Transplant International

POINT OF VIEW

Organ transplants of the future: planning for innovations including xenotransplantation

Emanuele Cozzi[1], Stefan Schneeberger[2], Maria Irene Bellini[3,4], Erik Berglund[5], Georg Böhmig[6], Kevin Fowler[7], Martin Hoogduijn[8], Ina Jochmans[9], Georg Marckmann[10], Lorna Marson[11], James Neuberger[12], Rainer Oberbauer[13], Richard N. Pierson III[14], Bruno Reichart[15], Linda Scobie[16], Colin White[17] & Maarten Naesens[18], for ESOT Workstream 1 of the TLJ (Transplantation Learning Journey) Project

1 Department of Cardiac, Thoracic and Vascular Sciences and Public Health, Transplant Immunology Unit, Padua University Hospital, Padua, Italy
2 Department of Visceral, Transplant and Thoracic Surgery, Innsbruck Medical University, Innsbruck, Austria
3 Department of Surgical Sciences, Sapienza University of Rome, Rome, Italy
4 Department of Emergency Medicine and Surgery, Azienda Ospedaliera San Camillo Forlanini, Rome, Italy
5 Department of Clinical Science, Intervention and Technology (CLINTEC), Division of Transplantation Surgery, Karolinska Institute and ITB-MED, Stockholm, Sweden
6 Division of Nephrology and Dialysis, Medical University Vienna, Vienna, Austria
7 The Voice of the Patient, Inc., Chicago, IL, USA
8 Department of Internal Medicine, Erasmus MC Transplant Institute, University Medical Center Rotterdam, Rotterdam, The Netherlands
9 Transplantation Research Group, Department of Microbiology, Immunology and Transplantation, KU Leuven, Leuven, Belgium
10 Institute of Ethics, History and Theory of Medicine, Ludwig-Maximilians-University Munich, Munich, Germany
11 The Edinburgh Transplant Centre, Royal Infirmary of Edinburgh, Edinburgh, UK

SUMMARY

The future clinical application of animal-to-human transplantation (xenotransplantation) is of importance to society as a whole. Favourable preclinical data relevant to cell, tissue and solid organ xenotransplants have been obtained from many animal models utilizing genetic engineering and protocols of pathogen-free husbandry. Findings have reached a tipping point, and xenotransplantation of solid organs is approaching clinical evaluation, the process of which now requires close deliberation. Such discussions include considering when there is sufficient evidence from preclinical animal studies to start first-in-human xenotransplantation trials. The present article is based on evidence and opinions formulated by members of the European Society for Organ Transplantation who are involved in the Transplantation Learning Journey project. The article includes a brief overview of preclinical concepts and biology of solid organ xenotransplantation, discusses the selection of candidates for first-in-human studies and considers requirements for study design and conduct. In addition, the paper emphasizes the need for a regulatory framework for xenotransplantation of solid organs and the essential requirement for input from public and patient stakeholders.

Transplant International 2021; 34: 2006–2018

Key words
clinical study design, innovation, patient centred, organ shortage, endpoints, regulation

Received: 30 June 2021; Revision requested: 10 August 2021; Accepted: 24 August 2021;
Published online: 6 October 2021

doi:10.1111/tri.14031

12 Liver Unit, Queen Elizabeth Hospital, Birmingham, UK
13 Medical University of Vienna, Vienna, Austria
14 Center for Transplantation Sciences, Department of Surgery, Massachusetts General Hospital, Boston, MA, USA
15 Walter Brendel Center for Experimental Medicine, LMU Munich, Munich, Germany
16 Department of Biological and Biomedical Sciences, Glasgow Caledonian University, Glasgow, UK
17 Irish Kidney Association, Dublin, Ireland
18 Department of Microbiology, Immunology and Transplantation, KU Leuven, Leuven, Belgium

Correspondence
Maarten Naesens, Nephrology and Renal Transplantation Research Group, Department of Microbiology, Immunology and Transplantation, KU Leuven, Herestraat 49, 3000 Leuven, Belgium.
Tel.: + 3216344580;
fax: +3216344599;
e-mail: maarten.naesens@uzleuven.be

Introduction

Despite initiatives to improve access to transplantation, the worldwide demand for donor organs by far exceeds the current supply [1]. Without new approaches to transplantation, it is likely that a growing number of patients will continue to wait a very long time before receiving an organ, will remain on dialysis if they experience kidney failure or will die while waiting for a first or repeat solid organ transplant. Despite widespread use of expanded-criteria donors, donations after circulatory death, virus-positive donors and recipients, acceptable blood-group incompatibilities and desensitization protocols, transplant candidates – many of whom are broadly sensitized – accumulate on waiting lists, and the numbers of people listed (or delisted) have changed little over many years (Figs. 1 and 2) [2,3]. This is not only because of lack of supply: many candidate organs are discarded at evaluation, often because of impaired quality [2]. Although a key goal of national presumed consent schemes has been to increase the number of allografts, such strategies cannot fulfil the demand for organs [4]. Unfortunately, opt-in schemes may also have had unintended consequences, including a reduction in live donors and little improvement in public awareness of organ donation [5]. In addition, the COVID-19 pandemic has substantially disrupted transplantation programmes and lengthened waiting lists in many countries [6–8].

Undoubtedly, several innovations in technology and service delivery will be required if outcomes are to improve for patients with advanced-stage organ failure, including the development of artificial organs [9,10]; some relatively new processes are already in clinical use. For example, machine reperfusion is optimizing organ preservation, viability assessment and reconditioning [11–15]. Innovations in preclinical development have the potential to expand the donor-organ pool, improve graft function or increase the range of patients who are suitable for transplantation. Emerging technologies include recellularization of poorly functioning organs (for which proof of concept has been demonstrated [16]) and tissue generation, using pluripotent stem cells to create organoids with the structure (and, ultimately, we hope, the function) of solid organs, suitable for replacement [17,18]. These technologies, however, lie outside the scope of this article.

The present article discusses xenotransplantation, with a focus on heart and kidney xenotransplantation, both of which have a large evidence base in preclinical settings. We believe that the conceptual considerations, regulatory framework, clinical trial design and societal implications evident with xenotransplantation also apply to other technologies approaching clinical application in transplantation medicine [19–21]. The content is based on evidence and opinions formulated by participants who have discussed xenotransplantation as a workstream within the Transplantation Learning Journey (TLJ) project, which is an initiative from the European Society for Organ Transplantation (ESOT) [see Box 1]. ESOT initiatives evaluate innovations in transplantation research in preparation for their journey through preclinical, clinical and regulatory review. As TLJ and the workstream on xenotransplantation are ongoing projects, this article aims to stimulate further discussions and contributions from any interested stakeholders. There are many factors to evaluate, including (but not limited to) how clinical development might be structured to address the most informative questions, and investigate endpoints using the most appropriate cohorts for solid organ xenotransplantation. It is essential to involve patients and the general public in discussions about organ transplants of the future.

