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Review

FUTURE THERAPY FOR END-STAGE RENAL FAILURE: GENE-EDITED PIG KIDNEY XENOTRANSPLANTATION

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Summary

In suitable patients with end-stage renal disease, the transplantation of kidneys from living or deceased human donors offers a much-improved quality and length of life. However, the availability of donor kidneys is grossly inadequate. Gene-edited pigs might provide an alternative source of kidneys for clinical transplantation (xenotransplantation). However, there are major pathobiological barriers to successful pig kidney transplantation in human or nonhuman primate (NHP) recipients. These have steadily been overcome by a combination of (i) genetic engineering of the organ-source pig, and (ii) the administration of novel immunosuppressive agents.

Pig kidney transplants have now supported immunosuppressed (anephric) NHPs for periods in excess of a year, although this cannot yet be achieved consistently. Studies of pig kidney function after transplantation indicate that the pig kidney can likely fulfill all of the requirements of a human kidney. Potential infectious complications are likely to be similar to those seen in any immunosuppressed patient, and the potential risks of infection with a pig microorganism will be minimized by the breeding and housing of the organ-source pigs in biosecure 'clean' environments.

The attitudes of patients, their family members, healthcare providers, and the public appear to be positive towards xenotransplantation if it will be life-saving. For the first clinical trial, we suggest that patients on the wait-list aged 55-65 years in good physiological condition with blood group O or B (and who are possibly diabetic) who are unlikely ever to receive a deceased human donor kidney (because of death or the development of comorbidities that result in their removal from the waitlist) might accept a pig kidney if it will negate the need for dialysis for one or more years.

Key words: clinical trials, gene-editing, immunosuppressive therapy, kidney, pig, xenotransplantation

Abbreviations

HLA: human leukocyte antigen
NHP: nonhuman primate
PERV: porcine endogenous retrovirus
SLA: swine leukocyte antigen
TKO: triple-knockout

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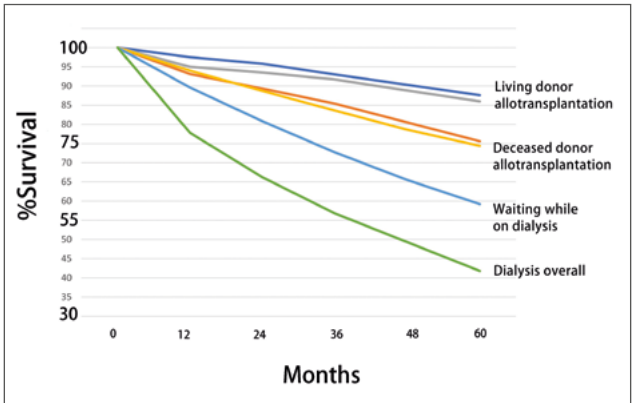
INTRODUCTION

Until now, patients with end-stage renal disease who need kidney transplantation continue to face a critical shortage of kidneys from deceased human donors. In the USA, the median time to receive a donor kidney is 3.9 years, but patients of blood groups B or O experience significantly longer waiting periods. Of waitlisted patients, approximately 45% will have been removed from the waitlist within 5 years (because of death or no longer being acceptable candidates) even with the help of dialysis and other adjuvant therapy (Fig. 1) [1-3].

The shortage of deceased donor organs is a worldwide problem, and alternatives to the transplantation of deceased human organs need to be explored. The most likely alternative is xenotransplantation (cross-species transplantation), specifically the transplantation of gene-edited pig kidneys into human recipients. Unlike the heart, with the exception of dialysis, mechanical replacement or support of the kidney is in its infancy. The major alternatives are regenerative medicine and/or stem cell therapy [4]. Given the complexity of the cellular functions in the kidney, bioengineering of new kidneys will be difficult and unlikely to provide a solution within the foreseeable future [5].



— All living donor allografts (USRDS annual data, 2017) [1].

— ALiving HLA-incompatible donor allograft (Orandi BJ, 2016) [2].

