

## **Regulation of Clinical Xenotransplantation – Time for a Reappraisal**

David K.C. Cooper, MD, PhD (1), Richard N. Pierson III, MD, PhD (2), Bernhard J. Hering, MD (3), Muhammad M. Mohiuddin, MD (4), Jay A. Fishman, MD (5), Joachim Denner, MD (6), Curie Ahn, MD, PhD (7), Agnes M. Azimzadeh, PhD (2), Leo H. Buhler, MD (8), Peter J. Cowan, MD (9), Wayne J. Hawthorne, MD, PhD (10), Takaaki Kobayashi, MD, PhD (11),  
David H. Sachs, MD (12)

(1) Thomas E. Starzl Transplantation Institute, Department of Surgery, University of Pittsburgh, Pittsburgh, PA, USA; (2) Division of Cardiac Surgery, Department of Surgery, University of Maryland, Baltimore VAMC, Baltimore, MD, USA; (3) Schultze Diabetes Institute, Department of Surgery, University of Minnesota, Minneapolis, MN, USA ; (4) National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, USA; (5) MGH Transplantation Center and Transplant Infectious Disease and Compromised Host Program, Massachusetts General Hospital/Harvard Medical School, Boston, MA, USA; (6) Robert Koch Institute, Berlin, Germany; (7) Transplantation Research Institute, College of Medicine, Seoul National University, Seoul, South Korea; (8) Department of Surgery, University Hospital Geneva, Geneva, Switzerland; (9) Immunology Research Centre, St Vincent's Hospital Melbourne, University of Melbourne, Melbourne, Victoria, Australia; (10) Department of Surgery, Westmead Clinical School, University of Sydney, Westmead Hospital, Westmead, NSW, Australia; (11) Department of Renal Transplant Surgery, Aichi Medical University School of Medicine, Nagakute, Japan; (12) Columbia University Medical Center, New York, NY, USA.

Address for correspondence:-

David K.C. Cooper, MD, PhD

Xenotransplantation Program/Department of Surgery

UAB, The University of Alabama at Birmingham

ZRB 701

703 19th Street S

Birmingham, AL 35233

Tel: 205-996-7772

Fax: 205-996-1043

E-mail: cooperdk@uab.edu

**Authors' contributions**

All authors participated in an initial discussion of the topic. The draft manuscript was put together by DKCC, RNP, BJH, MMM, and DHS, with further input from JAF and JD. All authors approved the final manuscript.

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**Abbreviations**

FDA = US Food and Drug Administration

PERV = porcine endogenous retrovirus

## **Abstract**

The continual critical shortage of organs and cells from deceased human donors has stimulated research in the field of cross-species transplantation (xenotransplantation), with the pig selected as the most suitable potential source of organs. Since the US Food and Drug Administration (FDA) concluded a comprehensive review of xenotransplantation in 2003, considerable progress has been made in the experimental laboratory to improve cell and organ xenograft survival in several pig-to-nonhuman primate systems that offer the best available models to predict clinical outcomes. Survival of heart, kidney, and islet grafts in nonhuman primates is now being measured in months or even years. The potential risks associated with xenotransplantation, eg, the transfer of an infectious microorganism, that were highlighted in the 2003 FDA guidance and subsequent World Health Organization consensus documents, have been carefully studied and shown to be either less likely than previously thought, or readily manageable by donor selection or recipient management strategies. In this context, we suggest that the national regulatory authorities worldwide should re-examine their guidelines and regulations regarding xenotransplantation, so as to better enable design and conduct of safe and informative clinical trials of cell and organ xenotransplantation when and as supported by the preclinical data. We identify specific topics that we suggest require reconsideration.

The continual critical shortage of organs and cells from deceased human donors has stimulated research in the field of cross-species transplantation (xenotransplantation), with the pig selected as the most suitable potential source of organs. Research has progressed rapidly in recent years, largely through the availability of an increasing number of genetically-engineered pigs and of novel immunosuppressive agents. Survival of heart, kidney, and islet grafts in nonhuman primates is now being measured in months or even years (<sup>1</sup> and see below).

