle illness within the herd
- Monitoring health of caretaking staff and others
- Isolation of pigs to be used as donor animals, to prevent transmission from other animals
- Validated sanitization processes

As stated in the CBER Xeno Guidance, FDA recommends that source animals used for xenotransplantation are bred from closed herds of documented origin and known infectious disease status. Prior to clinical use, FDA recommends that two or more generations of animals be maintained in Specific Pathogen-Free (SPF) conditions. This recommendation also applies to gamete donor animals[1].

To maintain appropriate health conditions for donor herds, screening and sentinel animal testing are expected, in addition to appropriate feed that is free of rendered animal materials, and appropriate health care. Animals used for xenotransplantation should be maintained in an appropriate, well-controlled animal facility that is pathogen-free, with appropriate trained staff and limited access. Procedures to minimize infectious disease risks should be in place. For example, donor animals are to be quarantined from the herd at least 3 weeks prior to transplant. Bio-secure transportation should be in place between the animal facility and the clinic to prevent the animal from acquiring infectious agents from the environment. Organs should be harvested in a well-controlled surgical environment [1].

7.0 Identity, Purity and Potency Requirements

Xenotransplantation products include human cells or tissues which have had *ex vivo* contact with animal cells, cellular or tissue products derived from animals, and whole organs derived from animals. Xenotransplantation products regulated as biologics must meet regulatory requirements for potency (21 CFR 610.10), sterility (21 CFR 610.12), purity (21 CFR 610.13), and identity (21CFR 610.14).

7.1 Cellular Xenotransplantation Products

For cellular xenotransplantation products, approaches like those used to characterize cellular therapy products derived from human cells can be used to assess the requirements for potency, sterility, purity and identity. Products must have acceptable limits and analytical methods to identify the product and to uniquely distinguish the product from other products made in the same facility. For example, a cellular therapy product may be evaluated by flow cytometry (immunophenotyping) for the presence of cell-specific markers. Furthermore, criteria to establish the quality attributes of each product lot of the final formulated cellular product should be developed. These lot release criteria should include specifications for the concentration and/or percentage of cell populations that comprise the final product (i.e., purity), including those not anticipated to have a therapeutic effect. Characterization studies to identify any additional cell subsets can be employed. Evaluation of purity also includes tests for endotoxins or pyrogens. A potency assay is used to assess the biological function of each lot of the product. Potency assays should be determined based on individual product attributes, including functional aspects of the product; thus, the adequacy of potency tests is evaluated on a case-by-case basis. For example, if the cellular therapy product is purported to exert its effects through production of cytokines, then a potency assay might examine cytokine production by cells.

7.2 Whole Organ Xenotransplantation Products

For whole organs, determination of identity, purity, potency, and sterility may be more challenging due to the complex architecture of the organ, the presence of multiple cell types within the organ, and the fact that organs rarely serve only one specific function in their host. Testing of the donor animal for pathogens and expected genetic makeup prior to removal of the organ will be required as part of safety and identity. Visual inspection of the organ, and, as outlined in the PHS Guideline and CBER Xeno Guidance, histology, performed on a retention sample or biopsy of the xenotransplantation product, can also be used to document quality attributes of the product. Evaluation of purity and potency for whole organs should be justified on a case-by-case basis, but should include evaluation of endotoxin, which may be introduced through the organ preservation fluids and containers. If extraneous materials such as transport fluid are used prior to transplant, their sterility and adequate removal will need to be documented. To assess potency, an *in vivo* evaluation of organ function prior to harvest from the donor animal could be used together with an assay evaluating organ function immediately prior to transplantation into the human recipient. Noninvasive, nondestructive methods that

can be demonstrated to be predictive of organ function in the recipient will need to be developed for whole organ xenotransplantation products, in an organ-specific manner.

8.0 Strategies to Control Rejection of Pig Organs

Xenotransplant rejection is one of the major hurdles to successful pig-to-human xenotransplantation. Early attempts to transplant pig organs into humans revealed immediate incompatibilities between pig organs and the human immune system due to the presence of preformed anti-pig antibodies in recipients' blood [36]. Within minutes to hours, hyperacute rejection occurs through ischemia/reperfusion injury, antibody-mediated rejection and modulation of T and B cell responses [37]. If hyperacute rejection can be prevented, rejection is caused by vascular injury due to coagulation incompatibility between the pig and human, platelet activation, and clot formation. This takes place within days post-transplant. Cell-mediated rejection takes place within weeks after transplantation and is hallmarked by NK cell, T cell, and macrophage activity. As with allotransplantation, chronic rejection taking place months to years post-transplantation is the result of immune responses to novel antigens in the graft. The two basic approaches to circumvent these immune functions: genetic modification of source pigs, and systemic targeted immune suppression in organ recipients, are outlined below.