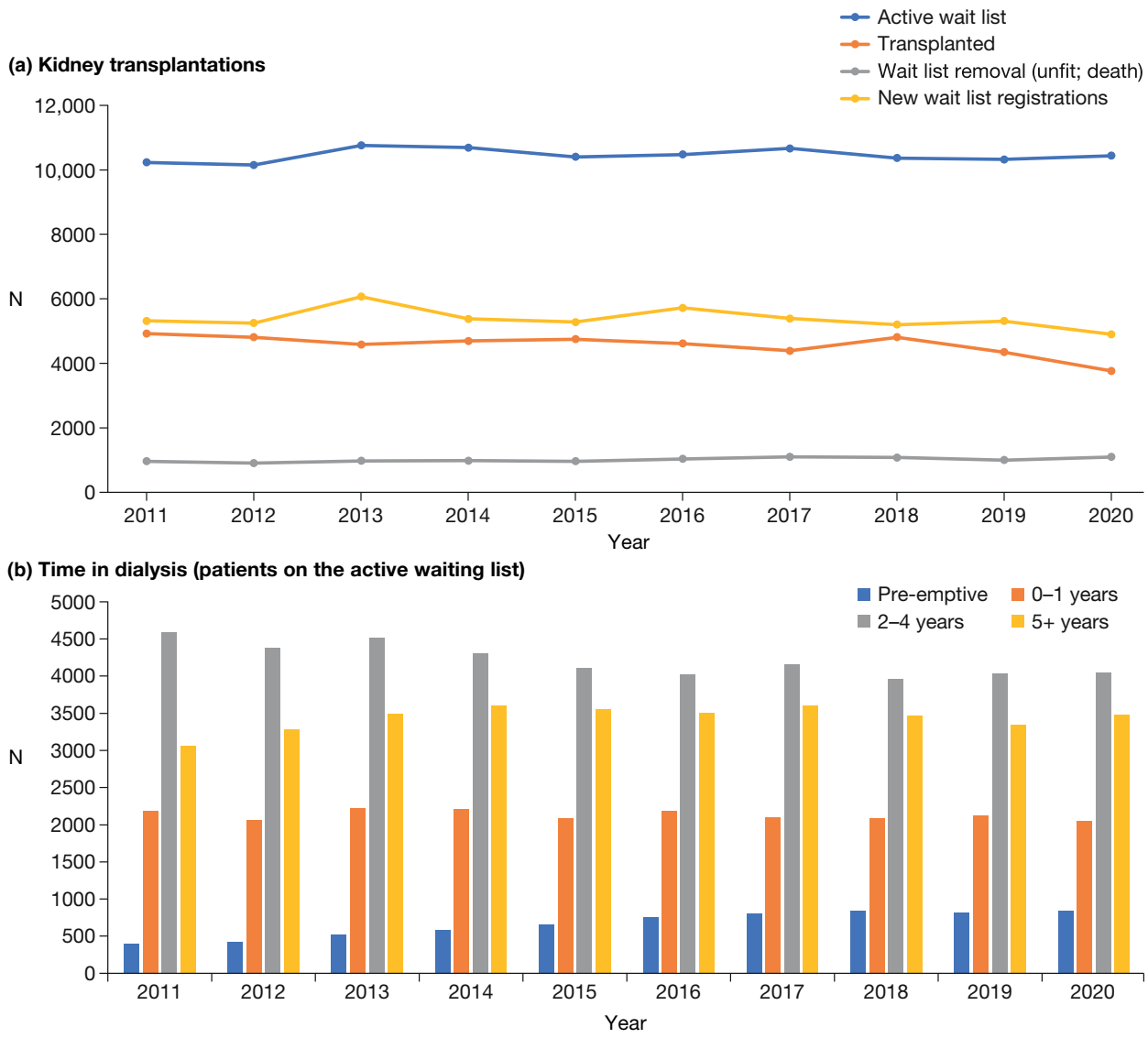


Figure 1 Eurotransplant data (2011–2020) for kidney transplantation [3]: (a) changes in waiting list, (b) active waiting list.

Xenotransplantation: history and current state of development

Xenotransplantation is defined as 'any procedure that involves the transplantation, implantation or infusion into a human recipient of live cells, tissues or organs from an animal source. It also includes human body fluids, cells, tissues or organs that have had *ex vivo* contact with live animal cells, tissues or organs' [22].

Previous attempts

Solid organ xenotransplantation is not a new concept: primate-to-human kidney, heart or liver transplants were performed from the 1960s until the 1980s [23–29], but generally failed soon after surgery. For example, a chimpanzee heart transplant failed in 1964 because of size mismatch between the primate and the adult [28]; chimpanzee and baboon xenotransplants conducted by Christian Barnard were unsuccessful, possibly because of heterotopic positioning of the xenotransplants [28]. As late as 1984, the case of Baby Fae was widely reported: she was a neonate with hypoplastic left-heart syndrome, who lived for 20 days after receiving a baboon heart. Again, the procedure failed, probably because of rejection [29]. However, 9 months' survival was reported for a female teacher who received a chimpanzee kidney in the 1960s [25], and in 1992, a patient lived for 70 days after receiving a baboon liver [26].

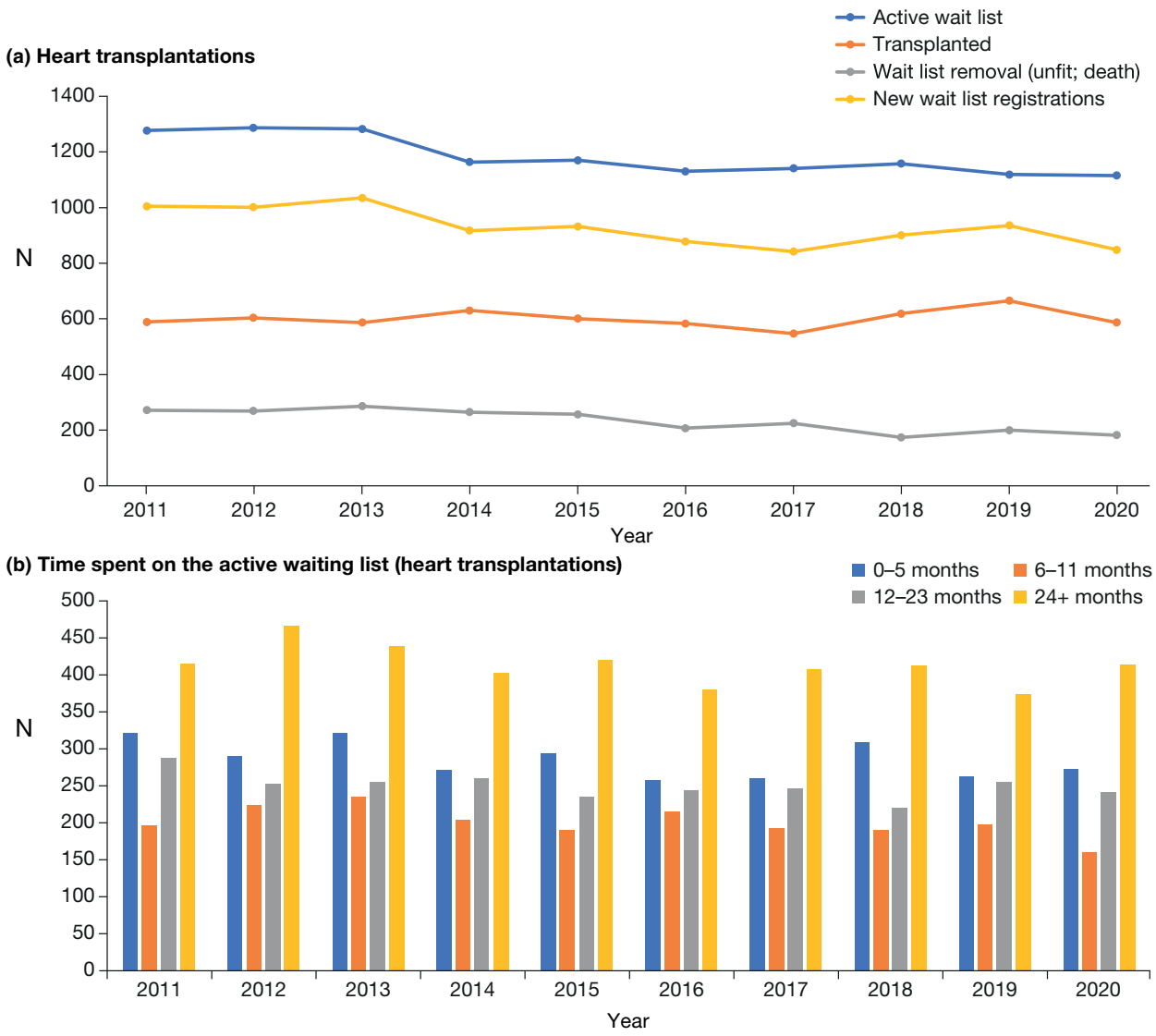


Figure 2 Eurotransplant data (2011–2020) for heart transplantation [3]: (a) changes in waiting list, (b) active waiting list.