Since the US Food and Drug Administration (FDA) concluded a comprehensive review of xenotransplantation in 2003 (<http://www.fda.gov/cber/guidelines.htm>), considerable progress has been made in the experimental laboratory to improve cell and organ xenograft survival in several pig-to-nonhuman primate systems that offer the best available models to predict clinical outcomes. Meanwhile, the increasing number of deceased human donor organs used for clinical transplantation has failed to keep pace with an expanding candidate wait list, and a significant number of waiting patients die without receiving a donor organ (<http://optn.transplant.hrsa.gov/>).

### **Risks**

The potential risks associated with xenotransplantation, eg, the transfer of an infectious microorganism, that were highlighted in the 2003 FDA guidance and subsequent WHO consensus documents, have been carefully studied and shown to be either less likely than previously thought, or readily manageable by donor selection or recipient management strategies (see below). As such, we consider that the risk-benefit ratio associated with pig-to-human transplantation of organs and tissues has changed dramatically since the FDA and other national

(UKXIRA, MedSafe, etc.) and international (WHO) regulatory bodies last completed their careful assessments in the first half of the last decade.

In this context, we suggest that the national regulatory authorities worldwide should re-examine their rules and regulations regarding xenotransplantation, so as to better enable design and conduct of safe and informative clinical trials of cell and organ xenotransplantation when and as supported by the preclinical data.

### **Unmet clinical needs**

We feel it is important to place this recommendation in context. Despite 5 decades of concerted effort, the gap between the supply of organ allografts and demand for them has widened significantly. Many initiatives to increase the number of human organs that are utilized for transplantation have succeeded, eg, the use of expanded donors or donors after circulatory death, organ pairing, etc., and have been widely adopted internationally as a consequence of progressive, culturally-sensitive policy and education initiatives. Unfortunately, even as donor management options and donor acceptance criteria have been significantly expanded, optimistic projections that waiting lists would shrink have not been realized.

The results associated with various mechanical devices as an alternative to transplantation have improved significantly over the past decade, especially in cardiac support, though there remain several short- and long-term problems.<sup>2,3</sup> However, while ventricular assist devices play an increased role in the management of patients with cardiac failure, even for that population there remains a large unmet need, and patients suffering from failure of other vital organs at present have no similar option.

Other options for overcoming the shortage of deceased human donors in general have not made as much progress as xenotransplantation.<sup>4</sup> Specifically, although there has been progress in stem cell research, tissue engineering and regenerative medicine, and blastocyst complementation, we believe that these technologies remain less advanced than xenotransplantation.<sup>4</sup> Although we support continued investment in each of these fields, given the major, well-defined barriers facing each of them, we do not expect that any of them will have significant clinical impact in the near future, and believe that xenotransplantation provides the best near-term solution to the organ shortage that limits organ transplantation.

### **Preclinical Progress**

Progress in xenotransplantation has been achieved by systematic study of the scientific barriers. Each identified barrier has been addressed, either by genetic engineering of the organ-source pig or by availability and application of novel immunosuppressive and anti-inflammatory agents (reviewed in <sup>1,5</sup>).

Genetically-engineered pig heart transplants have functioned in a heterotopic position in baboons for more than 2 years, only failing after all immunosuppressive therapy had been discontinued.<sup>6-9</sup> Genetically-engineered pig kidneys have supported life in baboons and monkeys for more than 6 months and in one case for almost a year<sup>10-12</sup>; Iwase H and Adams A, personal communications). Both genetically-engineered and wild-type pig islets have maintained insulin-independent normoglycemia in diabetic monkeys for periods of more than a year, and in one case for almost 3 years.<sup>13-17</sup> Genetically-engineered mesencephalic pig cells have reduced the physical features of a Parkinson-like disease in monkeys for greater than 1 year.<sup>18</sup> Even in the difficult pig-to-

baboon liver transplantation model there has been significant improvement in graft survival, to almost 1 month in 2 recent instances.<sup>19-21</sup>

Preclinical results are rapidly approaching consensus benchmarks intended to trigger consideration of clinical trials. Indeed, clinical trials of decellularized pig corneal transplantation<sup>22</sup> and encapsulated pig islet transplantation<sup>23,24</sup> are already underway, and consideration is being given to the selection of patients for initial clinical trials of pig solid organ xenotransplantation.<sup>25</sup>