8.1 Intentional Genomic Alterations to Source Pigs

Strategies to alter the pig genome to overcome immune incompatibilities have been a longstanding goal in xenotransplantation. The primary immune incompatibility stems from reaction to α Gal, a carbohydrate moiety found on proteins and lipids in most mammals, with the exception Old World nonhuman primates and humans. Humans produce high titers of anti- α -Gal antibodies induced by gut microflora, making α Gal the major contributor to hyperacute rejection of pig organs. The pig genome contains an enzyme, α 1,3 galactosyltransferase (GGTA1) that catalyzes the synthesis of α Gal. Inactivation of the *GGTA1* gene removes the problem of hyperacute rejection.

However, when hyperacute rejection is eliminated, delayed xenograft rejection by other more minor pig antigens occurs [38]. These additional pre-formed antibodies to non- α Gal carbohydrate antigens were removed by genetic modification. This revealed additional immune mechanisms (e.g., thrombosis and inflammation) that contributed to xenotransplant rejection. This allowed for the development of further approaches to counteract these issues during a time of rapid advancement in genome modification technology. The recent development of targeted high efficiency genome editing, and improved methods to increase the size and number of transgenes, as well as improvements in somatic cell nuclear technology, have allowed the production of genetically modified pigs with improved efficiency. Strategies include inactivating endogenous genes in source animals, or inserting exogenous DNA sequences, i.e., human genes, with the aim of making materials from source animals more compatible

immunologically with human recipients. The application of clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein (Cas9) endonuclease [39] to the production of transgenic animals has enabled developers to rapidly create novel genome modifications in source animals. Gene editing has many advantages, but it also has inherent disadvantages. Although gene editing enables the targeting of specific DNA sequences and creation of multiple intentional genomic alterations (either sequentially or simultaneously) into cell lines and/or animals, the associated nucleases introduce double-stranded DNA breaks which often are repaired by error-prone DNA repair mechanisms, such as non-homologous end-joining [40]. In addition, while the modifications can be made to target specific DNA sequences, modifications at other targets (off-target effects) occur and can cause unintended genetic modifications in animals. Therefore, developers are continuously refining their approaches to enhance the consistency/predictability of introducing multiple intentional genomic alterations.

Various alterations to the pig genome to prevent rejection, avert PERV transmission, and regulate organ growth post-transplant are being tested and are presented in Table 1. Developers have produced genetically modified pigs lacking the enzymes that catalyze the addition of carbohydrate antigens responsible for hyperacute and delayed rejection. In addition to the deletion of the gene synthesizing α Gal, described above, the genes encoding the enzymes cytidine monophosphate-N-acetylneuraminic acid hydroxylase (CMAH), which catalyzes the addition of N-glycolylneuraminic acid (Neu5Gc), and β 1,4 N-acetyl-galactosaminyl transferase (β 4GalNT2) [41], which catalyzes the terminal addition of N-acetylgalactosamine to a sialic acid--modified lactosamine to produce GalNAc β 4 [Neu5Ac α 2,3] Gal β 4GlcNAc β 3Gal, the Sd^a blood group antigen [42], have both been deleted.

A complementary strategy has been to create pigs expressing human proteins to “cloak” the organ to prevent activation of various immune pathways. Transgenes expressing human complement regulatory proteins (CD55, also known as complement decay-accelerating factor (DAF); CD46 also known as membrane cofactor protein (MCP); CD59, also known as membrane inhibitor of reactive lysis (MIRL) have been introduced into pig genomes. Expression of any one or more of these is associated with a reduction in cytotoxicity of pig cells exposed to human serum. Pig xenografts often fail due to thrombotic microangiopathy. Creation of pigs that, in addition to the above-described genetic modifications, also expressed anticoagulation/antithrombotic proteins such as human thrombomodulin (TBM) or human endothelial protein C receptor (EPCR) reduced platelet aggregation *in vitro* and contributed to improved survival in a pig-to-baboon model for kidney xenotransplantation. Addition of transgenes bearing antiapoptotic and anti-inflammatory proteins, such as human tumor necrosis factor -alpha-induced protein 3 (A20) and human heme oxygenase-1 (HO-1) are also under study. Human CD47 expression in pig cells has been shown to prevent macrophage -mediated destruction of pig-derived cells and organs [44, 45]. Currently, the number and types of genomic alterations that would create the best environment to prevent rejection is unknown. It is also unclear if all modifications are equally suitable or necessary for all organs or cells being considered for xenotransplantation. Other genetic modifications to the

pig have been made to reduce the risk of PERV transmission [21] by deleting all PERV sequences within the pig genome. An additional concern is overgrowth of pig hearts once transplanted. This has been observed in pig hearts transplanted into baboons. Growth hormone receptor knockout pigs have been produced to reduce the growth of pig hearts post-transplant [45]. This genetic modification may alleviate concerns about potential rapid growth of pig organs transplanted into humans.