— ADeceased donor allograft while on dialysis (USRDS annual data, 2017) [1].

— ADeceased donor allograft while on dialysis (Orandi BJ, 2016) [2].

— AWaiting for a deceased donor allograft while on dialysis (Orandi BJ, 2016) [2].

— AAll patients on dialysis (USRDS annual data, 2017) [1].

Figure 1. Percentage survival of patients with end-stage renal disease by treatment modality (from Jagdale et al., 2019, mod. [3], and based on data from two sources (i) USRDS 2017 [1], and (ii) Orandi 2016 [2]).

PIONEERING CLINICAL ATTEMPTS OF XENOTRANSPLANTATION

In the early 20th century, both before and after the pioneering surgical work of Alexis Carrel, who developed the technique of blood vessel anastomosis, several attempts at nonhuman kidney transplantation were carried out, with little or no success [6]. In the 1960s, Keith Reemtsma carried out six kidney transplants from chimpanzees to patients in terminal renal failure [7]. Reemtsma believed that this experimental approach was ethically justified because chronic dialysis was available to only a very small number of patients at that time, and the number of deceased human donor kidneys that became available was very limited.

Five of Reemtsma's six patients died from organ rejection or infection within approximately 10 weeks. However, one patient lived for 9 months, and returned to work as a schoolteacher. Her sudden death was thought to be from an electrolyte disturbance. At autopsy, the chimpanzee kidneys showed no features of rejection, and the patient's native kidneys were clearly very diseased (Fig. 2). It is perhaps remarkable that even this one patient did so relatively well with the primitive immunosuppressive therapy available at the time (azathioprine and corticosteroids). Other surgeons soon followed Reemtsma's lead, carrying out clinical kidney xenotransplantation from nonhuman primates (NHPs) [6].

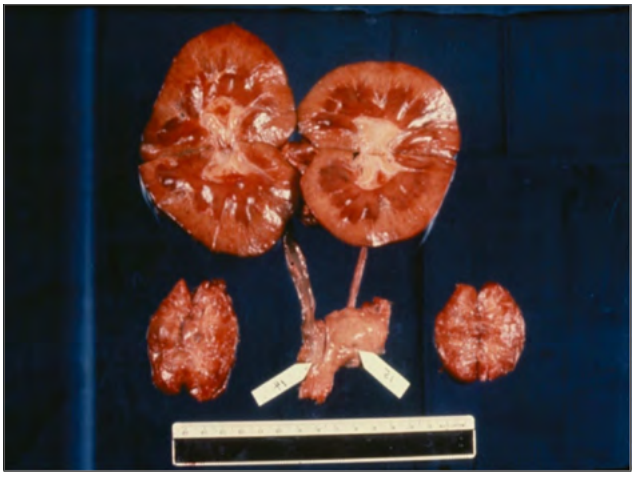


Figure 2. Macroscopic appearance of the two chimpanzee kidneys (top) and the two native kidneys (bottom) at necropsy 9 months after transplantation. The chimpanzee kidneys were macroscopically normal, and microscopically showed no features of rejection.

REASONS WHY THE PIG (RATHER THAN A NHP) HAS BEEN SELECTED AS THE POTENTIAL ORGAN-SOURCE FOR HUMANS

From an immunologic perspective, NHPs would be the preferred sources of organs for transplantation into humans, but virtually all of these species are either endangered or are too small to provide organs suitable for transplantation into large adult humans. Furthermore, concerns have been raised about the transmission of infectious agents from NHPs to humans, particularly since most NHPs are either wild-caught or have been housed under colony conditions for relatively few generations. The time and expense of breeding these animals in captivity are also prohibitive, as is a lack of experience in genetically modifying them. In addition, many members of the public would object to the use of NHPs on ethical grounds [8].

The pig has been identified as the species most likely to be the source of organs for clinical xenotransplantation, and in recent years research efforts have been directed toward pig-to-NHP transplantation. There are several advantages for using the pig as an organ-source [9]. However, a major disadvantage is that the human and NHP immune response to organs from wild-type (i.e., genetically-unmodified) pigs is rapid and intense, resulting in hyperacute rejection.

