Article:
Thom, R., Ayres, D., Cooper, D. et al. (11 more authors) (2023) Update on the ethical, legal, and technical challenges of translating xenotransplantation. Journal of Medical Ethics. ISSN 0306-6800

https://doi.org/10.1136/jme-2023-109298

Journal of
Medical Ethics

An update on the ethical, legal, and technical challenges of translating xenotransplantation

Journal:	*Journal of Medical Ethics*
Manuscript ID	jme-2023-109298.R1
Article Type:	Current controversy
Keywords:	Ethics, Tissue and Organ Procurement, Legislation, Policy

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

An update on the ethical, legal, and technical challenges of translating xenotransplantation

Abstract

This manuscript reports on a landmark symposium on the ethical, legal and technical challenges of xenotransplantation in the UK. King's College London, with endorsement from the British Transplant Society (BTS), and the European Society of Organ Transplantation (ESOT), brought together a group of experts in xenotransplantation science, ethics, and law to discuss the ethical, regulatory, and technical challenges surrounding translating xenotransplantation into the clinical setting. The symposium was the first of its kind in the UK for 20 years. This paper summarises the content of the expert lectures showcasing the progress which has been made in xenotransplantation including - the history of xenotransplantation, advances in gene edited animals and progress towards clinical xenotransplantation. We then set out the ethical and legal issues still to be resolved. Finally we report the themes of the roundtable discussion highlighting areas of consensus and controversy. While the detail of the legal discussion was directed towards the UK, the principles and summary reported here are intended to be applicable to any jurisdiction seeking to implement clinical xenotransplantation.

Background

The history of xenotransplantation is peppered with optimistic predictions about its imminence and feasibility. In 1969 the Nobel winning transplant immunologist Peter Medawar hypothesised that-

> "We should solve the problem [of organ transplantation] by using heterografts [xenografts] one day if we try hard enough, and maybe in less than 15 years[1]."

More than 20 years later the Nuffield Council on Bioethics predicted that the first pig to human xenotransplants would be performed in 1996[2]. What followed was almost 30 more years of intense research focused on the remaining immunological and infectious challenges. However, in 2022 xenotransplantation was thrust back into mainstream transplant discourse following two significant developments. First were the reports of two genetically-engineered (GE) pig kidneys transplanted into deceased human recipients, mechanically maintained after death by neurologic criteria (decedents)[3,4]. Secondly was the landmark GE porcine-to-living-human heart transplant conducted in Maryland [5–7]. This resurgence of activity, in the shadow of the COVID pandemic, comes at a time when public and professional anxieties about zoonosis, public health, and experimental treatments are all running high.

It was on this background that in late 2022 King's College London, with endorsement from the British Transplant Society (BTS), and the European Society of Organ Transplantation (ESOT), brought together a group of experts in xenotransplantation science, ethics, and law. This symposium, the first of it's kind in the UK for 20 years, aimed to discuss the ethical, regulatory, and technical challenges surrounding translating xenotransplantation into the clinical setting. Firstly, a series of three lectures showcased the progress which has been made in xenotransplantation including- the history of xenotransplantation, advances in gene edited animals and preclinical research activities. Following these were sessions introducing ethical and legal concerns. Finally, a roundtable discussion was held. This paper follows this structure firstly summarising the content of the expert lectures. Then we report on the broad themes identified in the ethical and legal sessions and the third and

final section reflects on the roundtable discussion, highlighting areas of consensus and controversy. While the detail of the legal discussion was directed towards the UK, the principles and summary reported here are intended to be applicable to any jurisdiction seeking to implement clinical xenotransplantation.

Part 1: Expert presentations - progress in xenotransplantation

A history of xenotransplantation

Dr David Cooper, a heart transplant surgeon and xenotransplantation research pioneer who has more than 50 years' experience in the field, opened the symposium with a lecture on the history of xenotransplantation. He noted that the idea of blending humans and animals can be seen as far back as ancient Greek, Hindu, and Mesopotamian iconography but that the first sustained attempts at a medical application of xenotransplantation was the use of non-human animal-to-human blood transfusions in the 17th century. Other unsuccessful historical uses of animal tissues through the 18th, 19th and 20th centuries have included skin grafts, corneal grafts and, in the 1920s, the transplantation of slices of baboon's or chimpanzee testicles into elderly men to improve their 'vitality'[1]. To the eyes of modern scientists these experiments may have been doomed to failure, but animal studies have provided significant contributions to the development of transplantation techniques, for example, the work of Alexis Carrel who performed the first vascular anastomosis[1]. It was surgical techniques such as these, first practiced on animals, which allowed the first human allotransplants to occur in the 1950s[8].

By the 1960s while both dialysis and human allotransplantation had been successfully demonstrated, neither was readily available to kidney failure patients due to a lack of donors and dialysis facilities. Xenotransplantation represented a proposed solution (Figure 1). To this end Keith Reemtsma transplanted chimpanzee kidneys into humans between 1963 and 1964 using

azathioprine and prednisolone as immune suppression[9]. One patient survived for 9 months but the remainder succumbed either to rejection or infections within weeks or short months. In 1964 James Hardy performed the first ever heart transplant using a chimpanzee's heart[10]. However, this too was unsuccessful with the recipient swiftly rejecting the organ and dying after just a few hours. Then in 1983 there was the widely publicised case of baby Fae who, at a time when no infant heart transplants were being performed, received a baboon's heart. While the reporting of her case did successfully raise the profile of the issue of the shortage of paediatric organ donors, she died just 20 days later[11].

By the mid 1980's it was becoming accepted that non-human primates (NHP) were not the ideal source of donor organs they were once imagined to be. Attention moved to pigs with the first transgenic pig created in 1992[1]. Three decades of subsequent research culminated in January 2022 with the first GE porcine-to-human heart transplant performed at the University of Maryland under the Food and Drug Administration's (FDA) compassionate use scheme. The recipient- David Bennett- was a man in his 50s who was critically unwell, requiring ECMO, and was only anticipated to live a few weeks. The organ was immediately life sustaining despite significant operative complications in the form of an aortic dissection. David lived for 60 days post-xenotransplant [5–7].

Engineering Pigs for Xenotransplantation Products

Implanting unmodified porcine organs results in hyperacute rejection in both non-human primates and humans. Dr David Ayares presented his lifetime's work researching overcoming this critical barrier. The primary porcine xeno-antibody is targeted against alpha-1, 3-galactose (GTA), a carbohydrate antigenic structure which is absent in both humans and NHPs[1]. In 2003 Dr Ayares' team created the first GTA knockout pigs with an inactive alpha gal transferase gene[12]. These single knockout 'GalSafe' pigs have been subjected to multiple safety studies and have been cleared by the FDA for use in decellularized products such as heart valves and for human consumption for

those with red meat allergy[13,14]. In addition to the GTA knockout modification, through selective breeding a line of pigs has been established that is of low risk for porcine endogenous retrovirus (PERV) activation. However, Dr Ayares contends that bioengineering will need to be more sophisticated to achieve successful xenotransplantation (Figure 2). A discussion about the application of bioengineering in the field was beyond the scope of the symposium, but several recent reviews have documented progress in this area [15–18].

To overcome these barriers, Dr Ayares' team have developed a line of pigs with multiple additional genetic modifications which both knock out porcine genes and insert human genes. These modifications include knocking out additional genes encoding important antigenic targets to make a 'triple knock out' (TKO) pig, adding human complement-regulatory genes aimed at reducing hyperacute rejection, and inserting human immune suppressor genes. Furthermore, to address the coagulation dysregulation seen in xenotransplant recipients, human anti-coagulant genes have been inserted. Finally, pig growth hormone receptor genes have been knocked out to prevent growth beyond that which will provide organs of a size suitable for human use. In total, the team have developed a line in which 10 genes have been edited - 4 knock out porcine genes and 6 inserted human genes[19]. Dr Ayares's team is currently undertaking preclinical trials and hopes to enter clinical trials from 2024.

Preclinical research in Xenotransplantation

Professor Jayme Locke presented some of the pre-clinical research in xenotransplantation which has been conducted by the team at the University of Alabama at Birmingham.