Revisiting xenotransplantation

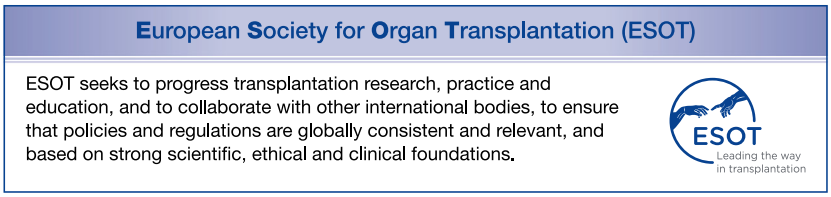
Lack of clinical and immunological success, concerns relating to supply and use of non-human organs and risk of zoonoses, together with advances in allograft transplantation, meant that xenotransplantation has been less of a focus for medical research in the past 20 years. However, the severe ongoing shortage of human donor organs, the continued (and largely increasing) waiting lists of patients in end-stage organ failure, advances in genetic engineering and our growing understanding of immunology [30] have rekindled interest and activity in preclinical xenotransplantation research.

Processes broadly applied to xenotransplantation now have the potential to provide animal-derived cells, tissues (e.g. islet or neuroendocrine grafts) and solid organs (e.g. kidney, heart, lung and liver) for clinical application. Rather than primates, which were initially utilized, donor animals are now expected to be defined pathogen-free (DPF) pigs [31] that are genetically modified [32,33], as outlined below. Organ supply from DPF pigs could effectively be unlimited, given their short gestation period, multiparity and rapid maturation that achieves organs of an appropriate size for human recipients (i.e., pigs weighing 70–90 kg); furthermore, donor organs could be procured on demand [20]. DPF pigs are reared in high-welfare conditions [29,34,35] where, unlike most human donors, there is low risk of illness or transmission of known human pathogens, and procured organs do not undergo the physiological stresses associated with trauma or brain death [36–39].

Box 1 European Society for Organ Transplantation (ESOT) and the Transplantation Learning Journey (TLJ) project.

Workstreams within the TLJ project help to achieve the primary aim of ESOT – to improve patient access to (and outcomes in) transplantation. TLJ workstreams facilitate objective discussion of scientific and clinical research, and expert opinion, to ensure that all perspectives on a topic are considered, with clinically relevant end goals in mind.

ESOT seeks to progress transplantation research, practice and education, and to collaborate with other international bodies, to ensure that policies and regulations are globally consistent and relevant, and based on strong scientific, ethical and clinical foundations.



Preclinical 'proof of concept'

Genome editing

Until 2018, the longest survival of a solid organ xenotransplant in a genetically modified knockout pig-to-primate model was ~180 days [40–43]. Subsequent improvements in genome editing make use of the programmable synthetic endonuclease system CRISPR/Cas9, which allows any gene to be inactivated or introduced; this has enabled pig lines with multiple genetic modifications to be created [44]. The Third WHO Global Consultation on Regulatory Requirements for Xenotransplantation Clinical Trials suggested that donor pigs should have a minimum number of 'essential' genetic modifications [33], a view that is supported by others [45]. Although the most appropriate combination of genetic modifications has yet to be defined, the most successful pig models developed to date lack the major swine carbohydrate xenoantigen Gal 1-3αGal (for humans, Neu5Gc, β4Gal or even swine leukocyte antigen (SLA) class I may also have to be omitted); they also express additional human genes that address cross-species physiological incompatibilities (at least one human complement regulator protein plus human thrombomodulin; Table 1) [44,46]. This combination of modifications apparently reduces the risk of post-transplant organ dysfunction [44,46–49].

Survival

Together with emerging therapeutic strategies for induction or maintenance immunosuppression [44,50–52] and donor-organ conditioning, genome editing has substantially improved long-term graft outcomes in primate recipient models [42,46,53,54]. For example, survival of (non-life-sustaining) abdominal heterotopic pig hearts (from α1,3-galactosyltransferase gene knockout pigs, with two genetic modifications) transplanted into baboons has reached up to 945 days, with grafts failing only after immunosuppression was diminished or discontinued [41]. To the authors' knowledge, to date, there has been no evidence that insurmountable physiological incompatibilities may preclude the successful clinical application of xenotransplantation.

Survival beyond 1 year was also reported following transplantation of life-supporting genetically modified kidney xenografts into macaques with low levels of pre-existing anti-pig antibodies, treated with anti-CD4 and anti-CD154 therapy [43]. In this study, xenografts were obtained from genetically engineered donor pigs lacking expression of Gal 1,3αGal and transgenic for a gene protective against the complement cascade [43].

Minimizing infection risk

Another study in the GalTKO.hCD46.hTBM pig-to-baboon life-supporting orthotopic heart transplantation model [40,50] reported consistent survival (6/8 baboons) for up to 6 months postoperatively. The two deaths were because of porcine cytomegalovirus (PCMV) infection, a complication that could have been avoided through the use of PCMV-negative donors. Therefore, it will be important to use pathogen-free animals in future [46,55]. In DPF-environments, infection avoidance is achieved primarily through good animal husbandry, and selecting

Table 1. Possible genetic modifications of the porcine genome, including gene knockouts and transgenes, have increased the potential for pig-to-human xenotransplantation by reducing the risk of rejection, thrombotic microangiopathy or inflammatory reactions post procedure [44,46]; for successful preclinical heart xenotransplantations, only three modifications were needed: α-1,3-galactosyltransferase knockout and overexpression of human CD46 and human thrombomodulin [42].

Pathophysiological target	Modification/mechanism of action
Elimination of porcine αGal (Neu5Gc and Sd(a)) epitopes, against which preformed antibodies exist in non-human primates (humans)	Disruption of genes encoding α-1,3-galactosyltransferase, cytidine monophosphate-N-acetylneuraminic acid hydroxylase and β-1,4-N-acetyl-galactosaminyl transferase 2
Complement regulation (inhibition of activation)	Transgenic expression of human complement regulatory proteins (hCD46, hCD55 and hCD59)
Anticoagulation (preventing thrombotic microangiopathy)	Transgenic expression of human coagulation regulatory proteins (thrombomodulin, tissue factor pathway inhibitor, EPCR, hCD39 and siRNA-mediated knockdown of tissue factor expression)
Anti-inflammation/anti-apoptosis	Transgenic expression of human TNFα-induced protein 3 (A20) and human heme oxygenase 1
Inhibition of T-cell activation	Transgenic expression of CTLA4-Ig, to block CD28-CD80/CD86 co-stimulatory pathway; expression of membrane-bound human PD-L1 inhibiting T-cell activation via the inhibitory PD1 receptor
Inhibition of NK cell activation	HLA-E or HLA-E/human β expression
Inhibition of macrophage activation	Transgenic expression of hCD47 and inhibition of macrophage activation via SIRP-α
Downregulation of MHC molecules (e.g. swine leukocyte antigen)	Strategies include CRISPR/cas-mediated mutagenesis of SLA class I heavy chain coding sequences or mutagenesis of the β2-microglobulin gene; SLA class II expression can be reduced by expression of a human dominant-negative class II transactivator transgene, mutagenesis of the *CIITA* gene or by expression of siRNA

donor strains at low risk for porcine endogenous retrovirus (PERV) transmission (pigs that carry low loads of PERV subgroups A and B, and no subgroup C), rather than via donor gene-editing strategies or prophylactic use of anti-infective therapies [34,35].