ADVANTAGES AND DISADVANTAGES OF XENOTRANSPLANTATION OVER ALLOTRANSPLANTATION

There are several potential advantages of xenotransplantation when compared to allotransplantation. Perhaps most important, xenotransplantation provides us with the first real opportunity (in > 70 years of clinical transplantation) of modifying the donor, rather than just treating the recipient. The more we can do to the donor, the less we will need to do to the recipient. This should eventually result in the need for minimal or no immunosuppressive drug therapy, leading to fewer adverse events.

OVERCOMING THE IMMUNE RESPONSE

The immunobiological response to a pig xenograft is complex and has been reviewed elsewhere in this issue. It has largely been overcome by (i) gene-editing of the organ-source pigs (to reduce the effect of the innate immune response), and (ii) the administration of novel immunosuppressive therapy (to suppress the adaptive immune response).

Gene-editing in pigs

The biotechnology of gene modification in pigs has seen a slow but steady evolution (Fig. 3) [10,11]. The most recent innovative genetic modification tool, CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats [CRISPR] and CRISPR-associated [Cas] proteins) [12], has greatly facilitated the process, making it less expensive and quicker, and has initiated an exciting new era of genetic engineering. Numerous gene-edited pigs have been introduced, and now 10 or more genetic modifications can be made simultaneously in a single pig [13]. These include (i) the deletion of expression of xenoantigens, and (ii) the transgenic introduction of human protective proteins expressed on pig cells. It is not yet certain how many genetic modifications are absolutely essential to protect the pig organ from the human innate immune response.

Immunosuppressive therapy

In 2000, Buhler and his colleagues were the first to clearly demonstrate that conventional (cyclosporine-based) immunosuppressive therapy did not prevent sensitization to pig xenoantigens from occurring [14]. However, they also demonstrated that this response to pig xenoantigens could be prevented (or at least delayed) by administration of an anti-CD154mAb to the NHP recipient. Importantly, blockade of the CD40/CD154 co-stimulation pathway was successful, but blockade of the B7/CD28 pathway, e.g., by CTLA4-Ig, was less so. Even after transplanting kidneys with up to 6 genetic manipulations, this conclusion has been confirmed (Fig. 4) [15]. Blockade of the CD40/CD154 co-stimulation pathway has formed the basis of all successful immunosuppressive regimens in xenotransplantation from 2000 until the present day [16,17].

The anti-CD154mAbs available at that time were found to be thrombogenic [18-20], resulting in their withdrawal for several years until the recent introduction of Fc-modified anti-CD154 agents that are not thrombogenic. Co-stimulation blockade is currently combined with a conventional agent, e.g., rapamycin or mycophenolate mofetil (MMF)(Tab. I) [21].

RECENT PROGRESS IN THE PRECLINICAL PIG-TO-NHP KIDNEY XENOTRANSPLANTATION MODEL

Based on (i) the innovative biotechnology for pig gene modification aimed at reducing the effect of the primate immune response to the xenograft, and (ii) the administration of novel immunosuppressive agents that block the CD40/CD154 co-stimulation pathway, significant progress has been made in the pig-to-NHP kidney xenotransplantation model [22-26]. These advances have led to prolonged survival of pig kidney grafts in NHPs, and today survival is being recorded in months or years.