Non-Human Primate Pre-Clinical Xenotransplantation Research

Pre-clinical xenotransplantation research relies on the use of non-human animal models. By selectively transplanting genetically modified porcine organs into NHPs they hope to address some

key safety concerns before moving to clinical trials. For instance, the impact of hormonal and electrolyte handling differences between porcine and human nephrons, establishing expected urine output and clotting or coagulation complications. To this end, Professor Locke reported that they have successfully shown that porcine kidneys can replicate physiological functions such as urine output, creatinine clearance and electrolyte homeostasis [20–22]. Throughout their studies on NHP the team have not noted any major haematological abnormalities when using the 10 gene edited porcine organs and through careful measurements, they aim to establish the weight and age of a pig needed to yield an organ the same size as a typical adult human kidney.

However, their studies have also served to delineate critical limitations of utilizing NHPs as a model for use in humans- these pigs have been augmented to evade the *human* but not the NHP immune responses. Professor Locke cited studies which have shown that sequential modifications to the porcine genome result in significant reductions in antibody binding from human plasma[23]. However in NHPs, while reactivity against single, double or triple knock out pigs reduces with each modification *all* still have a positive cross match[24]. This means that *all* pig-to-NHP transplants are undertaken against a positive crossmatch at high immunological risk of rejection. In addition, tacrolimus-based regimens, which have been so successful in improving outcomes of human allotransplantation, have proved ineffective in NHP models[20]. Research has shown that CD40/CD154 co-stimulation pathway blockade provides better results, and a novel anti CD40 agent was used in the 2022 porcine heart transplant[7] however it is yet to be determined if this will enable clinical xenotransplantation. In light of these immunological issues, NHP studies typically require more aggressive immunosuppressive regimens than those used as standard in human allotransplantation. Professor Locke argued that this has led to concerns that the intensity of immunosuppression needed may result in higher infectious complications and diminish the ability of NHP studies to prove long term organ function.

Human Decedent Pre-Clinical Xenotransplantation Research – the "Parsons Model"

In light of the limitations inherent with NHP research, some in the field argue that we have reached as far as we can with animal models, and that to progress further it is essential to move to human recipients. Professor Locke also presented one proposed solution to this difficulty – the human decedent or 'Parsons Model' – which utilises brain dead humans as initial research participants. In this way, Professor Locke argued, that as well as addressing any remaining safety concerns which cannot be resolved in the NHP model, the 'Parsons Model' offers the opportunity for key technology to be tested (Figure 3). She emphasised that for xenotransplantation to be a credible alternative to conventional allotransplantation it will be essential that xenotransplantation protocols adhere as closely as possible to the existing standards of care. For example, cross matching, interpreting of biopsy findings and infectious disease testing will all need to be re-validated in a xenotransplant setting.

Professor Locke presented the findings of their two human decedent studies. Institutional research ethics boards are traditionally tasked with overseeing research conducted only on living human subjects[25], however Professor Locke emphasised that ethics oversight and authorisation was obtained prior to these studies being undertaken. Consent was also gained from the families of the deceased[3]. The immediate results included that the decedents were hemodynamically stable through reperfusion and no hyperacute was rejection observed. The decedents were maintained until the study was terminated after 3 days. In this time the kidneys appeared viable, produced urine, and no transmission of PERV was detected. However, in the first decedent a thrombotic microangiopathy of unknown cause was noted on biopsy on day one, and this led to the administration of eculizumab (C5 complement inhibitor, anti-C5 antibody) during the second study. In neither study was creatinine clearance restored[3]. A detailed summary of the study involving the first recipient has been published[3].

Part 2: Ethical and legal issues in translating xenotransplantation

Professor Michael Gusmano and Professor Brendan Parent are two leading ethicists in xenotransplantation in the USA[26–29]. Their presentations reported on work currently being undertaken by their respective Universities on the ethical and legal issues around xenotransplantation. The following commentary summarises the central themes emerging from their work (Figure 4), and the discussion which followed their thought provoking lectures.

Regulation

Transplantation is one of the most highly scrutinised areas of medicine. Protecting the rights of donors and recipients, ensuring the quality and safety of organs used for transplantation and ensuring the equitable and effective allocation of scarce resources are all vital roles. Professor Parent observed that in the USA, the mechanisms for transplant oversight apply only to human organs. In the UK, as in most European jurisdictions, there is no legislation in place which prohibits xenotransplantation. However there is also no framework which sanctions and regulates its practice in a form comparable to human allotransplantation. There is no universal form of legal instrument to achieve this, but lessons could be drawn from the implementation of other transplant practices that have been contentious during their evolution - such as non-directed living altruistic donation, directed deceased donation or kidney sharing schemes. In addition, guidelines issued by regulators require regularly updating to ensure that they are compatible with the latest scientific and social developments. Professor Gusmano highlighted that those currently in use in the USA contain ambiguous language such as “sufficient” or “adequate” to describe the necessary pre-clinical safety and efficacy data to move into clinical trials.

Recipient selection

While advances in the last 50 years have made definitive progress on resolving issues of xeno-organ compatibility, there remains little consensus on which patients should be included in the first living

human clinical xenotransplant trials. On the one hand you have those who argue that patients who are already too ill to recover from their underlying illness should be among the first enrolled. However, such patients are not necessarily the optimal choice when trying to assess the therapeutic potential of porcine organs. Studying transplants (regardless of organ source) in such recipients is unlikely to provide reliable answers to key research or safety questions and their inclusion could therefore compromise the integrity of research conclusions. Furthermore, while these patients are sometimes framed as having 'nothing to lose' they may also have little to gain personally from a xenotransplant. For these patients, some of those present argued that palliation is an existing alternative to xenotransplantation which can sometimes be overlooked in this discussion.

Target organ system

There is also a debate about which organ system provides the best model for initial trials. Kidney transplantation has by some measure the largest waiting list. This therefore presents the opportunity to benefit the greatest number of people should trials prove successful. An alternative view could be that initial work is better focused on paediatric heart transplantation because many paediatric patients are not even wait-listed due to the lack of available human donor organs. Much like the participants in early xenotransplant experiments, paediatric cardiac patients, without an option for a human allotransplant continue to have extremely limited available treatment options.

Eligibility for human allotransplant

The question which logically follows from "which patients and which organs should first be included" is, whether and if so to what extent, eligibility for human allotransplant should determine eligibility for xenotransplant? In this case it can again be argued either that xenotransplant research participants should have been deemed not to be eligible for human allotransplant so that they are not forgoing an established lifesaving treatment for an experimental one. Or the counter position, which is that patients *must* be suitable for an allotransplant, because if we do not anticipate an allotransplant to succeed why should we expect a xenotransplant to?

Risks and benefits including xenozoonotic disease transmission

While the potential benefits of xenotransplant are self-evident – continued life, freedom from dialysis, reduction in organ waiting lists– there are also significant risks. Some of these risks are established facts, for example, the operative risks of any transplant procedure. Other risks are unknown, and some may not yet have been conceived (unknown unknowns). The most challenging issue perhaps is risks which are theoretically known but the likelihood and magnitude of the risk is not fully understood. This category of risk includes – rejection of the organ, sensitisation to future human allotransplantation, and zoonotic infection.

Zoonosis is also important in considering the balance between private benefits and public risk. In this case the argument is that the benefit of xenotransplantation is principally to those individuals whose lives are saved, but the risks taken are those which, should they be realised, could be at the expense of the wider public if a virus of zoonotic origin were transmittable to the recipient's contacts and beyond. Against this is the argument that by not pursuing xenotransplantation we must weigh the risk of an ever-increasing waiting list with the attendant suffering and harm to those waiting and their communities. Exemplifying the dynamic nature of risk and its perception Professor Gusmano noted that in attitudes surveys on xenotransplantation a frequently encountered response is "I wouldn't mind being, you know, the 50th person to receive this. I wouldn't want to be the 1st".

Informed consent

Uncertainty of the benefits and risks of clinical xenotransplantation will present difficulties for ethics committees determining the permissibility of trials, but it will also make it challenging to satisfy the 'information' criterion of 'informed consent'. For example, xenotransplant is sometimes discussed as a possible 'bridge' to an allotransplant. However, we currently do not understand how or if a xenotransplant would sensitise a recipient to future allotransplant[30] which would undoubtedly be considered material to a recipient's decision making. Furthermore, patients without viable curative

treatment alternatives or who are critically unwell may be in a state of vulnerability which could compromise their ability to give free and informed consent.

Equitable subject selection

It is recognised that under the current human allotransplant system some groups experience disadvantage. For example, Black patients wait significantly longer than their white counterparts for an organ for transplantation. This might lead some to argue that one aim of a xenotransplantation programme should be to reduce this inequity. However, the egregious history of exploitation of Black participants in clinical trials highlights the importance that the inclusion of disadvantaged or 'vulnerable' groups is carefully and sensitively undertaken. Empirical research has suggested that non-white respondents are six-times less likely to consider a xenotransplant[31].