Current strategies involve the production of pigs with a large number of genetic modifications. Developers are actively characterizing these genetic modifications with respect to whether the modifications actually ameliorate the risks of organ rejection. These developers will need to establish the long-term genetic stability of these modifications, and describe the effects on the health of the genetically modified animals and their organs. To date, submission of these types of characterization data to the Agency have been minimal and represent an information gap that needs to be addressed to (1) determine the utility of the multiple genetic modifications, (2) allow the Agency to provide constructive input to developers in designing studies to support the licensing of these products, and (3) allow further advancement of the field of xenotransplantation.

8.2 Immune Modulation in the Recipient

An alternative strategy to prevent rejection, to be used in concert with intentional genomic alterations, is to systemically administer targeted immunomodulatory drugs to xenotransplant recipients. Organ transplantation requires maintenance of strong immunosuppression with calcineurin-inhibiting drugs like tacrolimus or cyclosporine to suppress the T and B cell compartments of the immune system. Other T and B cell immunosuppressants like anti-thymocyte globulin (ATG) and rituximab are also routinely used. However, methods to provide early, targeted immunosuppression are under study in allotransplantation, and also in pig-to-primate models of xenotransplantation. Blocking costimulatory pathways such as CD28/B7, cytotoxic T-lymphocyte-associated protein 4 (CTLA4), and CD40/CD154, with monoclonal antibodies in pig-to-primate models has shown some promise [39, 46, 47]. Targeted suppression of the complement pathway is another focus of the systemic immune suppression approach. Monoclonal antibodies that neutralize the C5 complement component have been used in humans [49] and in pig-to-primate renal transplantation [49]. Induction of tolerance, by either mixed hematopoietic chimerism or porcine thymic transplantation, is an additional approach under study [38]. Successful xenotransplantation will most likely require a combination of a specific genomic alteration approach with appropriate targeted suppression of immune compartments in the recipient. This may be further complicated by organ-specific requirements with tailored approaches for each type of transplant.

9.0 Additional Considerations

Per Title 21 of the Code of Federal Regulations (CFR), 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.” Well-designed preclinical studies are critical to provide data to support clinical development of a product. The appropriate animal model(s) to evaluate a xenotransplantation product for a specific indication can provide insight regarding activity and toxicity relationships. These data will allow for the most reliable assessment of the therapeutic potential of the product, including the potential duration of clinical effect.

Preclinical studies are particularly valuable to gain insight into safety issues that cannot be evaluated in human recipients for ethical or practical reasons. Definitive preclinical animal studies should be prospectively designed to evaluate long-term graft survival and safety. These definitive studies should use the intended clinical product with the same immunosuppression regimen and surgical procedure as in the clinic, if feasible. Studies should include a robust number of animals and demonstrate consistent, stable, long-term, functional graft survival post-transplant.

Preclinical study design is a critical issue for successful development of xenotransplantation products. However, the preclinical development program for each xenotransplantation product is individualized, and discussion of preclinical studies is generally outside the scope of this Advisory Committee meeting.

10.0 Table 1:

Examples of Genetic Modifications of Pigs for Xenotransplantation

Genetic Modification	Function
GGTA1 knock-out	Deletion of Gal xenoantigen [50, 51]
CMAH knock-out	Deletion of Neu5Gc xenoantigen [52]
B4GALNT2 knock-out	Deletion of SDa xenoantigen [41]
Expression of human CD55 gene	Complement regulation [53]
Expression of human CD46 gene	Complement regulation [54]
Expression of human CD59 gene	Complement regulation [55]
Expression of human TBM gene	Coagulation regulation [56]
Expression of human EPCR gene	Coagulation regulation [57]
Expression of human TFPI gene	Coagulation regulation [58]
Expression of human CD39 gene	Coagulation regulation [59]
Expression of human HO-1 gene	Anti-inflammatory/anti-apoptotic [60]
Expression of human A20 gene	Anti-inflammatory/anti-apoptotic [61]
Expression of HLA-E	Regulation of NK cell-mediated responses [63, 64]
ULBP1 knockout	Regulation of NK cell-mediated responses [64]
Expression of human CD47 gene	Regulation of macrophage responses [65]
Expression of human CTLA4-Ig	Regulation of T cell-mediated responses [66]
SLA class 1 knock-out	Regulation of T cell-mediated responses [67]
PERV inactivation	Xenozoonosis [21]
GHR- knock-out	Pig Growth Hormone Receptor [45]

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