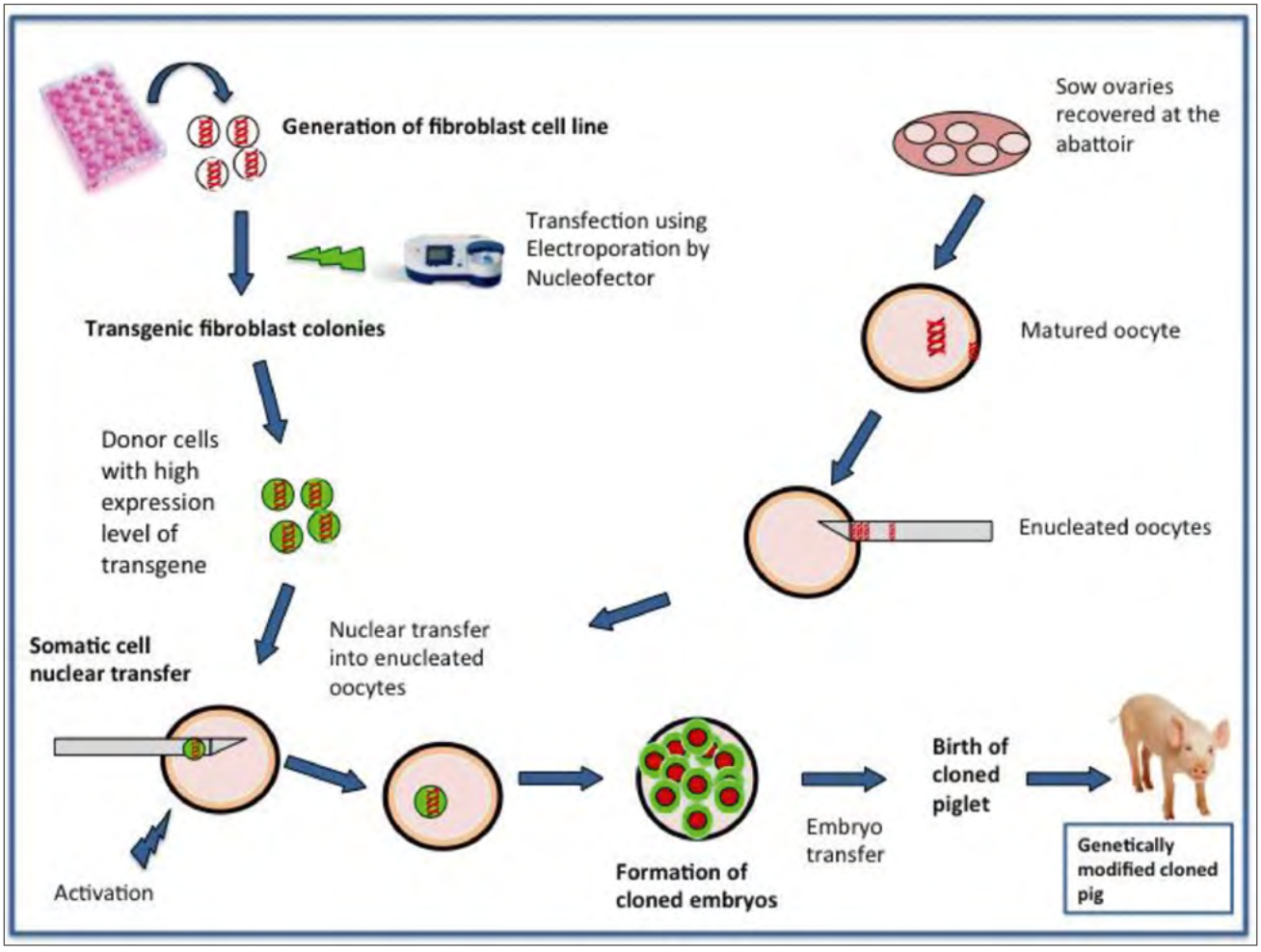


Figure 3. Steps involved in somatic cell nuclear transfer (SCNT). (Reprinted with permission from Eyestone, 2020) [11].

Table I. Representative immunosuppressive and adjunctive agents administered in pig-to-NHP kidney transplantation experiments.