Religious and Cultural beliefs

Reluctance on the part of recipients to accept a xenotransplant may not just relate to suspicions regarding the intentions of researchers. Religious and cultural beliefs will undoubtedly play a role in an individual's assessment as to if certain animals' organs are viewed as acceptable [31–36]. The current narrow focus on porcine organs may be perceived as commercial pragmatism by some but as discriminatory by those who object to the use of pigs in medical products for cultural or religious reasons.

Compassionate use

The expanded access or 'compassionate use' scheme which facilitated David Bennett's heart transplant is designed to enable access to investigational treatments or products when the patient is critically ill and there is "no comparable or satisfactory alternative therapy options[37]". Although drugs and treatments available through this scheme may be undergoing clinical trials, the terms under which they are offered are quite different. There are not strict guidelines regarding the design of the intervention as there would be in a clinical trial, nor is there an obligation to publish findings.

Regardless, compassionate use xenotransplants should be subject to the same safety standards and monitoring for zoonotic infection as for those performed under the auspices of a clinical trial and, data generated should be published and shared.

Alternatives to xenotransplantation and non-human animal welfare

Finally, concerns have been raised about the compatibility of xenotransplantation with established agreements on the reduction, refinement, and replacement of non-human animals in scientific research. Professor Parent finished his lecture with a challenge for all involved in this field to consider if there are alternatives to xenotransplantation which might in the long term be immunologically preferable *and* more sustainable. Specifically, if bioengineered organs are equally likely to provide an unlimited supply of organs for transplantation but without harm to sentient and cognitively complex non-human animals or the environmental impact of continued reliance on animal products.

Part 3: Round table discussion – consensus and controversy

Several of this symposiums' speakers contended that the sequential progress made from laboratory, NHP and human decedent research, combined with the 'diminishing returns' from NHP studies, mean that clinical trials are now indicated. These lectures provided a platform for a thought-provoking discussion between the experts in attendance, during which, some agreement was formed on the practical next steps needed, and identified key priorities for resolution.

Establishing a regulator

Establishing an appropriate regulator is a key priority. In the 1990s the UK was at the forefront of xenotransplantation research and regulation. The formation of the United Kingdom Xenotransplantation Interim Regulatory Authority (UKXIRA) in 1997 gave the UK one of the most robust and forward-thinking regulatory environments in the world. However, when scientific progress failed to materialise, the UKXIRA was disbanded in 2006 and there is currently no permanent regulatory structure for xenotransplantation in place[38]. Nevertheless, the UKXIRA's short tenure demonstrated the sorts of qualities that a good regulator should possess, including multidisciplinary representation - clinical, scientific, ethical, legal, religious, and animal welfare expertise are all required. A regulatory body should also be capable of undertaking the necessary public consultation. Crucially, links with government departments and statutory bodies overseeing agriculture, animal welfare, health, and organ transplantation will be required for the regulatory body to be effective. For example, in the UK human allotransplantation is regulated by the Human Tissue Authority (HTA) which at present has no remit to oversee xenotransplantation activities.

Practical requirements: infrastructure and cost

As the experience of the US sites conducting xenotransplantation research shows, the infrastructure needed to facilitate xenotransplantation is significant. In addition to identifying a source of genetically engineered pigs, a bio-secure (referred to as 'pathogen free' in US federal regulations)

facility capable of housing, testing and euthanising these animals, at a cost of tens of millions of pounds is required. To ensure security of supply, two such facilities are preferred to one. As Professor McGregor said, “these are essential components, and they are all expensive”.

Determining an Immune Suppression Regimen

An optimal immune suppression regimen avoids both over immunosuppression, with subsequent infectious complications and under immunosuppression, which precipitates rejection. There is currently significant worldwide variation on immunosuppressive regimens used in human allograft transplantation although there is more consensus with regards to maintenance therapy than induction[39]. When considering xenotransplantation all that is certain is that we have not yet reached a consensus regarding what an optimal regimen will include. However at least some of the agents used in the human decedent model or which are proposed for use in future xenotransplantation studies use are likely to be familiar to transplant physicians and their patients (Figure 5).

Zoonoses

In human allotransplantation infectious complications are a significant driver of mortality and morbidity. In xenotransplantation the usual human pathogens will need to be considered, but in addition zoonotic infections. However, the likelihood of transmission, the effect of this on the recipient and the likelihood and threat of wider contagion to their close contacts or the wider public are all unknown. For example, cytomegalovirus (CMV) is routinely tested for in human donors and allograft recipients. Porcine CMV has therefore been flagged as a key concern in xenotransplant. The donor animal for the xeno-heart transplant tested negative on several occasions for porcine CMV prior to implantation, however Mr Bennett subsequently tested positive[7]. There is ongoing research into any relevance this may have had to his clinical course and this finding has stimulated the development of more sensitive testing methods. This case exemplifies the need not just for

rigorous testing regimes but also for reliable and robust methods which are able to respond to new risks as they emerge.

Additional ethical considerations

In addition to the major issues identified by Professor Gusmano and Professor Parent outlined in the previous section, during the roundtable discussion several additional ethical issues were proposed by our panel of experts and attendees. These included – ensuring equity of access to successful treatments, the burden of xenotransplantation monitoring requirements on patients, their close contacts and clinical personnel, and the need for cross border agreements to cover the prospect of recipients receiving initial treatment in one jurisdiction but moving to another. The breadth of issues raised during this discussion were too wide ranging to be adequately covered in a single event and show that the legal and ethical discourse on xenotransplantation may yet uncover new challenges that will need to be addressed prior to the start of clinical trials.

Moving forward – Closing Comments

This symposium combined a state-of-the-art science update with a wide-ranging discussion on the ethical regulatory and technical challenges to implementing clinical xenotransplantation. There was consensus among the speakers that xenotransplantation, now more so than in any of the previous decades, is an imminent possibility. It was beyond the remit of this symposium to generate and ratify formal recommendations on how the UK should proceed however a few key messages could be taken away. A regulatory framework and the appropriate technical resources will need to be established to facilitate a clinical xenotransplantation programme. Moreover, in the wake of COVID, in an ever more connected world of international travel, an *international* monitoring and regulatory framework is essential. There remain significant legal and ethical questions regarding recipient

selection, risk benefit analysis and the allocation of resources, which will require detailed discussion.

Above all up-to-date public consultation is essential to finding acceptable resolutions.

References

1 Cooper DKC. A Brief History of Cross-Species Organ Transplantation. *Baylor University Medical Center Proceedings* 2012;**25**:49–57. doi:10.1080/08998280.2012.11928783

2 Nairne P, Nuffield Council on Bioethics, editors. *Animal-to-human transplants: the ethics of xenotransplantation*. London: : Nuffield Council on Bioethics 1996.

3 Porrett PM, Orandi BJ, Kumar V, *et al.* First clinical-grade porcine kidney xenotransplant using a human decedent model. *American Journal of Transplantation* 2022;**22**:1037–53. doi:10.1111/ajt.16930

4 Montgomery RA, Stern JM, Lonze BE, *et al.* Results of Two Cases of Pig-to-Human Kidney Xenotransplantation. *N Engl J Med* 2022;**386**:1889–98. doi:10.1056/NEJMoa2120238

5 Reardon S. First pig-to-human heart transplant: what can scientists learn? *Nature* 2022;**601**:305–6. doi:10.1038/d41586-022-00111-9

6 Wang W, He W, Ruan Y, *et al.* First pig-to-human heart transplantation. *The Innovation* 2022;**3**:100223. doi:10.1016/j.xinn.2022.100223

7 Griffith BP, Goerlich CE, Singh AK, *et al.* Genetically Modified Porcine-to-Human Cardiac Xenotransplantation. *N Engl J Med* 2022;**387**:35–44. doi:10.1056/NEJMoa2201422

8 Merrill JP, Murray JE, Harrison JH, *et al.* Successful homotransplantation of the human kidney between identical twins. *J Am Med Assoc* 1956;**160**:277–82. doi:10.1001/jama.1956.02960390027008

9 Reemtsma K, Mccracken BH, Schlegel JU, *et al.* Renal Heterotransplantation in Man. *Ann Surg* 1964;**160**:384–410. doi:10.1097/00000658-196409000-00006

10 Hardy JD, Kurrus FD, Chavez CM, *et al.* Heart Transplantation in Man. Developmental Studies and Report of a Case. *JAMA* 1964;**188**:1132–40.