It is of interest that other investigators have proposed targeted inactivation of PERV-proviruses present in donor animals [48] – a strategy that might be associated with a high risk of genomic rearrangements [56]. However, confidence can be extrapolated from the lack of evidence of PERV and/or pathogen infection, or indeed other adverse events, in human recipients of cellular and skin xenotransplants [57–59]. It can be argued that solid organ xenotransplants could be considered a higher risk because of function and site; while no single method can fully eliminate the theoretical risk that PERV presents, a matrix of preventive monitoring and therapeutic measures is a powerful rational basis to now support the clinical application of solid organ xenotransplantation [60,61].

In addition, it is important to note that, in the case of cardiac xenotransplantation, survival data in animal models [40,41,44,50] now generally exceed the internationally suggested threshold for considering a research transition into clinical development (namely 60% survival for ≥3 months, with ≥10 animals surviving for this minimum period) [32,62].

It is now time to examine whether these achievements allow human trials that align with current clinical outcomes and expectations to begin.

Regulations and guidelines

In the European Union (EU), clinical trials are authorized at the national level [63]; similarly, the US Food and Drug Administration (FDA) approves studies conducted in the United States [64]. The European Medicines Agency (EMA) and FDA websites provide guidance on the conduct of first-in-human studies and are responsible for marketing authorization, once a drug or device completes preregistration trials, and data review [64,65]. The process is collaborative, offering many opportunities for investigators to seek guidance from the agencies about any aspect of a study or its data analysis prior to submission [66].

Many influential bodies have drafted guidance on safety, quality, processing, record-keeping, monitoring and traceability relating to the clinical application of animal-derived technologies [33,36,67–74]. These initiatives address xenotransplantation guidance, according to

its widest definition. For example, the EMA and EU define cells, tissues and organs, including any components that are genetically modified, as advanced therapy medicinal products (ATMPs) [73]. The EMA's Committee for Advanced Therapies already conducts scientific assessment of ATMPs, and numerous EU-funded research projects involving tissue or cell therapies (outside the solid organ transplant field) have been carried out or are in progress [75,76]. However, different regions use different terminology. For example, the term 'ATMP' is only used in Europe [77]. In the United States, a xenotransplantation product is considered a biological product, but a xenograft (e.g. a xenogeneic heart valve) is described as an acellular or decellularized product, regardless of process. In contrast, the equivalent of a xenograft in Europe is considered a medical device and subsequently an ATMP, according to the regulations [77,78].

Clinical development planning for xenotransplantation will undoubtedly have such publications at its foundation, although new guidance could aim for global harmonization of the terminology, particularly with respect to the definition of the product.

Predictable risks

From the initial stages of clinical trial planning, regulatory guidance focuses on how to minimize the predictable risks of using a new technology, rather than the technology itself or its mode of action. This approach aims to meet the end goals of improving patient outcomes and achieving alignment between regulatory guidance and later stages of the data review process [66]. Predictable risks relate to aspects covered in international guidelines, including how the source herd was managed and monitored; traceability; procedures for quarantine and transport of animals and organs; organ procurement (including preparation, characterization and microbiological status); how the recipient was selected, educated, consented, treated and followed up; and record-keeping throughout the process [79,80]. All such factors impact on clinical trial design and conduct (Fig. 3). Fortunately,

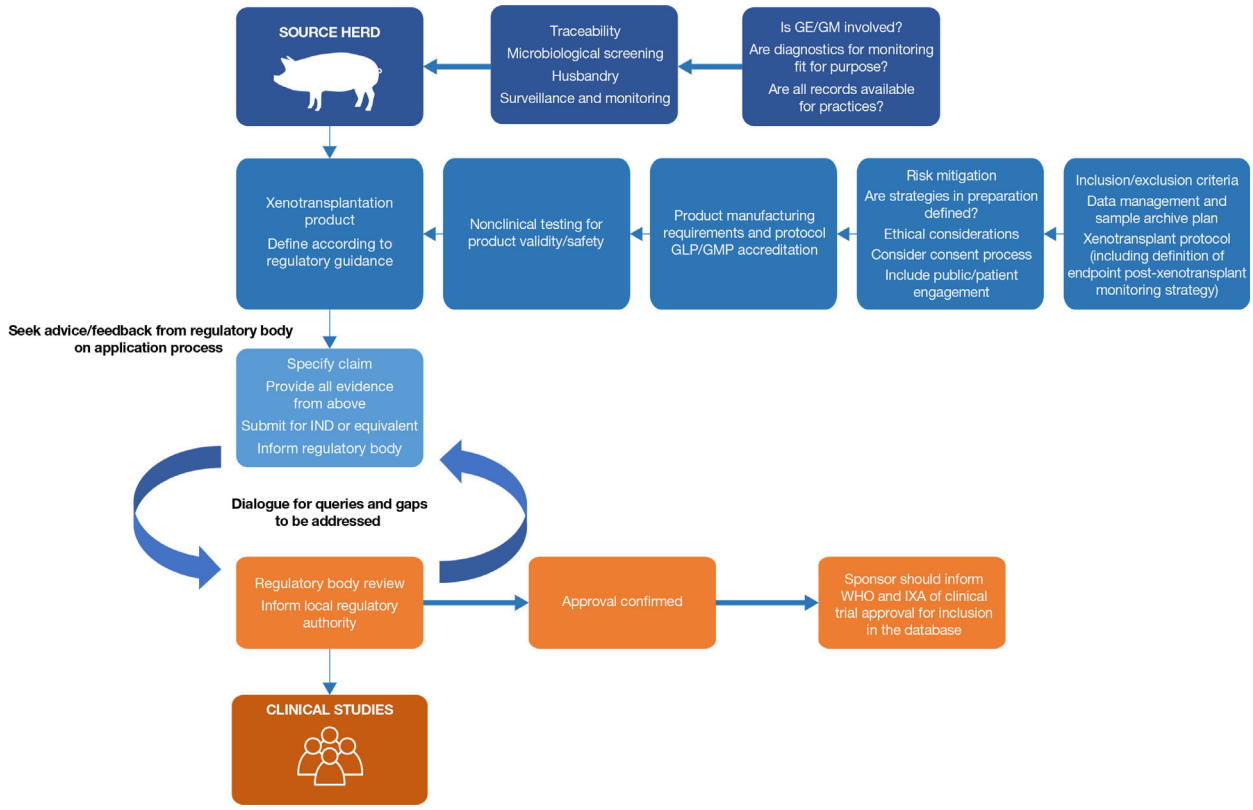


Figure 3 Key factors and processes to be considered to achieve clinical xenotransplantation. Summary organization of data flow required for a clinical trial application. Definition of the product is paramount and varies according to location. It is important to categorize accurately in order to follow the correct regulatory process. Advice from the regulatory body must be sought prior to application, to ensure that the definitions and data submitted meet all the criteria specified. Traceability and record-keeping should be key points throughout the entire process and preparation. Once the trial has received approval, it is important to list on the global inventory https://www.humanxenotransplant.org/.

Transplant International 2021; 34: 2006–2018

these challenges are not that difficult to overcome. They are, however, time consuming.

Public and patient stakeholder contributions

The need to build trust and awareness of xenotransplantation among the general public [81] is just as important as collaborative approaches for nonclinical and first-in-human trials. Building this trust requires early engagement and ongoing dialogue throughout clinical development.