Agent	Dose (duration)
Induction	
Thymoglobulin (ATG)	5 mg/kg i.v. (days -3 and -1) (to reduce the $CD3^+$T cell count to < 500/mm^3)
i-CD20mAb (rituximab)	10 mg/kg i.v. (day -2)
C1-esterase inhibitor	17.5U/kg i.v. on days 0 and 2
Maintenance	
Anti-CD40 OR anti-CD154 monoclonal antibody (mAb)	25-50 mg/kg (days 0, 2, 7, 10, 14, and weekly)
Rapamycin	0.02-0.06 mg/kg i.m. x2/day (target trough 6-10 ng/ml) beginning on day -5
Methylprednisolone	10 mg/kg/d on day 0, tapering to 0.25 mg/kg/d by day 7
Adjunctive	
Aspirin	40 mg p.o. (alternate days), beginning on day 4
Erythropoietin	2,000 U i.v. x1-2 weekly (if Hct < 25)
Ganciclovir	5 mg/kg/d i.v., when the baboon is sedated for blood draws (x 2 weekly)
Valganciclovir	15 mg/kg/d p.o., beginning on day 15

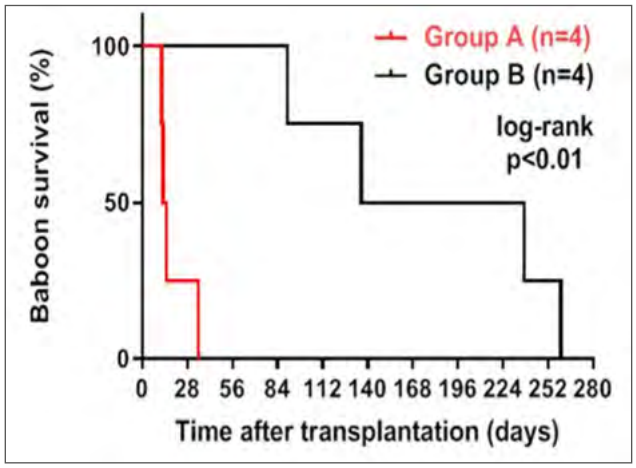


Figure 4. Pig kidney graft survival in baboons receiving either conventional (tacrolimus-based; Group A) or anti-CD40mAb-based (Group B) immunosuppressive therapy. Median pig kidney graft survival in Group B (186 days) was significantly longer than in Group A (13 days) ($p < 0.01$). (Reproduced with permission from Yamamoto, 2019) [15].

POTENTIAL RISK OF INFECTION, INCLUDING PORCINE ENDOGENOUS RETROVIRUSES (PERV)

The current opinion of experts in the infectious complications that occur in immunosuppressed patients with allografts is that the incidence and nature of these complications is likely to be similar after xenotransplantation [27]. The organ-source pigs will be bred and housed in biosecure, environmentally-controlled conditions, and should be free of all relevant pathogenic microorganisms, e.g., cytomegalovirus. Indeed, from an infection perspective, they should be preferable sources of organs than most deceased human donors.

The single topic that has given most concern over the past 30 years is the presence of porcine endogenous retroviruses (PERVs) within the genome of every pig cell, and which therefore will inevitably be transferred to the recipient with the organ [28-30]. Although humans have similar species-specific viruses and virus particles in every cell, which do not appear to be pathogenic in the hosts, the question has been raised of whether PERVs will be pathogenic in humans. Although PERVs can be transmitted from pig cells to human cells under special laboratory conditions [29,31], there has been no evidence that humans exposed to pig tissues, e.g., spleen cells, skin grafts, etc. or NHPs with functioning pig organ grafts have experienced any complications from PERV [32]. However, although expert opinion is that the risk is low, a conclusive answer will not be known until clinical trials with long-term follow-up take place.

If necessary, genetic engineering can be utilized to negate this potential problem by either inactivating the PERVs [33,34] or by deleting them from the pig [35]. Indeed, pigs are currently available in which PERVs have been inactivated [35].

NON-IMMUNOLOGICAL ASPECTS OF PIG KIDNEY XENOTRANSPLANTATION

Rapid post-transplant growth of the pig kidney

As long ago as 2000, it was observed that a pig kidney grew rapidly in the first few weeks after transplantation into a NHP (as if it were still in a rapidly-growing pig) [36]. The cause was uncertain, but this phenomenon has been more recently confirmed by others [23,37,38]. After approximately 3 months, its rate of growth may reduce to equate with that of the recipient baboon. It is presumed that there is an innate factor that results in this early growth.