11 Bailey LL, Nehlsen-Cannarella SL, Concepcion W, *et al.* Baboon-to-human cardiac xenotransplantation in a neonate. *JAMA* 1985;**254**:3321–9.

12 Phelps CJ, Koike C, Vaught TD, *et al.* Production of α1,3-Galactosyltransferase-Deficient Pigs. *Science* 2003;**299**:411–4. doi:10.1126/science.1078942

13 Food and Drug Administration. Freedom of Information Summary - Origional New animal drug application. 2020.https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadFoi/10168

14 Food and Drug Administration. FDA Approves First-of-its-Kind Intentional Genomic Alteration in Line of Domestic Pigs for Both Human Food, Potential Therapeutic Uses. https://www.fda.gov/news-events/press-announcements/fda-approves-first-its-kind-intentional-genomic-alteration-line-domestic-pigs-both-human-food

15 Lei T, Chen L, Wang K, *et al.* Genetic engineering of pigs for xenotransplantation to overcome immune rejection and physiological incompatibilities: The first clinical steps. *Front Immunol* 2022;**13**:1031185. doi:10.3389/fimmu.2022.1031185

16 Cooper DKC, Raza SS, Chaban R, *et al.* Shooting for the moon: Genome editing for pig heart xenotransplantation. *J Thorac Cardiovasc Surg* 2023;**166**:973–80. doi:10.1016/j.jtcvs.2022.04.032

17 Chan JCY, Chaban R, Chang SH, *et al.* Future of Lung Transplantation: Xenotransplantation and Bioengineering Lungs. *Clin Chest Med* 2023;**44**:201–14. doi:10.1016/j.ccm.2022.11.003

18 Ibi Y, Nishinakamura R. Kidney Bioengineering for Transplantation. *Transplantation* 2023;**107**:1883–94. doi:10.1097/TP.0000000000004526

19 Mohiuddin MM, Goerlich CE, Singh AK, *et al.* Progressive genetic modifications of porcine cardiac xenografts extend survival to 9 months. *Xenotransplantation* 2022;**29**. doi:10.1111/xen.12744

20 Cooper DKC, Hara H, Iwase H, *et al.* Pig kidney xenotransplantation: Progress toward clinical trials. *Clin Transplant* 2021;**35**. doi:10.1111/ctr.14139

21 Hansen-Estruch C, Bikhet MH, Javed M, *et al.* Renin-angiotensin-aldosterone system function in the pig-to-baboon kidney xenotransplantation model. *American Journal of Transplantation* 2023;**23**:353–65. doi:10.1016/j.ajt.2022.11.022

22 Hansen-Estruch C, Bikhet MH, Shaik IH, *et al.* Assessment of glomerular filtration and tubular secretion in baboons with life-supporting pig kidney grafts. *Xenotransplantation* 2023;**30**:e12795. doi:10.1111/xen.12795

23 Martens GR, Reyes LM, Butler JR, *et al.* Humoral Reactivity of Renal Transplant-Waitlisted Patients to Cells From GGTA1/CMAH/B4GalNT2, and SLA Class I Knockout Pigs. *Transplantation* 2017;**101**:e86–92. doi:10.1097/TP.0000000000001646

24 Adams AB, Kim SC, Martens GR, *et al.* Xenoantigen Deletion and Chemical Immunosuppression Can Prolong Renal Xenograft Survival. *Annals of Surgery* 2018;**268**:564–73. doi:10.1097/SLA.0000000000002977

25 Parent B, Gelb B, Latham S, *et al.* The ethics of testing and research of manufactured organs on brain-dead/recently deceased subjects. *J Med Ethics* 2020;**46**:199–204. doi:10.1136/medethics-2019-105674

26 Parent B. Research Involving the Newly Deceased Following Death by Neurologic Criteria: Ethical Justification and Guidelines. *Transplantation* 2022;**106**:2275–7. doi:10.1097/TP.0000000000004141

27 Caplan A, Parent B. Ethics and the emerging use of pig organs for xenotransplantation. *The Journal of Heart and Lung Transplantation* 2022;**41**:1204–6. doi:10.1016/j.healun.2022.06.008

28 Gusmano MK. Xenotransplantation Clinical Trials and the Need for Community Engagement. *Hastings Center Report* 2022;**52**:42–3. doi:10.1002/hast.1420

29 Maschke, Karen, Gordon, Elisa, Gusmano MK. Opinion: After the pig-to-human heart transplant, the FDA, clinicians and insurers have some catching up to do. *The Washington Post*

2022.https://www.washingtonpost.com/opinions/2022/01/13/what-comes-after-pig-to-human-transplant-breakthrough/

30 Cooper DKC, Habibabady Z, Kinoshita K, *et al.* The respective relevance of sensitization to alloantigens and xenoantigens in pig organ xenotransplantation. *Human Immunology* 2023;**84**:18–26. doi:10.1016/j.humimm.2022.06.003

31 Padilla LA, Hurst DJ, Jang K, *et al.* Racial differences in attitudes to clinical pig organ Xenotransplantation. *Xenotransplantation* 2021;**28**. doi:10.1111/xen.12656

32 Hurst DJ, Padilla LA, Cooper DK, *et al.* The attitudes of religious group leaders towards xenotransplantation: A focus group study. *Xenotransplantation* 2022;**29**. doi:10.1111/xen.12777

33 Padilla LA, Rhodes L, Sorabella RA, *et al.* Attitudes toward xenotransplantation: A survey of parents and pediatric cardiac providers. *Pediatr Transplant* 2021;**25**. doi:10.1111/petr.13851

34 Padilla LA, Hurst D, Lopez R, *et al.* Attitudes to Clinical Pig Kidney Xenotransplantation among Medical Providers and Patients. *Kidney360* 2020;**1**:657–62. doi:10.34067/KID.0002082020

35 DeLaura I, Anwar IJ, Ladowski J, *et al.* Attitudes of patients with renal disease on xenotransplantation: A systematic review. *Xenotransplantation* 2023;**30**:e12794. doi:10.1111/xen.12794

36 Mitchell C, Lipps A, Padilla L, *et al.* Meta-analysis of public perception toward xenotransplantation. *Xenotransplantation* 2020;**27**. doi:10.1111/xen.12583

37 Food and Drug Administration. Food and Drug Administration Expanded Access. https://www.fda.gov/news-events/public-health-focus/expanded-access

38 McLean S, Williamson L. The demise of UKXIRA and the regulation of solid-organ xenotransplantation in the UK. *Journal of Medical Ethics* 2007;**33**:373–5. doi:10.1136/jme.2007.020768

39 Nelson J, Alvey N, Bowman L, *et al.* Consensus recommendations for use of maintenance immunosuppression in solid organ transplantation: Endorsed by the American College of Clinical Pharmacy, American Society of Transplantation, and the International Society for Heart and Lung Transplantation. *Pharmacotherapy* 2022;**42**:599–633. doi:10.1002/phar.2716

Figure legends

Figure 1. Proposed advantages of xenotransplantation over human allotransplantation

Figure 2. Barriers to porcine-to-human xenotransplantation

Figure 3. Key advantages and disadvantages of the human decedent model.

Figure 4. Key Outstanding Ethical and Legal Issues

Figure 5. Comparison of immune suppression agents across historical xenotransplant, current allotransplant and future xenotransplant

An update on the ethical, legal, and technical challenges of translating xenotransplantation

Abstract

This manuscript reports on a landmark symposium on the ethical, legal and technical challenges of xenotransplantation in the UK. King's College London, with endorsement from the British Transplant Society (BTS), and the European Society of Organ Transplantation (ESOT), brought together a group of experts in xenotransplantation science, ethics, and law to discuss the ethical, regulatory, and technical challenges surrounding translating xenotransplantation into the clinical setting. The symposium was the first of its kind in the UK for 20 years. This paper summarises the content of the expert lectures showcasing the progress which has been made in xenotransplantation including - the history of xenotransplantation, advances in gene edited animals and progress towards clinical xenotransplantation. We then set out the ethical and legal issues still to be resolved. Finally we report the themes of the roundtable discussion highlighting areas of consensus and controversy. While the detail of the legal discussion was directed towards the UK, the principles and summary reported here are intended to be applicable to any jurisdiction seeking to implement clinical xenotransplantation.

Background

The history of xenotransplantation is peppered with optimistic predictions about its imminence and feasibility. In 1969 the Nobel winning transplant immunologist Peter Medawar hypothesised that-

> "We should solve the problem [of organ transplantation] by using heterografts [xenografts] one day if we try hard enough, and maybe in less than 15 years[1]."