Public opinion

Research investigating attitudes to xenotransplantation reveals a heterogeneous landscape. In many cases, xenotransplantation is cautiously welcomed [82–85]; the most positive findings emerged from studies conducted in health professionals or students [82,84,85]. Research conducted in German citizens who initially were rather critical of xenotransplantation, identifying more risks than opportunities, illustrated how opinions could change over time [86]. Following appropriate education and deliberation on the subject (involving xenotransplantation experts), most participants changed their views and concurred that the expected benefits of xenotransplantation outweighed the potential risks. Eventually, most participants considered xenotransplantation, a reasonable treatment option, and recommended to continue research and development in the field [86]. Importantly, these findings suggest that, although public surveys exploring attitudes to xenotransplantation provide useful information, such findings are misleading if they permit misinterpretation of the information provided; measures to prevent misinterpretation must be considered.

Public engagement

Certainly, engaging and involving all public and patient stakeholders in xenotransplantation – from the earliest stages of clinical planning – is of critical importance. Studies will need to include adequate numbers of participants, with each subject, their caregivers and close contacts properly informed and consented. Again, xenotransplantation can learn from (and adapt) existing strategies for participant engagement. For example, the Kidney Health Initiative (KHI) has created frameworks that help to build trust across communities through multistakeholder collaboration, review and endorsement of projects [87,88]. Patient and public engagement in novel procedures can be addressed systematically, with the process stratified into four levels: at level 1, the patient is disengaged and overwhelmed; at level 2, the patient becomes aware of the concept, but struggles with it; at level 3, the patient is ready for 'action', and at level 4, the patient maintains the behaviours (or beliefs) and pushes the concept further [89]. A similar systematic approach could be beneficial for patient and public engagement in relation to xenotransplantation.

Components of xenotransplantation clinical trials

The xenotransplantation workstream outlined several aspects for consideration in clinical trials, detailed below. The next phase aims to identify areas for expansion and crystallize the discussion points.

Animal welfare/husbandry

The goal was to maintain the highest possible standards of animal welfare and hygiene, while minimizing the use of prophylactic drug regimens. Facilities for rearing specific pathogen-free (SPF) or DPF pigs follow high welfare and safety standards and have the potential to provide solid organs, including hearts and kidneys, for early clinical trials [34]. To maintain levels of animal husbandry that incorporate biosecurity, quality, safety and traceability when production is upscaled for larger studies and beyond, animals must be reared in centralized facilities, with organs distributed rapidly across countries or even an entire continent. Specialized farms that are capable of this shift in delivery are being developed [34,90]. Guidance, consensus statements and documented evidence of operation of these facilities are available [33,34,64,91–93].

Patient selection for first-in-human trials

Although preclinical xenotransplantation models consistently demonstrate reproducible long-term graft survival, the implementation of animal-to-human procedures presents specific challenges and obligations.

First-in-human studies should be conducted in patients who have a very high risk of poor outcomes without timely organ transplantation. For cardiac xenotransplantations, initial candidates would be patients in terminal heart failure [94,95] who are not suitable for mechanical assist devices, or adolescents with congenital cardiac lesions and a persisting single (right) heart circulation. For end-stage kidney disease, the co-authors of

this paper cannot presently reach consensus as to who would be prime candidates for first-in-human xenotransplantations. Some co-authors suggest that candidates could include high-risk haemodialysis patients (e.g. people with poor predicted 1-year survival) or patients with dialysis access problems, although others argue that human kidneys would usually be available to such patients. The option of selecting extremely sensitized patients (i.e., cPRA >99%) seems at first sight to be logical, as these people have little to no chance of receiving HLA-compatible human organs. Some co-authors argue, however, that selecting such recipients could increase immunological risks after xenotransplantation, linked to the development of cross-reactive antibodies that would injure the xenograft. It should be noted, however, that the risk of HLA-I/SLA-I cross-reactivity could be mitigated by generating donor organs lacking SLA class I expression on a swine carbohydrate triple-knockout background. In theory, such organs could be acceptable for transplantation even in extremely sensitized human recipients (Table 1).

Informed consent

Given the complexity and potential risks of participation in clinical trials, patients and advocates should be involved throughout the planning of each study. The processes of defining and obtaining informed consent should be determined in clinical trial protocols, but before consent can be 'informed', public- and patient-focused programmes should build awareness and knowledge of solid organ xenotransplantation across the community [77,83,86–88,96].

Providing informed consent to first-in-human xenotransplantation studies will require detailed consultations with participants because of remaining uncertainties regarding the unintended effects of xenotransplantation, such as the potentially serious consequences of incompatibility, or emerging zoonoses [97,98]. Participants should receive up-to-date information about preclinical and clinical experiences and be supported in balancing the expected benefits, burdens and risks of participating in an early xenotransplantation trial based on their own individual values. Safeguarding the participants' free and voluntary informed consent is especially important, given the difficult circumstances created by life-threatening illness, absence of good treatment alternatives and uncertain outcomes with experimental xenograft technology. Consequently, study participants should be sufficiently able to understand the concept of xenotransplantation and its expected benefits and potential burdens and risks, including the unavoidable uncertainties.

Endpoints for clinical studies

The co-authors of this paper have a range of opinions as to the possible structure and endpoints for first-in-human clinical trials in solid organ xenotransplantation. The following are speculative concepts that reflect our collective views, and other options will likely emerge.

Endpoints will be standard survival (patient and graft), short- and long-term safety, documentation of adverse events, tolerability (drug interactions, infections, including adventitious agent's assays) and patient-reported outcomes, including health-related quality of life.

Although noninferiority trials appear to represent the ideal clinical studies for comparing any novel therapy in transplantation with current clinical outcomes, the cohorts needed to report clinically or statistically meaningful data would be so large that they are unlikely to be feasible in practice. For breakthrough innovations such as xenotransplantation, first-in-human pilot studies will be required. After achieving success in such trials, noninferiority trials might be possible, although it is unclear what the best control group(s) would be.

Several co-authors of this paper indicate that initial xenotransplantation studies could involve organ recipients who are extremely sensitized, as discussed above. The most likely comparators could be outcomes for current standards of care in allogeneic transplantation (e.g., extremely sensitized patients on dialysis, waitlisted for allotransplantation). Alternatively, parameters could compare the xenograft recipient's pre- and post-transplant state of health and well-being.

Some co-authors of this paper express concern about using extremely sensitized patients as the first candidates when clinical xenotransplantation trials restart. They consider that this strategy could compromise the fair evaluation of xenotransplantation, because of issues relating to heightened risk of cross-reactivity of antibodies against human and porcine MHC, as mentioned above [52,99]. At the beginning of clinical development, when few donor animals will be available, and the choice of immunotherapy regimens will be experimental [44,50–52], finding matching porcine organs will be a cumbersome process. Over the longer term, however, the challenges of cross-reactive antibodies will be resolvable by scaling up the animal rearing and husbandry processes, and by genetic elimination of SLA, as previously discussed.

All that can be confirmed at present is that xenotransplantation study design will require detailed

Transplant International 2021; 34: 2006–2018

consideration by stakeholders, in particular collaboration between regulatory bodies and research groups. Studies must include the participant groups, evaluations and endpoints that are clinically meaningful and also appropriate for market authorization applications. Characterizing the first patients for inclusion in clinical trials of xenotransplantation will be a decisive step, in order to balance ethical acceptability with immunological risk. This is particularly important if trials start with extremely sensitized patients, not least because of the risk that clinical xenotransplantation might end before it has really begun, similar to the situation that occurred with early human heart transplantation in the late 1960s.

Final considerations

Restarting clinical trials in animal-to-human organ transplantation raises questions of importance to society, science and clinical practice. Taking a transparent, patient-centred, protocol- and data-driven approach, from the earliest phase of clinical trial planning, is a good starting point that helps to ensure that evaluations of xenotransplantation remain fair. Clinical trials will be complex and not risk free, given their pioneering nature, and will require high levels of trust, engagement and openness among all stakeholders. By identifying and acknowledging points of uncertainty or potential conflict, different perspectives can be understood. Collaborative approaches overcome hurdles.