## **PERV**

More recent experience has suggested that the risk of porcine endogenous retrovirus (PERV) infection in human recipients is less than anticipated.<sup>26-28</sup> Based on the molecular sequencing of PERV, both genomic screening and quantitative assays for circulating PERV have been developed.<sup>29</sup> These advances have allowed development of testing methods for source animals, organs, and human recipients.<sup>30</sup> While persistent microchimerism in xenograft recipients may pose some risk of delayed donor-derived infection, no transmission to human xenograft recipients or in preclinical pig-to-primate studies has been demonstrated.<sup>31,32</sup> Burn patients treated with wild-type skin transplants did not develop evidence of infection.<sup>27</sup> Available antiviral agents also have activity against PERV.<sup>33-35</sup> Multiple intrinsic mechanisms appear to further limit the infectivity of PERV for human cells despite the presence of PERV receptors.<sup>36</sup> A variety of other approaches have been suggested including the selection of pigs with reduced PERV loci, including those used in a New Zealand clinical trial without evidence of PERV transmission, though this trial was in non-immunosuppressed patients.<sup>28</sup> The same observation

was made following the transplantation of encapsulated pig islets in patients in a second clinical trial in Argentina.<sup>37</sup> It is possible that newer molecular techniques including siRNA technology<sup>38-41</sup> or the generation of PERV knockout swine using CRISPR-Cas9 technology<sup>42,43</sup> could limit or completely exclude PERV transmission.

## Proposals

On the basis of these considerations, we would propose the following topics as candidates for reconsideration by national and international regulatory authorities.

1. *The archiving of samples from both source-pig and human recipient to enable investigation in the event of an unexpected complication following a xenotransplant*

It was originally suggested that archiving of tissues should be maintained for up to 50 years. Considering the much lower risk now envisioned for PERV-induced disease<sup>26,32,44,45</sup> and the unwieldy logistics and high cost of such prolonged archiving, we believe that this requirement should be relaxed. It is anticipated that the majority of Infections associated with the presence of exogenous microorganisms will occur early after transplantation, but it is unknown how, when, or whether a PERV-related complication might present. However, new technologies may be applied to archived specimens (eg, high-throughput sequencing) to detect organisms not originally noted in screening assays for donor animals or not detected in non-immunosuppressed hosts.

Further clarification and guidelines are required on a number of points that include the following. Who will be responsible for maintaining the archives? Will it be

the academic or clinical center carrying out the clinical trial or a company sponsoring a trial? Will the national regulatory authorities play any role in this archiving? Where will the tissues be archived and under what level of security? Who will bear the cost of storage of the archived samples?

2. *The monitoring of patients and their relatives and close friends after a xenotransplant*

For the same reasons as described for archiving above, and because life-long monitoring, even if deemed advantageous, would be difficult and not likely enforceable, we suggest that such prolonged monitoring may be neither necessary nor advisable, and therefore should be reconsidered.

3. *Pigs with multiple genetic modifications.*

It needs to be made clear whether a pig with multiple genetic modifications will be considered as a single 'product' or whether each individual genetic modification needs to be assessed and approved separately. Our present understanding is that, in the USA, a pig with multiple genetic modifications will be considered as a single entity. Separate assessment will almost certainly delay the clinical introduction of this potentially life-saving form of therapy. A related concern that should be clarified is whether a pig with a specific pattern of genetic modifications will be approved as a source of 1 specific organ, or of all organs.

4. *The inclusion in the immunosuppressive treatment regimen of a drug not yet clinically approved by the national regulatory authority.*

Guidance is sought about what circumstances might make it possible to use an investigational drug together with a genetically-engineered pig, neither of which has yet been approved. For example, could islets from a genetically-engineered source-pig, presumably one with multiple hitherto unapproved genetic modifications, be combined with an investigational T cell costimulation blockade agent or other investigational drug or device (eg, for immunoisolation)? This is not unprecedented in that numerous islet allotransplant trials have included investigational islet products and off-label use of immunosuppressants.<sup>46</sup> If adequately supported by preclinical efficacy and safety data, might a drug that is approved for other indications be used for off-label use in combination with an investigational genetically-engineered pig organ?

Progress in xenotransplantation research is now relatively rapid. As such, we believe it is timely for the above points – and others that may emerge – to be reappraised as the basis for informing clinical trials of xenotransplantation.

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