More than 20 years later the Nuffield Council on Bioethics predicted that the first pig to human xenotransplants would be performed in 1996[2]. What followed was almost 30 more years of intense research focused on the remaining immunological and infectious challenges. However, in 2022 xenotransplantation was thrust back into mainstream transplant discourse following two significant developments. First were the reports of two genetically-engineered (GE) pig kidneys transplanted into deceased human recipients, mechanically maintained after death by neurologic criteria (decedents)[3,4]. Secondly was the landmark GE porcine-to-living-human heart transplant conducted in Maryland [5–7]. This resurgence of activity, in the shadow of the COVID pandemic, comes at a time when public and professional anxieties about zoonosis, public health, and experimental treatments are all running high.

It was on this background that in late 2022 King's College London, with endorsement from the British Transplant Society (BTS), and the European Society of Organ Transplantation (ESOT), brought together a group of experts in xenotransplantation science, ethics, and law. This symposium, the first of it's kind in the UK for 20 years, aimed to discuss the ethical, regulatory, and technical challenges surrounding translating xenotransplantation into the clinical setting. Firstly, a series of three lectures showcased the progress which has been made in xenotransplantation including- the history of xenotransplantation, advances in gene edited animals and preclinical research activities. Following these were sessions introducing ethical and legal concerns. Finally, a roundtable discussion was held. This paper follows this structure firstly summarising the content of the expert lectures. Then we report on the broad themes identified in the ethical and legal sessions and the third and

final section reflects on the roundtable discussion, highlighting areas of consensus and controversy. While the detail of the legal discussion was directed towards the UK, the principles and summary reported here are intended to be applicable to any jurisdiction seeking to implement clinical xenotransplantation.

Part 1: Expert presentations - progress in xenotransplantation

A history of xenotransplantation

Dr David Cooper, a heart transplant surgeon and xenotransplantation research pioneer who has more than 50 years' experience in the field, opened the symposium with a lecture on the history of xenotransplantation. He noted that the idea of blending humans and animals can be seen as far back as ancient Greek, Hindu, and Mesopotamian iconography but that the first sustained attempts at a medical application of xenotransplantation was the use of non-human animal-to-human blood transfusions in the 17th century. Other unsuccessful historical uses of animal tissues through the 18th, 19th and 20th centuries have included skin grafts, corneal grafts and, in the 1920s, the transplantation of slices of baboon's or chimpanzee testicles into elderly men to improve their 'vitality'[1]. To the eyes of modern scientists these experiments may have been doomed to failure, but animal studies have provided significant contributions to the development of transplantation techniques, for example, the work of Alexis Carrel who performed the first vascular anastomosis[1]. It was surgical techniques such as these, first practiced on animals, which allowed the first human allotransplants to occur in the 1950s[8].

By the 1960s while both dialysis and human allotransplantation had been successfully demonstrated, neither was readily available to kidney failure patients due to a lack of donors and dialysis facilities. Xenotransplantation represented a proposed solution (Figure 1). To this end Keith Reemtsma transplanted chimpanzee kidneys into humans between 1963 and 1964 using

azathioprine and prednisolone as immune suppression[9]. One patient survived for 9 months but the remainder succumbed either to rejection or infections within weeks or short months. In 1964 James Hardy performed the first ever heart transplant using a chimpanzee's heart[10]. However, this too was unsuccessful with the recipient swiftly rejecting the organ and dying after just a few hours. Then in 1983 there was the widely publicised case of baby Fae who, at a time when no infant heart transplants were being performed, received a baboon's heart. While the reporting of her case did successfully raise the profile of the issue of the shortage of paediatric organ donors, she died just 20 days later[11].

By the mid 1980's it was becoming accepted that non-human primates (NHP) were not the ideal source of donor organs they were once imagined to be. Attention moved to pigs with the first transgenic pig created in 1992[1]. Three decades of subsequent research culminated in January 2022 with the first GE porcine-to-human heart transplant performed at the University of Maryland under the Food and Drug Administration's (FDA) compassionate use scheme. The recipient- David Bennett- was a man in his 50s who was critically unwell, requiring ECMO, and was only anticipated to live a few weeks. The organ was immediately life sustaining despite significant operative complications in the form of an aortic dissection. David lived for 60 days post-xenotransplant [5–7].

Engineering Pigs for Xenotransplantation Products

Implanting unmodified porcine organs results in hyperacute rejection in both non-human primates and humans. Dr David Ayares presented his lifetime's work researching overcoming this critical barrier. The primary porcine xeno-antibody is targeted against alpha-1, 3-galactose (GTA), a carbohydrate antigenic structure which is absent in both humans and NHPs[1]. In 2003 Dr Ayares' team created the first GTA knockout pigs with an inactive alpha gal transferase gene[12]. These single knockout 'GalSafe' pigs have been subjected to multiple safety studies and have been cleared by the FDA for use in decellularized products such as heart valves and for human consumption for

those with red meat allergy[13,14]. In addition to the GTA knockout modification, through selective breeding a line of pigs has been established that is of low risk for porcine endogenous retrovirus (PERV) activation. However, Dr Ayares contends that bioengineering will need to be more sophisticated to achieve successful xenotransplantation (Figure 2). A discussion about the application of bioengineering in the field was beyond the scope of the symposium, but several recent reviews have documented progress in this area [15–18].

To overcome these barriers, Dr Ayares' team have developed a line of pigs with multiple additional genetic modifications which both knock out porcine genes and insert human genes. These modifications include knocking out additional genes encoding important antigenic targets to make a 'triple knock out' (TKO) pig, adding human complement-regulatory genes aimed at reducing hyperacute rejection, and inserting human immune suppressor genes. Furthermore, to address the coagulation dysregulation seen in xenotransplant recipients, human anti-coagulant genes have been inserted. Finally, pig growth hormone receptor genes have been knocked out to prevent growth beyond that which will provide organs of a size suitable for human use. In total, the team have developed a line in which 10 genes have been edited - 4 knock out porcine genes and 6 inserted human genes[19]. Dr Ayares's team is currently undertaking preclinical trials and hopes to enter clinical trials from 2024.

Preclinical research in Xenotransplantation

Professor Jayme Locke presented some of the pre-clinical research in xenotransplantation which has been conducted by the team at the University of Alabama at Birmingham.

Non-Human Primate Pre-Clinical Xenotransplantation Research

Pre-clinical xenotransplantation research relies on the use of non-human animal models. By selectively transplanting genetically modified porcine organs into NHPs they hope to address some

key safety concerns before moving to clinical trials. For instance, the impact of hormonal and electrolyte handling differences between porcine and human nephrons, establishing expected urine output and clotting or coagulation complications. To this end, Professor Locke reported that they have successfully shown that porcine kidneys can replicate physiological functions such as urine output, creatinine clearance and electrolyte homeostasis [20–22]. Throughout their studies on NHP the team have not noted any major haematological abnormalities when using the 10 gene edited porcine organs and through careful measurements, they aim to establish the weight and age of a pig needed to yield an organ the same size as a typical adult human kidney.

However, their studies have also served to delineate critical limitations of utilizing NHPs as a model for use in humans- these pigs have been augmented to evade the *human* but not the NHP immune responses. Professor Locke cited studies which have shown that sequential modifications to the porcine genome result in significant reductions in antibody binding from human plasma[23]. However in NHPs, while reactivity against single, double or triple knock out pigs reduces with each modification *all* still have a positive cross match[24]. This means that *all* pig-to-NHP transplants are undertaken against a positive crossmatch at high immunological risk of rejection. In addition, tacrolimus-based regimens, which have been so successful in improving outcomes of human allotransplantation, have proved ineffective in NHP models[20]. Research has shown that CD40/CD154 co-stimulation pathway blockade provides better results, and a novel anti CD40 agent was used in the 2022 porcine heart transplant[7] however it is yet to be determined if this will enable clinical xenotransplantation. In light of these immunological issues, NHP studies typically require more aggressive immunosuppressive regimens than those used as standard in human allotransplantation. Professor Locke argued that this has led to concerns that the intensity of immunosuppression needed may result in higher infectious complications and diminish the ability of NHP studies to prove long term organ function.