To prevent the rapid growth of the organ-source pig and of its organs after xenotransplantation, growth hormone receptor gene-knockout (GHRKO) was undertaken, as suggested by Hinrichs et al. [37,39]. After pig heart transplantation in NHPs [40], this proved successful, and thus this manipulation was included in the pig used in the clinical heart xenotransplant in Maryland [41].

Although perhaps not essential in pigs to be sources of kidneys (as there is more space in the abdomen) whether GHRKO is indicated remains uncertain. The transplantation of kidneys from GHRKO pigs into NHP recipients was initially associated with a high incidence of ureteric complications (Cooper DKC, unpublished), the cause of which is not yet fully understood. Nevertheless, post-transplant growth of the kidneys was reduced.

An alternative is to select a miniature pig, e.g., Yucatan, as the source of the organs. When this is done, growth of the kidney after transplantation is no longer problematic [22].

Pig kidney function

Recent data from NHPs in which the immune response appeared to have been well-controlled have indicated normal serum creatinine levels for months or even years in some cases, with a low level or an absence of proteinuria, and maintenance of normal levels of serum albumin, but with low levels of serum phosphate and high levels of calcium [42,43]. We have recently measured glomerular filtration and tubular secretion in a small series of baboons with life-supporting pig kidneys and find it to be within the normal range [44].

The renin-angiotensinogen-aldosterone system (RAAS)

A syndrome of hypovolemia/dehydration has been described in baboons with a well-functioning pig kidney graft, in which, even though fluid intake appears to be adequate, the baboon becomes fluid-depleted [45]. As a result,

the serum creatinine rises (in the absence of any features of rejection either clinically, e.g., proteinuria, or on renal biopsy). The intravenous or subcutaneous administration of normal saline brings about an almost immediate normalization of the serum creatinine.
It was considered possible that this may result from dysfunction of the renin-angiotensinogen-aldosterone system, as it had been suggested that pig renin does not function in primates [46]. However, recent studies suggest that the renin-angiotensin-aldosterone system remains functional, though angiotensin II levels are somewhat reduced [47].

Erythropoietin

There has been doubt as to whether pig erythropoietin functions adequately in primates, or whether the human (or NHP) recipient of a pig kidney would become anemic [42]. However, NHPs with a life-supporting pig kidney do not become anemic even when no recombinant erythropoietin is administered (Adams AB, personal communication). This appears to be convincing evidence that pig erythropoietin is sufficient to prevent anemia from developing in primates. In clinical kidney xenotransplantation, the native kidneys are left in situ, which may be a source of human erythropoietin. If proved essential, the pig could be genetically - engineered to produce human erythropoietin.
The fact that numerous NHPs have remained healthy and active for > 1 year after pig kidney transplantation, in one case for > 4 years (Adams AB, personal communication), surely confirms that the pig kidney function is sufficient to support an immunosuppressed NHP, and therefore probably a human recipient.

WHAT RESULTS DO WE NEED TO ACHIEVE IN THE PIG-TO-NHP MODEL TO JUSTIFY A CLINICAL TRIAL?

It has been suggested that the optimal pigs for clinical kidney transplantation today are TKO (Gal, Neu5Gc and Sda knock-out) with expression of six human protective proteins, including complement-regulatory [CD46, CD55], coagulation-regulatory [thrombomodulin, endothelial protein C receptor], the anti-inflammatory/antiapoptotic peptide, hemeoxygenase-1 [HO-1], and CD47 [48].
Using these (or similar) pigs as sources of organs, there is increasing evidence from studies in NHPs that, when combined with an effective immunosuppressive regimen, prolonged pig kidney graft survival in human recipients would be likely.
We would therefore suggest that survival of 6 of 8 or 10 NHPs supported by a pig kidney for at least 6 months, with some remaining healthy for 12 months in the absence of features of graft rejection or other life-threatening complication, e.g., infection, malignancy, would be sufficient to initiate a limited initial clinical trial.