Advances in gene-editing and high-throughput technologies increase the chance of success for clinical xenotransplantation of solid organs. Xenotransplantation offers the potential to improve health outcomes for thousands of people worldwide who require donor organs, but whose needs cannot be met by current practices. The alternatives for these people are further deterioration of their health (and health-related quality of life) while they continue on dialysis, or a high risk of dying if they have terminal heart failure. Xenotransplantation might not be a panacea, but it is a rapidly progressing approach that shows great clinical potential for treating people with end-stage organ failure.

We encourage all groups with an interest in the clinical application of solid organ xenotransplantation to reflect on the end goals of preclinical research. Animal-to-human transplantation is a concept for society to consider at levels far deeper than whether it is clinically feasible. It is a matter of balancing risks and benefits, and ensuring that the benefits outweigh the risks. If the risks of acute rejection of the xenogeneic organ and the transmission of zoonoses are low, the net benefit of xenotransplantation will rise. In addition, the risks of the transplantation procedure itself must be taken into account. However, such risks should be similar (or even lower) than those associated with standard allotransplantation procedures, because of optimum planning and preparation of the novel surgery. Prerequisites are stable genetic modification, donor-organ treatment (perfusion), donor growth control and immunosuppression with co-stimulation blockade. This article opens discussions for everyone with an interest in transplantation, to optimize information sharing and education, and clinical development planning. Much more detail is required, with contributions from ethicists, clinical trial experts, health authority representatives, patients, advocates, academia, engineers and industry bodies; in other words, society at large. Very wide and open interactions should begin immediately. Good planning and collaboration will help establish a pathway for clinical research – one that is ready to address any needs that emerge as the innovative area of xenotransplantation develops.

Authorship

This article has been developed following two webinars conducted by the European Society for Organ Transplantation (ESOT), Workstream (WS)01, *The promise of xenotransplantation* (6 July 2020; https://www.youtube.com/watch?v=Eed4BxANPls) and *Solving the hurdles for disruptive technologies* (26 October 2020; https://www.youtube.com/watch?v=rh1J-Qdgpyo), together with related presentations at the ESOT TLJ2.0 online congress in November 2020.

Aspects of access to donor organs and organ quality assessment, and emerging technologies in transplantation, were considered. PubMed literature was searched from 1900 to 2021 to obtain articles relating to xenotransplantation for evidence and review. In addition, input into the opinions and content was provided from all ESOT WS01 members, via e-mail and virtual discussions at webinars and ESOT teleconferences. All WS01 members have considerable expertise and interest in the study and application of new technologies in transplant research and clinical practice; discussions relating to this WS also included representatives from international regulatory and patient organizations.

The fully written and referenced article was circulated to all WS members and other co-authors for review by e-mail and teleconference. The document was finalized by EC, SS, BR, LS and MN, and circulated to all co-authors for approval before submission for publication.

Funding

This initiative was supported by the European Society for Organ Transplantation.

Conflict of interest

MIB, GB, MH, GM, LM, JN, MN, RO, RNP and CW report no conflicts of interest. SS received grants/research support from Novartis, Sandoz, Bridge to Life, Chiesi, Neovii and Organ Recovery; speakers' bureaux/honoraria from Astellas, Novartis, BMS, Sanofi, OrganOx and Chiesi; and consulting fees from Astellas, Novartis, Teva, Sandoz, Merck, Atara and NefroHealth. EC reports a consulting agreement with Hansa BioPharma. EB is a shareholder in ITB-MED. KF reports consulting agreements with Hansa BioPharma, Talaris, Otsuka, Palladio Biosciences, Travere Therapeutics, Bayer, Gilead, eGenesis and Veloxis. IJ has received speaker fees from XVIVO Perfusion. BR is CEO of xTransplant. LS is a consulting scientist on safety for XenoTherapeutics and has received funding from MVD Diagnostics for research and development of veterinary diagnostics.

Acknowledgements

The authors thank Anna-Pia Papageorgiou, Policy Officer, European Commission – Research Directorate General, Brussels, Belgium, for her contributions to discussions relating to this work. The authors are also grateful to all members of the European Society for Organ Transplantation (ESOT) for their comments and suggestions in the development of this article. Medical writing support was provided by Linda Edmondson, independent medical writer, and funded by ESOT.

Ethical approval

This article does not report a clinical study in human subjects, and therefore, ethical approval was not required.

REFERENCES

1. Lewis A, Koukoura A, Tsianos G-I, Gargavanis AA, Nielsen AA, Vassiliadis E. Organ donation in the US and Europe: The supply vs demand imbalance. *Transplant Rev (Orlando)* 2020; **35**: 100585.
2. US Department of Health and Human Services. OPTN/SRTR 2018 Annual data report: Kidney. Available from: https://srtr.transplant.hrsa.gov/annual_reports/2018/Kidney.aspx. Accessed August 6, 2021.
3. Eurotransplant. Yearly statistics. Available from: https://www.eurotransplant.org/statistics/yearly-statistics/. Accessed August 6, 2021.
4. DeRoos LJ, Marrero WJ, Tapper EB, *et al.* Estimated association between organ availability and presumed consent in solid organ transplant. *JAMA Netw Open* 2019; **2**: e1912431.
5. Arshad A, Anderson B, Sharif A. Comparison of organ donation and transplantation rates between opt-out and opt-in systems. *Kidney Int* 2019; **95**: 1453.
6. Danziger-Isakov L, Blumberg EA, Manuel O, Sester M. Impact of COVID-19 in solid organ transplant recipients. *Am J Transplant* 2021; **21**: 925.
7. Bellini MI, Pengel L, Potena L, Segantini L. ESOT COVID-19 Working Group. COVID-19 and education: restructuring after the pandemic. *Transpl Int* 2021; **34**: 220.
8. Naeem SB, Bhatti R. The Covid-19 "infodemic": a new front for information professionals. *Health Info Libr J* 2020; **37**: 233.
9. Mehrotra R. Advancing American kidney health: an introduction. *Clin J Am Soc Nephrol* 2019; **14**: 1788.
10. Himmelfarb J, Vanholder R, Mehrotra R, Tonelli M. The current and future landscape of dialysis. *Nat Rev Nephrol* 2020; **16**: 573.
11. Consortium for Organ Preservation in Europe (COPE). List of approved research proposals for the liver trial (WP2). Available from: https://cope-eu.com/research/approved%20proposals-2. Accessed August 6, 2021.
12. Pinezich M, Vunjak-Novakovic G. Bioengineering approaches to organ preservation ex vivo. *Exp Biol Med (Maywood)* 2019; **244**: 630.
13. Jochmans I, Brat A, Davies L, *et al.* Oxygenated versus standard cold perfusion preservation in kidney transplantation (COMPARE): a randomised, double-blind, paired, phase 3 trial. *Lancet* 2020; **396:** 1653. [Erratum in *Lancet* 2021; **396:** 1978.]
14. Hosgood SA, Hoff M, Nicholson ML. Treatment of transplant kidneys during machine perfusion. *Transpl Int* 2021; **34**: 224.
15. Weissenbacher A, Vrakas G, Nasralla D, Ceresa CDL. The future of organ perfusion and re-conditioning. *Transpl Int* 2019; **32**: 586.
16. Leuning DG, Witjas FMR, Maanaoui M, *et al.* Vascular bioengineering of scaffolds derived from human discarded transplant kidneys using human pluripotent stem cell-derived endothelium. *Am J Transplant* 2019; **19**: 1328.
17. Homan KA, Gupta N, Kroll KT, *et al.* Flow-enhanced vascularization and maturation of kidney organoids in vitro. *Nat Methods* 2019; **16**: 255.
18. Hoogduijn MJ, Montserrat N, van der Laan LJW, *et al.* The emergence of regenerative medicine in organ transplantation: 1st European Cell Therapy and Organ Regeneration Section meeting. *Transpl Int* 2020; **33**: 833.
19. Ekser B, Cooper DKC, Tector AJ. The need for xenotransplantation as a source of organs and cells for clinical transplantation. *Int J Surg* 2015; **23**(Pt B): 199.
20. Ekser B, Li P, Cooper DKC. Xenotransplantation: past, present, and future. *Curr Opin Organ Transplant* 2017; **22**: 513.
21. Zhang X, Li X, Yang Z, *et al.* A review of pig liver xenotransplantation: current problems and recent progress. *Xenotransplantation* 2019; **26**: e12497.
22. US Food and Drug Administration. Xenotransplantation. 2021. Available from:

https://www.fda.gov/vaccines-blood-biologics/xenotransplantation Accessed August 6, 2021 .
23. Cooper DKC, Ekser B, Tector AJ. A brief history of clinical xenotransplantation. *Int J Surg* 2015; **23**(Pt B): 205.
24. Reemtsma K, McCracken BH, Schlegel JU, *et al*. Renal heterotransplantation in man. *Ann Surg* 1964; **160**: 384.
25. Starzl TE, Fung J, Tzaki A, *et al*. Baboon-to-human liver transplantation. *Lancet* 1993; **341**: 65.
26. Knott GJ, Doudna JA. CRISPR-Cas guides the future of genetic engineering. *Science* 2018; **361**: 866.
27. Morris T. The art of medicine. James Hardy and the first heart transplant. *The Lancet* 2017; **389**: 2280.
28. Murthy R, Bajona P, Bhama JK, *et al*. Heart xenotransplantation: historical background, experimental progress, and clinical prospects. *Ann Thorac Surg* 2016; **101**: 1605.
29. Bailey LL, Nehlsen-Cannarella SL, Concepcion W, *et al*. Baboon-to-human cardiac xenotransplantation in a neonate. *JAMA* 1985; **254**: 3321.
30. Davis MM, Brodin P. Rebooting human immunology. *Annu Rev Immunol* 2018; **36**: 843.
31. Schuurman HJ. Regulatory aspects of clinical xenotransplantation. *Int J Surg* 2015; **23**(Pt B): 312.
32. Pierson RN 3rd, Dorling A, Ayares D, *et al*. Current status of xenotransplantation and prospects for clinical application. *Xenotransplantation* 2009; **16**: 263.
33. Hawthorne WJ, Cowan PJ, Bühler LH, *et al*. Third WHO global consultation on regulatory requirements for xenotransplantation clinical trials, Changsha, Hunan, China December 12–14, 2018: "The 2018 changsha communiqué" The 10-year anniversary of the international consultation on xenotransplantation. *Xenotransplantation* 2019; **26**: e12513.
34. Noordergraaf J, Schucker A, Martin M, *et al*. Pathogen elimination and prevention within a regulated, Designated Pathogen Free, closed pig herd for long-term breeding and production of xenotransplantation materials. *Xenotransplantation* 2018; **25**: e12428.
35. Powell EJ, Charley S, Boettcher AN, *et al*. Creating effective biocontainment facilities and maintenance protocols for raising specific pathogen-free, severe combined immunodeficient (SCID) pigs. *Lab Anim* 2018; **52**: 402.
36. Cooper DKC, Cowan P, Fishman JA, *et al*. Joint fda-ixa symposium, september 20, 2017. *Xenotransplantation* 2017; **24**: e12365.
37. Cooper DKC, Pierson RN 3rd, Hering BJ, *et al*. Regulation of clinical xenotransplantation: time for a reappraisal. *Transplantation* 2017; **101**: 1766.
38. Cooper DKC, Wijkstrom M, Hariharan S, *et al*. Selection of patients for initial clinical trials of solid organ xenotransplantation. *Transplantation* 2017; **101**: 1551.
39. Cooper DKC, Hara H, Iwase H, *et al*. Pig kidney xenotransplantation: progress toward clinical trials. *Clin Transplant* 2021; **35**: e14139.
40. Tseng YL, Kuwaki K, Dor FJ, *et al*. alpha1,3-Galactosyltransferase gene-knockout pig heart transplantation in baboons with survival approaching 6 months. *Transplantation* 2005; **80**: 1493.
41. Mohiuddin MM, Singh AK, Corcoran PC, *et al*. Chimeric 2C10R4 anti-CD40 antibody therapy is critical for long-term survival of GTKO.hCD46.hTBM pig-to-primate cardiac xenograft. *Nat Commun* 2016; **7**: 11138.
42. Längin M, Mayr T, Reichart B, *et al*. Consistent success in life-supporting porcine cardiac xenotransplantation. *Nature* 2018; **564**: 430.
43. Kim SC, Mathews DV, Breeden CP, *et al*. Long-term survival of pig-to-rhesus macaque renal xenografts is dependent on CD4 T cell depletion. *Am J Transplant* 2019; **19**: 2174.
44. Meier RPH, Muller YD, Balaphas A, *et al*. Xenotransplantation: back to the future? *Transpl Int* 2018; **31**: 465.
45. Kemter E, Schnieke A, Fischer K, *et al*. Xeno-organ donor pigs with multiple genetic modifications – the more the better? *Curr Opin Genet Dev* 2020; **64**: 60.
46. Reichart B, Längin M, Denner J, *et al*. Pathways to clinical cardiac xenotransplantation. *Transplantation* 2021; **105**: 1930–1943.
47. Cooper DKC, Hara H, Iwase H, *et al*. Justification of specific genetic modifications in pigs for clinical organ xenotransplantation. *Xenotransplantation* 2019; **26**: e12516.
48. Yue Y, Xu W, Kan Y, *et al*. Extensive germline genome engineering in pigs. *Nat Biomed Eng* 2021; **5**: 134.
49. Wolf E, Reichart B. Xenotransplantation solider Organe. *Nephrologe* 2021; **16**: 138. [in German].
50. Adams AB, Lovasik BP, Faber DA, *et al*. Anti-C5 antibody tesidolumab reduces early antibody-mediated rejection and prolongs survival in renal xenotransplantation. *Ann Surg* 2021; **274**: 473.
51. Meier RPH, Longchamp A, Mohiuddin M, *et al*. Recent progress and remaining hurdles toward clinical xenotransplantation. *Xenotransplantation* 2021; **28**: e12681.
52. Martens GR, Reyes LM, Li P, *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.
53. Kemter E, Denner J, Wolf E. Will genetic engineering carry xenotransplantation of pig islets to the clinic? *Curr Diab Rep* 2018; **18**: 103.
54. Reichart B, Längin M, Radan J, *et al*. Pig-to-non-human primate heart transplantation: the final step toward clinical xenotransplantation? *J Heart Lung Transplant* 2020; **39**: 751.
55. Scobie L, Denner J. Theme issue on infections and safety: an introduction. *Xenotransplantation* 2018; **25**: e12447.
56. Leibowitz ML, Papathanasiou S, Doerfler PA, *et al*. Chromothripsis as an on-target consequence of CRISPR–Cas9 genome editing. *Nat Genet* 2021; **53**: 895.
57. Scobie L, Padler-Karavani V, Le Bas-Bernardet S, *et al*. Long-term IgG response to porcine Neu5Gc antigens without transmission of PERV in burn patients treated with porcine skin xenografts. *J Immunol* 2013; **191**: 2907.
58. Matsumoto S, Wynyard S, Giovannangelo M, *et al*. Long-term follow-up for the microbiological safety of clinical microencapsulated neonatal porcine islet transplantation. *Xenotransplantation* 2020; **27**: e12631.
59. Paradis K, Langford G, Long Z, *et al*. Search for cross-species transmission of porcine endogenous retrovirus in patients treated with living pig tissue. The XEN 111 Study Group. *Science* 1999; **285**: 1236.
60. McGregor CGA, Takeuchi Y, Scobie L, *et al*. PERVading strategies and infectious risk for clinical. *Xenotransplantation* 2018; **25**: e12402.
61. Denner J, Scobie L, Schuurman HJ. Is it currently possible to evaluate the risk posed by PERVs for clinical xenotransplantation? *Xenotransplantation* 2018; **25**: e12403.
62. Cooper DK, Keogh AM, Brink J, *et al*. Xenotransplantation Advisory Committee of the International Society for Heart and Lung Transplantation. Report of the Xenotransplantation Advisory Committee of the International Society for Heart and Lung Transplantation: the present status of xenotransplantation and its potential role in the treatment of end-stage cardiac and pulmonary diseases. *J Heart Lung Transplant* 2000; **19**: 1125.
63. EudraCT. Registering a trial in EudraCT and updating its information on EU CTR. Available from: https://eudract.ema.europa.eu/protocol.html. Accessed August 10, 2021.