Human Decedent Pre-Clinical Xenotransplantation Research – the "Parsons Model"

In light of the limitations inherent with NHP research, some in the field argue that we have reached as far as we can with animal models, and that to progress further it is essential to move to human recipients. Professor Locke also presented one proposed solution to this difficulty – the human decedent or 'Parsons Model' – which utilises brain dead humans as initial research participants. In this way, Professor Locke argued, that as well as addressing any remaining safety concerns which cannot be resolved in the NHP model, the 'Parsons Model' offers the opportunity for key technology to be tested (Figure 3). She emphasised that for xenotransplantation to be a credible alternative to conventional allotransplantation it will be essential that xenotransplantation protocols adhere as closely as possible to the existing standards of care. For example, cross matching, interpreting of biopsy findings and infectious disease testing will all need to be re-validated in a xenotransplant setting.

Professor Locke presented the findings of their two human decedent studies. Institutional research ethics boards are traditionally tasked with overseeing research conducted only on living human subjects[25], however Professor Locke emphasised that ethics oversight and authorisation was obtained prior to these studies being undertaken. Consent was also gained from the families of the deceased[3]. The immediate results included that the decedents were hemodynamically stable through reperfusion and no hyperacute was rejection observed. The decedents were maintained until the study was terminated after 3 days. In this time the kidneys appeared viable, produced urine, and no transmission of PERV was detected. However, in the first decedent a thrombotic microangiopathy of unknown cause was noted on biopsy on day one, and this led to the administration of eculizumab (C5 complement inhibitor, anti-C5 antibody) during the second study. In neither study was creatinine clearance restored[3]. A detailed summary of the study involving the first recipient has been published[3].

Part 2: Ethical and legal issues in translating xenotransplantation

Professor Michael Gusmano and Professor Brendan Parent are two leading ethicists in xenotransplantation in the USA[26–29]. Their presentations reported on work currently being undertaken by their respective Universities on the ethical and legal issues around xenotransplantation. The following commentary summarises the central themes emerging from their work (Figure 4), and the discussion which followed their thought provoking lectures.

Regulation

Transplantation is one of the most highly scrutinised areas of medicine. Protecting the rights of donors and recipients, ensuring the quality and safety of organs used for transplantation and ensuring the equitable and effective allocation of scarce resources are all vital roles. Professor Parent observed that in the USA, the mechanisms for transplant oversight apply only to human organs. In the UK, as in most European jurisdictions, there is no legislation in place which prohibits xenotransplantation. However there is also no framework which sanctions and regulates its practice in a form comparable to human allotransplantation. There is no universal form of legal instrument to achieve this, but lessons could be drawn from the implementation of other transplant practices that have been contentious during their evolution - such as non-directed living altruistic donation, directed deceased donation or kidney sharing schemes. In addition, guidelines issued by regulators require regularly updating to ensure that they are compatible with the latest scientific and social developments. Professor Gusmano highlighted that those currently in use in the USA contain ambiguous language such as “sufficient” or “adequate” to describe the necessary pre-clinical safety and efficacy data to move into clinical trials.

Recipient selection

While advances in the last 50 years have made definitive progress on resolving issues of xeno-organ compatibility, there remains little consensus on which patients should be included in the first living

human clinical xenotransplant trials. On the one hand you have those who argue that patients who are already too ill to recover from their underlying illness should be among the first enrolled. However, such patients are not necessarily the optimal choice when trying to assess the therapeutic potential of porcine organs. Studying transplants (regardless of organ source) in such recipients is unlikely to provide reliable answers to key research or safety questions and their inclusion could therefore compromise the integrity of research conclusions. Furthermore, while these patients are sometimes framed as having 'nothing to lose' they may also have little to gain personally from a xenotransplant. For these patients, some of those present argued that palliation is an existing alternative to xenotransplantation which can sometimes be overlooked in this discussion.

Target organ system

There is also a debate about which organ system provides the best model for initial trials. Kidney transplantation has by some measure the largest waiting list. This therefore presents the opportunity to benefit the greatest number of people should trials prove successful. An alternative view could be that initial work is better focused on paediatric heart transplantation because many paediatric patients are not even wait-listed due to the lack of available human donor organs. Much like the participants in early xenotransplant experiments, paediatric cardiac patients, without an option for a human allotransplant continue to have extremely limited available treatment options.

Eligibility for human allotransplant

The question which logically follows from "which patients and which organs should first be included" is, whether and if so to what extent, eligibility for human allotransplant should determine eligibility for xenotransplant? In this case it can again be argued either that xenotransplant research participants should have been deemed not to be eligible for human allotransplant so that they are not forgoing an established lifesaving treatment for an experimental one. Or the counter position, which is that patients *must* be suitable for an allotransplant, because if we do not anticipate an allotransplant to succeed why should we expect a xenotransplant to?

Risks and benefits including xenozoonotic disease transmission

While the potential benefits of xenotransplant are self-evident – continued life, freedom from dialysis, reduction in organ waiting lists– there are also significant risks. Some of these risks are established facts, for example, the operative risks of any transplant procedure. Other risks are unknown, and some may not yet have been conceived (unknown unknowns). The most challenging issue perhaps is risks which are theoretically known but the likelihood and magnitude of the risk is not fully understood. This category of risk includes – rejection of the organ, sensitisation to future human allotransplantation, and zoonotic infection.

Zoonosis is also important in considering the balance between private benefits and public risk. In this case the argument is that the benefit of xenotransplantation is principally to those individuals whose lives are saved, but the risks taken are those which, should they be realised, could be at the expense of the wider public if a virus of zoonotic origin were transmittable to the recipient's contacts and beyond. Against this is the argument that by not pursuing xenotransplantation we must weigh the risk of an ever-increasing waiting list with the attendant suffering and harm to those waiting and their communities. Exemplifying the dynamic nature of risk and its perception Professor Gusmano noted that in attitudes surveys on xenotransplantation a frequently encountered response is "I wouldn't mind being, you know, the 50th person to receive this. I wouldn't want to be the 1st".

Informed consent

Uncertainty of the benefits and risks of clinical xenotransplantation will present difficulties for ethics committees determining the permissibility of trials, but it will also make it challenging to satisfy the 'information' criterion of 'informed consent'. For example, xenotransplant is sometimes discussed as a possible 'bridge' to an allotransplant. However, we currently do not understand how or if a xenotransplant would sensitise a recipient to future allotransplant[30] which would undoubtedly be considered material to a recipient's decision making. Furthermore, patients without viable curative

treatment alternatives or who are critically unwell may be in a state of vulnerability which could compromise their ability to give free and informed consent.

Equitable subject selection

It is recognised that under the current human allotransplant system some groups experience disadvantage. For example, Black patients wait significantly longer than their white counterparts for an organ for transplantation. This might lead some to argue that one aim of a xenotransplantation programme should be to reduce this inequity. However, the egregious history of exploitation of Black participants in clinical trials highlights the importance that the inclusion of disadvantaged or 'vulnerable' groups is carefully and sensitively undertaken. Empirical research has suggested that non-white respondents are six-times less likely to consider a xenotransplant[31].

Religious and Cultural beliefs

Reluctance on the part of recipients to accept a xenotransplant may not just relate to suspicions regarding the intentions of researchers. Religious and cultural beliefs will undoubtedly play a role in an individual's assessment as to if certain animals' organs are viewed as acceptable [31–36]. The current narrow focus on porcine organs may be perceived as commercial pragmatism by some but as discriminatory by those who object to the use of pigs in medical products for cultural or religious reasons.

Compassionate use

The expanded access or 'compassionate use' scheme which facilitated David Bennett's heart transplant is designed to enable access to investigational treatments or products when the patient is critically ill and there is "no comparable or satisfactory alternative therapy options[37]". Although drugs and treatments available through this scheme may be undergoing clinical trials, the terms under which they are offered are quite different. There are not strict guidelines regarding the design of the intervention as there would be in a clinical trial, nor is there an obligation to publish findings.

Regardless, compassionate use xenotransplants should be subject to the same safety standards and monitoring for zoonotic infection as for those performed under the auspices of a clinical trial and, data generated should be published and shared.

Alternatives to xenotransplantation and non-human animal welfare

Finally, concerns have been raised about the compatibility of xenotransplantation with established agreements on the reduction, refinement, and replacement of non-human animals in scientific research. Professor Parent finished his lecture with a challenge for all involved in this field to consider if there are alternatives to xenotransplantation which might in the long term be immunologically preferable *and* more sustainable. Specifically, if bioengineered organs are equally likely to provide an unlimited supply of organs for transplantation but without harm to sentient and cognitively complex non-human animals or the environmental impact of continued reliance on animal products.