SELECTION OF PATIENTS FOR THE INITIAL CLINICAL TRIALS

We suggest that the results of the initial pig kidney xenotransplant clinical trials should be compared with those for comparable patients maintained on chronic dialysis, but not with those receiving kidney allografts. In order to provide the patient with a realistic chance of benefitting from pig kidney transplantation and to assess the potential of xenotransplantation, the patient should be fully acceptable for kidney allotransplantation with an absence of major comorbidities or chronic infection. To select patients who are unlikely to survive after receiving an allograft (e.g., from general frailty, chronic infection, or previous or current neoplasia) would not prove to be an adequate trial of xenotransplantation, as the patient would be equally unlikely to survive.

Patients on the waitlist who will likely never receive an allograft

We have suggested that pig kidney transplantation should be offered to patients who are unable to receive a timely allograft [49]. It would be ethical to select patients whose life expectancy is less than the time it will take for them to obtain a deceased human donor organ. In the USA, the median waiting period for a patient with end-stage renal disease to obtain a human donor kidney is approximately 4 years [1]. Approximately 35% of transplant candidates may have died or been removed from the waitlist within this period of time (Fig. 1). Those of blood group B or O may spend a significantly longer period on the waitlist, sometimes > 7 years, even if the candidate has no antibodies directed to human leukocyte antigens (HLA) [1].
We therefore suggest that the patient should be of an age where it is unlikely that he/she will survive until a deceased donor becomes available. Patients in their early sixties, if in a good physiological state and without comorbidities (except possibly diabetes), may prove to be the candidates who are most likely to benefit from a pig kidney transplant.
For example, in USA, if the patient is aged 55-65 at the time, there is a realistic possibility that he/she will either have died or, because of the development of comorbidities, become an unacceptable candidate for a kidney transplant before a deceased human donor kidney becomes available for them (Fig. 1). If the patient is also diabetic, after only 2 years on the waitlist, many patients have a greater chance of dying than of obtaining a kidney allograft. We therefore suggest that patients aged 60-65 years of blood groups O or B who are diabetic should be

offered pig kidney xenotransplantation. At present, the FDA recommends that a patient should only be considered for a pig organ transplant if his or her life expectancy is anticipated to be < 2 years, but many patients who are in this category have already been supported by dialysis for several years and have developed comorbidities that make them no longer ideal transplant candidates.

Patients in whom vascular access for dialysis is becoming difficult could be considered, but again many of these will have been undergoing dialysis for a prolonged period of time and, for this and other reasons, may not be suitable candidates for inclusion in an initial clinical trial. Selection of the initial patients, therefore, will require very careful consideration. A method of predicting the potential benefit of a pig kidney transplant would be valuable. This could possibly be indicated by a similar method to that suggested by Bae et al. [50]. On the basis of the Kidney Donor Profile Index and the Estimated Post-Transplant Survival, this group calculated the predicted survival of a patient on the waitlist and compared it with the predicted survival after kidney allotransplantation, A similar approach is worth exploration in relation to xenotransplantation.

The HLA-sensitized patient

Many patients with antibodies to HLA do not appear to be at any increased risk
of rejection of a pig kidney graft [51-53]. However, in a small number of patients there may be cross-reactivity between anti-HLA antibodies and swine leukocyte antigens (SLA) [51-53], although the incidence of this is low [51]. Therefore, if patients with anti-HLA antibodies that do not cross-react with SLA are identified by in vitro assays [54], then these patients should be acceptable for the initial clinical trials. Methods are being developed to delete or replace specific SLA against which there might be cross-reactivity [55,56].