64. US Food and Drug Administration. Clinical trials guidance documents. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/clinical-trials-guidance-documents. Accessed August 6, 2021.
65. European Medicines Agency. Clinical trials in human medicines. Available from: https://www.ema.europa.eu/en/human-regulatory/research-development/clinical-trials-human-medicines. Accessed August 10, 2021.
66. Schuurman H-J, Hoogendoorn K. Solid organ xenotransplantation at the interface between research and clinical development: regulatory aspects. *Xenotransplantation* 2020; **27**: e12608.
67. European Medicines Agency. Xenogenic cell-based medicinal products. Available from: https://www.ema.europa.eu/en/xenogeneic-cell-based-medicinal-products. Accessed August 6, 2021.
68. US Food and Drug Administration. Guidance for Industry: Source animal, product, preclinical, and clinical issues concerning the use of xenotransplantation products in humans; December 2016. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/source-animal-product-preclinical-and-clinical-issues-concerning-use-xenotransplantation-products. Accessed August 6, 2021.
69. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. Available from: https://www.ich.org/. Accessed August 6, 2021.
70. International Xenotransplantation Association 2016 consensus statement issue in Xenotransplantation. Available from: https://onlinelibrary.wiley.com/toc/13993089/2016/23/1. Accessed August 6, 2021.
71. World Health Organization, Department of Communicable Disease Surveillance and Response. WHO guidance on xenogeneic infection/disease surveillance and response: a strategy for international cooperation and coordination. Document WHO/CDS/CSR/EPH/2001.2; 2001. Available from: https://apps.who.int/iris/handle/10665/68888. Accessed August 6, 2021.
72. European Medicines Agency. Legal framework: Advanced therapies. Available from: https://www.ema.europa.eu/en/human-regulatory/overview/advanced-therapies/legal-framework-advanced-therapies. Accessed August 6, 2021.
73. organtrans.eu. Accessed August 6, . 2021.
74. Boráň T, Menezes-Ferreira M, Reischl I, *et al.* Clinical development and commercialization of advanced therapy medicinal products in the European Union: how are the product pipeline and regulatory framework evolving? *Hum Gene Ther Clin Dev* 2017; **28**: 126.
75. Xenoislet.eu. Project description. Available from: http://xenoislet.eu/project_description. Accessed August 6, 2021.
76. Denner J. By definition. . .. *Xenotransplantation* 2020; **27**: e12599.
77. Godehardt AW, Tönjes RR. Xenotransplantation of decellularized pig heart valves: regulatory aspects in Europe. *Xenotransplantation* 2020; **27**: e12609.
78. Rayat GR, Gazda LS, Hawthorne WJ, *et al.* First update of the International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes - Chapter 3: Porcine islet product manufacturing and release testing criteria. *Xenotransplantation* 2016; **23**: 38.
79. Hering BJ, O'Connell PJ. First update of the International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes-Chapter 6: patient selection for pilot clinical trials of islet xenotransplant. *Xenotransplantation* 2016; **23**: 60.
80. Sobbrio P, Jorqui M. An overview of the role of society and risk in xenotransplantation. *Xenotransplantation* 2014; **21**: 523.
81. Abalovich A, Matsumoto S, Wechsler CJ, *et al.* Level of acceptance of islet cell and kidney xenotransplants by personnel of hospitals with and without experience in clinical xenotransplantation. *Xenotransplantation* 2017; **24**: e12315.
82. Amin L, Hashim H, Mahadi Z, Che Ngah A, Ismail K. Determinants of stakeholders' attitudes to xenotransplantation. *Xenotransplantation* 2018; **25**: e12430.
83. Shimoda M, Matsumoto S. Update regarding xenotransplantation in Japan. *Xenotransplantation* 2019; **26**: e12491.
84. Martínez-Alarcón L, Ríos A, Santainés-Borredá E, *et al.* Student nurses at Spanish universities and their attitude toward xenotransplantation. *Xenotransplantation* 2019; **26**: e12507.
85. Padilla LA, Sorabella RA, Carlo WF, *et al.* Attitudes to cardiac xenotransplantation by pediatric heart surgeons and physicians. *World J Pediatr Congenit Heart Surg* 2020; **11**: 426.
86. Kögel J, Marckmann G. "Xenotransplantation challenges us as a society": what well-informed citizens think about xenotransplantation. *EMBO Rep* 2020; **21**: e50274.
87. Kidney Health Initiative. Engaging the patient voice. Available from: https://khi.asn-online.org/pages/?ID=1. Accessed August 6, 2021.
88. Kidney Health Initiative. Overcoming barriers to innovation. Available from: https://khi.asn-online.org/pages/?ID=37. Accessed August 6, 2021.
89. Nair D, Cavanaugh KL. Measuring patient activation as part of kidney disease policy: are we there yet? *J Am Soc Nephrol* 2020; **31**: 1435.
90. Wang W, Liang Q, Nie W, Zhang J, Chen C. Biosafety barrier to xenotransplantation. Open access peer-reviewed chapter, published November 7, 2019. Available from: https://www.intechopen.com/books/xenotransplantation-comprehensive-study/biosafety-barrier-to-xenotransplantation. Accessed August 6, 2021.
91. Fishman JA. Prevention of infection in xenotransplantation: Designated pathogen-free swine in the safety equation. *Xenotransplantation* 2020; **27**: e12595.
92. Hartline CB, Conner RL, James SH, *et al.* Xenotransplantation panel for the detection of infectious agents in pigs. *Xenotransplantation* 2018; **25**: e12427.
93. Schuurman H-J. Chapter 2: Source pigs. *Xenotransplantation* 2009; **16**: 215
94. Pierson RN 3rd, Burdorf L, Madsen JC, Lewis GD, D'Alessandro DA. Pig-to-human heart transplantation: who goes first? *Am J Transplant* 2020; **20**: 2669.
95. Pierson RN 3rd, Fishman JA, Lewis GD, *et al.* Progress toward cardiac xenotransplantation. *Circulation* 2020; **142**: 1389.
96. Kögel J, Thiersch S, Ludwig B, *et al.* What does it take to consent to islet cell xenotransplantation? Insights from an interview study with type 1 diabetes patients and review of the literature. *BMC Medical Ethics* 2021; **22**: 37.
97. Cecka JM, Calculated PRA. (CPRA): The new measure of sensitization for transplant candidates. *Am J Transplant* 2010; **20**: 26.
98. Knoll MF, Cooper DKC, Bottino R. How the COVID-19 pandemic may impact public support for clinical xenotransplantation in the United States? *Xenotransplantation* 2020; **27**: e12623.
99. Hundrieser J, Hein R, Pokoyski C, *et al.* Role of human and porcine MHC DRB1 alleles in determining the intensity of individual human anti-pig T-cell responses. *Xenotransplantation* 2019; **26**: e12523.