Part 3: Round table discussion – consensus and controversy

Several of this symposiums' speakers contended that the sequential progress made from laboratory, NHP and human decedent research, combined with the 'diminishing returns' from NHP studies, mean that clinical trials are now indicated. These lectures provided a platform for a thought-provoking discussion between the experts in attendance, during which, some agreement was formed on the practical next steps needed, and identified key priorities for resolution.

Establishing a regulator

Establishing an appropriate regulator is a key priority. In the 1990s the UK was at the forefront of xenotransplantation research and regulation. The formation of the United Kingdom Xenotransplantation Interim Regulatory Authority (UKXIRA) in 1997 gave the UK one of the most robust and forward-thinking regulatory environments in the world. However, when scientific progress failed to materialise, the UKXIRA was disbanded in 2006 and there is currently no permanent regulatory structure for xenotransplantation in place[38]. Nevertheless, the UKXIRA's short tenure demonstrated the sorts of qualities that a good regulator should possess, including multidisciplinary representation - clinical, scientific, ethical, legal, religious, and animal welfare expertise are all required. A regulatory body should also be capable of undertaking the necessary public consultation. Crucially, links with government departments and statutory bodies overseeing agriculture, animal welfare, health, and organ transplantation will be required for the regulatory body to be effective. For example, in the UK human allotransplantation is regulated by the Human Tissue Authority (HTA) which at present has no remit to oversee xenotransplantation activities.

Practical requirements: infrastructure and cost

As the experience of the US sites conducting xenotransplantation research shows, the infrastructure needed to facilitate xenotransplantation is significant. In addition to identifying a source of genetically engineered pigs, a bio-secure (referred to as 'pathogen free' in US federal regulations)

facility capable of housing, testing and euthanising these animals, at a cost of tens of millions of pounds is required. To ensure security of supply, two such facilities are preferred to one. As Professor McGregor said, “these are essential components, and they are all expensive”.

Determining an Immune Suppression Regimen

An optimal immune suppression regimen avoids both over immunosuppression, with subsequent infectious complications and under immunosuppression, which precipitates rejection. There is currently significant worldwide variation on immunosuppressive regimens used in human allograft transplantation although there is more consensus with regards to maintenance therapy than induction[39]. When considering xenotransplantation all that is certain is that we have not yet reached a consensus regarding what an optimal regimen will include. However at least some of the agents used in the human decedent model or which are proposed for use in future xenotransplantation studies use are likely to be familiar to transplant physicians and their patients (Figure 5).

Zoonoses

In human allotransplantation infectious complications are a significant driver of mortality and morbidity. In xenotransplantation the usual human pathogens will need to be considered, but in addition zoonotic infections. However, the likelihood of transmission, the effect of this on the recipient and the likelihood and threat of wider contagion to their close contacts or the wider public are all unknown. For example, cytomegalovirus (CMV) is routinely tested for in human donors and allograft recipients. Porcine CMV has therefore been flagged as a key concern in xenotransplant. The donor animal for the xeno-heart transplant tested negative on several occasions for porcine CMV prior to implantation, however Mr Bennett subsequently tested positive[7]. There is ongoing research into any relevance this may have had to his clinical course and this finding has stimulated the development of more sensitive testing methods. This case exemplifies the need not just for

rigorous testing regimes but also for reliable and robust methods which are able to respond to new risks as they emerge.

Additional ethical considerations

In addition to the major issues identified by Professor Gusmano and Professor Parent outlined in the previous section, during the roundtable discussion several additional ethical issues were proposed by our panel of experts and attendees. These included – ensuring equity of access to successful treatments, the burden of xenotransplantation monitoring requirements on patients, their close contacts and clinical personnel, and the need for cross border agreements to cover the prospect of recipients receiving initial treatment in one jurisdiction but moving to another. The breadth of issues raised during this discussion were too wide ranging to be adequately covered in a single event and show that the legal and ethical discourse on xenotransplantation may yet uncover new challenges that will need to be addressed prior to the start of clinical trials.

Moving forward – Closing Comments

This symposium combined a state-of-the-art science update with a wide-ranging discussion on the ethical regulatory and technical challenges to implementing clinical xenotransplantation. There was consensus among the speakers that xenotransplantation, now more so than in any of the previous decades, is an imminent possibility. It was beyond the remit of this symposium to generate and ratify formal recommendations on how the UK should proceed however a few key messages could be taken away. A regulatory framework and the appropriate technical resources will need to be established to facilitate a clinical xenotransplantation programme. Moreover, in the wake of COVID, in an ever more connected world of international travel, an *international* monitoring and regulatory framework is essential. There remain significant legal and ethical questions regarding recipient

selection, risk benefit analysis and the allocation of resources, which will require detailed discussion.

Above all up-to-date public consultation is essential to finding acceptable resolutions.

References

1 Cooper DKC. A Brief History of Cross-Species Organ Transplantation. *Baylor University Medical Center Proceedings* 2012;**25**:49–57. doi:10.1080/08998280.2012.11928783

2 Nairne P, Nuffield Council on Bioethics, editors. *Animal-to-human transplants: the ethics of xenotransplantation*. London: : Nuffield Council on Bioethics 1996.

3 Porrett PM, Orandi BJ, Kumar V, *et al.* First clinical-grade porcine kidney xenotransplant using a human decedent model. *American Journal of Transplantation* 2022;**22**:1037–53. doi:10.1111/ajt.16930

4 Montgomery RA, Stern JM, Lonze BE, *et al.* Results of Two Cases of Pig-to-Human Kidney Xenotransplantation. *N Engl J Med* 2022;**386**:1889–98. doi:10.1056/NEJMoa2120238

5 Reardon S. First pig-to-human heart transplant: what can scientists learn? *Nature* 2022;**601**:305–6. doi:10.1038/d41586-022-00111-9

6 Wang W, He W, Ruan Y, *et al.* First pig-to-human heart transplantation. *The Innovation* 2022;**3**:100223. doi:10.1016/j.xinn.2022.100223

7 Griffith BP, Goerlich CE, Singh AK, *et al.* Genetically Modified Porcine-to-Human Cardiac Xenotransplantation. *N Engl J Med* 2022;**387**:35–44. doi:10.1056/NEJMoa2201422

8 Merrill JP, Murray JE, Harrison JH, *et al.* Successful homotransplantation of the human kidney between identical twins. *J Am Med Assoc* 1956;**160**:277–82. doi:10.1001/jama.1956.02960390027008

9 Reemtsma K, Mccracken BH, Schlegel JU, *et al.* Renal Heterotransplantation in Man. *Ann Surg* 1964;**160**:384–410. doi:10.1097/00000658-196409000-00006

10 Hardy JD, Kurrus FD, Chavez CM, *et al.* Heart Transplantation in Man. Developmental Studies and Report of a Case. *JAMA* 1964;**188**:1132–40.

11 Bailey LL, Nehlsen-Cannarella SL, Concepcion W, *et al.* Baboon-to-human cardiac xenotransplantation in a neonate. *JAMA* 1985;**254**:3321–9.

12 Phelps CJ, Koike C, Vaught TD, *et al.* Production of α1,3-Galactosyltransferase-Deficient Pigs. *Science* 2003;**299**:411–4. doi:10.1126/science.1078942

13 Food and Drug Administration. Freedom of Information Summary - Origional New animal drug application. 2020.https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadFoi/10168

14 Food and Drug Administration. FDA Approves First-of-its-Kind Intentional Genomic Alteration in Line of Domestic Pigs for Both Human Food, Potential Therapeutic Uses. https://www.fda.gov/news-events/press-announcements/fda-approves-first-its-kind-intentional-genomic-alteration-line-domestic-pigs-both-human-food

15 Lei T, Chen L, Wang K, *et al.* Genetic engineering of pigs for xenotransplantation to overcome immune rejection and physiological incompatibilities: The first clinical steps. *Front Immunol* 2022;**13**:1031185. doi:10.3389/fimmu.2022.1031185

16 Cooper DKC, Raza SS, Chaban R, *et al.* Shooting for the moon: Genome editing for pig heart xenotransplantation. *J Thorac Cardiovasc Surg* 2023;**166**:973–80. doi:10.1016/j.jtcvs.2022.04.032

17 Chan JCY, Chaban R, Chang SH, *et al.* Future of Lung Transplantation: Xenotransplantation and Bioengineering Lungs. *Clin Chest Med* 2023;**44**:201–14. doi:10.1016/j.ccm.2022.11.003

18 Ibi Y, Nishinakamura R. Kidney Bioengineering for Transplantation. *Transplantation* 2023;**107**:1883–94. doi:10.1097/TP.0000000000004526

19 Mohiuddin MM, Goerlich CE, Singh AK, *et al.* Progressive genetic modifications of porcine cardiac xenografts extend survival to 9 months. *Xenotransplantation* 2022;**29**. doi:10.1111/xen.12744

20 Cooper DKC, Hara H, Iwase H, *et al.* Pig kidney xenotransplantation: Progress toward clinical trials. *Clin Transplant* 2021;**35**. doi:10.1111/ctr.14139

21 Hansen-Estruch C, Bikhet MH, Javed M, *et al.* Renin-angiotensin-aldosterone system function in the pig-to-baboon kidney xenotransplantation model. *American Journal of Transplantation* 2023;**23**:353–65. doi:10.1016/j.ajt.2022.11.022

22 Hansen-Estruch C, Bikhet MH, Shaik IH, *et al.* Assessment of glomerular filtration and tubular secretion in baboons with life-supporting pig kidney grafts. *Xenotransplantation* 2023;**30**:e12795. doi:10.1111/xen.12795

23 Martens GR, Reyes LM, Butler JR, *et al.* Humoral Reactivity of Renal Transplant-Waitlisted Patients to Cells From GGTA1/CMAH/B4GalNT2, and SLA Class I Knockout Pigs. *Transplantation* 2017;**101**:e86–92. doi:10.1097/TP.0000000000001646

24 Adams AB, Kim SC, Martens GR, *et al.* Xenoantigen Deletion and Chemical Immunosuppression Can Prolong Renal Xenograft Survival. *Annals of Surgery* 2018;**268**:564–73. doi:10.1097/SLA.0000000000002977

25 Parent B, Gelb B, Latham S, *et al.* The ethics of testing and research of manufactured organs on brain-dead/recently deceased subjects. *J Med Ethics* 2020;**46**:199–204. doi:10.1136/medethics-2019-105674

26 Parent B. Research Involving the Newly Deceased Following Death by Neurologic Criteria: Ethical Justification and Guidelines. *Transplantation* 2022;**106**:2275–7. doi:10.1097/TP.0000000000004141

27 Caplan A, Parent B. Ethics and the emerging use of pig organs for xenotransplantation. *The Journal of Heart and Lung Transplantation* 2022;**41**:1204–6. doi:10.1016/j.healun.2022.06.008

28 Gusmano MK. Xenotransplantation Clinical Trials and the Need for Community Engagement. *Hastings Center Report* 2022;**52**:42–3. doi:10.1002/hast.1420

29 Maschke, Karen, Gordon, Elisa, Gusmano MK. Opinion: After the pig-to-human heart transplant, the FDA, clinicians and insurers have some catching up to do. *The Washington Post*

2022.https://www.washingtonpost.com/opinions/2022/01/13/what-comes-after-pig-to-human-transplant-breakthrough/

30 Cooper DKC, Habibabady Z, Kinoshita K, *et al.* The respective relevance of sensitization to alloantigens and xenoantigens in pig organ xenotransplantation. *Human Immunology* 2023;**84**:18–26. doi:10.1016/j.humimm.2022.06.003

31 Padilla LA, Hurst DJ, Jang K, *et al.* Racial differences in attitudes to clinical pig organ Xenotransplantation. *Xenotransplantation* 2021;**28**. doi:10.1111/xen.12656

32 Hurst DJ, Padilla LA, Cooper DK, *et al.* The attitudes of religious group leaders towards xenotransplantation: A focus group study. *Xenotransplantation* 2022;**29**. doi:10.1111/xen.12777

33 Padilla LA, Rhodes L, Sorabella RA, *et al.* Attitudes toward xenotransplantation: A survey of parents and pediatric cardiac providers. *Pediatr Transplant* 2021;**25**. doi:10.1111/petr.13851

34 Padilla LA, Hurst D, Lopez R, *et al.* Attitudes to Clinical Pig Kidney Xenotransplantation among Medical Providers and Patients. *Kidney360* 2020;**1**:657–62. doi:10.34067/KID.0002082020

35 DeLaura I, Anwar IJ, Ladowski J, *et al.* Attitudes of patients with renal disease on xenotransplantation: A systematic review. *Xenotransplantation* 2023;**30**:e12794. doi:10.1111/xen.12794

36 Mitchell C, Lipps A, Padilla L, *et al.* Meta-analysis of public perception toward xenotransplantation. *Xenotransplantation* 2020;**27**. doi:10.1111/xen.12583

37 Food and Drug Administration. Food and Drug Administration Expanded Access. https://www.fda.gov/news-events/public-health-focus/expanded-access

38 McLean S, Williamson L. The demise of UKXIRA and the regulation of solid-organ xenotransplantation in the UK. *Journal of Medical Ethics* 2007;**33**:373–5. doi:10.1136/jme.2007.020768

39 Nelson J, Alvey N, Bowman L, *et al.* Consensus recommendations for use of maintenance immunosuppression in solid organ transplantation: Endorsed by the American College of Clinical Pharmacy, American Society of Transplantation, and the International Society for Heart and Lung Transplantation. *Pharmacotherapy* 2022;**42**:599–633. doi:10.1002/phar.2716

Figure legends

Figure 1. Proposed advantages of xenotransplantation over human allotransplantation

Figure 2. Barriers to porcine-to-human xenotransplantation

Figure 3. Key advantages and disadvantages of the human decedent model.

Figure 4. Key Outstanding Ethical and Legal Issues

Figure 5. Comparison of immune suppression agents across historical xenotransplant, current allotransplant and future xenotransplant

Figure 1. Proposed advantages of xenotransplantation over human allotransplantation

- Potentially unlimited supply of donor organs.
- The opportunity to augment the donor to increase compatibility and not just reduce rejection from the recipient.
- Organs can be made available electively and surgery planned accordingly.
- Donors can be confirmed as infection free.
- Possibility to treat many recipients with one donor (particularly if using cells e.g. islet cells)
- Avoids the negative effects of brain death or unplanned circulatory death on donor organs.
- Avoids some of the cultural objections to deceased human donor organ transplantation.
- In borderline candidates for human allotransplantation, it may be considered more acceptable to give recipients a xenotransplant.

Figure 2. Barriers to porcine-to-human xenotransplantation

- Hyperacute rejection
- Longer term humoral rejection
- Coagulation dysregulation
- Organ size mismatch

Figure 3. Key advantages and disadvantages of the human decedent model.

Advantages	Limitations
• No harm to living persons. • Reduced harm to non-human animals • The opportunity to test supporting technology needed for transplantation – crossmatch, biopsies, vascular integrity etc. • Ability to test novel immune suppression regimens or agents in a human prior to use in living humans. • Ability to test for porcine disease transmission. • Ability to test for chimerism and migration of porcine cells to blood stream or distant tissues following transplant.	• Ethical objections to the use of brain-dead (deceased) humans in research. • The physiological instability of brain-dead humans impairing the reliability of studies and interfering with some key observation parameters. • Limited to short term studies. • Lack of a clear regulatory framework (although studies could be conducted under the rubric of Institutional Review Boards as at UAB). • Unresolved ethical questions regarding acceptable duration, roles of supporting clinical staff, appropriate venue for research, etc.

Figure 4. Key Outstanding Ethical and Legal Issues

Ethical	**Legal**
Informed consent	
Target organ system and recipient selection Eligibility for human allotransplant Equitable subject selection Risk and benefits - including xenozoonotic disease transmission Religious and cultural beliefs Alternatives to xenotransplantation and nonhuman animal welfare	Structure, type and scope of legal oversight Designation of a regulatory body Liability Monitoring and surveillance requirements

Figure 5. Comparison of immune suppression agents across historical xenotransplant, current allotransplant and future xenotransplant

Historical Xenotransplant	Current Human Allotransplantation	Agents proposed for use in future Xenotransplantation studies
Azathioprine Irradiation Steroids	Induction may include – Steroid (Methylprednisolone) + Interleukin-2 receptor blocker or Anti Thymocyte Globulin or Anti CD52	Induction may include – Steroid (Methylprednisolone) + Anti Thymocyte Globulin +/- Anti-CD20 monoclonal antibody, +/- Compliment inhibition +/- (C5 esterase and / or C1 esterase agent)
	Maintenance may include – Anti-metabolite Calcineurin Inhibitor mTOR Inhibitors Stimulation Blockade Steroids +/-	Maintenance may include – Anti-metabolite Calcineurin Inhibitor mTOR Inhibitors Novel anti-CD40/CD154 blockade Steroids