Of considerable importance, if a patient receives a pig kidney that is rejected with the development of new anti-pig antibodies, e.g., against SLA. the current (limited) evidence is that this will not preclude subsequent successful allotransplantation [51]. In clinical trials, therefore, although intended as destination therapy, the pig kidney graft could effectively act as a bridge to allotransplantation.

Although a pre-emptive transplant might be most beneficial, for the first clinical trial we suggest that recipient selection should be limited to those already on dialysis. This removes the additional variable of native renal function from the interpretation of the study results. Furthermore, if the patient is already requiring dialysis, there will be no doubt that renal failure had advanced enough to warrant a kidney transplant; the patient and his/her family will be convinced that kidney failure had progressed to the point where death would have occurred if dialysis had not been initiated.

Our current proposed inclusion and exclusion criteria for patients for a clinical trial of pig kidney xenotransplantation are similar in many respects to those used in the selection of patients for allotransplantation, though possibly somewhat more vigorous and with some xenotransplantation-specific exclusion criteria [49]. We anticipate that, when clinical pig kidney transplantation is proved successful, the selection criteria will steadily be relaxed.

Other patients who could be considered as possible candidates for xenotransplantation are those with an underlying disease that has recurred in a second or even third allograft [57]. It is unknown whether any of these diseases will recur in a pig kidney but, even if they do, the xenograft will have allowed for the allocation of an allograft to another recipient where it may have been better utilized. For the first clinical trial, however, we suggest that these patients, particularly those in whom the disease might recur rapidly, e.g., focal segmental glomerulosclerosis (FSGS), may not be ideal candidates.

Provision of realistic information on prognosis for waitlisted patients

In the USA, the median waiting time for a deceased human kidney (approximately 4 years) indicates the time it will take for half of the potential recipients to undergo kidney transplantation. Patients removed from the waitlist are not included in this calculation. Importantly, information on median waiting time obscures the fact that, because of death while on the waitlist or being removed from the waitlist because they are no longer considered to be acceptable recipients, a majority of candidates on the waitlist do not receive an allograft. We suggest that patients on hemodialysis, as well as their physicians, may be too optimistic with regard to their likelihood of survival. We recommend that, as part of their counseling, patients should be provided with a more accurate prediction of outcome so that they can make a more informed decision as to whether to take the risk of accepting a pig kidney graft.

PUBLIC AND PROFESSIONAL (DOCTORS AND NURSES) ATTITUDES TO CLINICAL XENOTRANSPLANTATION

There have been numerous surveys of relatively small groups within the community, and also of members of the healthcare professions [58-61]. The following points summarize the observations made from these studies: (i) There is considerable public support for use of pig organs for transplantation, as long as the results are likely to be comparable to those of allotransplantation (which, of course, cannot be guaranteed and indeed is currently unlikely). Support waned if this was not anticipated to be the case; (ii) Patients awaiting organ transplantation and their family members were

much more positive about xenotransplantation than those with no personal need for this form of therapy; (iii) The attitude of many patients surveyed indicated that, if there is no realistic alternative therapy, xenotransplantation would be fully acceptable; (iv) Religious influences were not as strong as we anticipated, and many Christians, Jews, and Muslims saw no reason why xenotransplantation should not be acceptable if it would be life-saving; (v) African-Americans were less likely to support a clinical trial than Caucasians; (vi) Considerable education of the public will be required before there is a general acceptance of xenotransplantation as a treatment option. The attitudes of doctors and nurses towards xenotransplantation were generally favorable [60,61].

ETHICAL ASPECTS OF CLINICAL XENOTRANSPLANTATION

Ethical considerations in xenotransplantation have been discussed for many years [7]. Many are similar to those raised with regard to allotransplantation, and others relate to animal welfare or to the role of the biotechnology industry. However, the significant advantages of xenotransplantation over allotransplantation must not be overlooked, e.g., negating the illegal trade in organs from living human donors, and eliminating the (small) risk associated with the excision of kidneys from healthy altruistic living donors. The question of whether the recipient of a pig organ, who will need to be monitored for potential pig-related complications throughout life, can withdraw from a clinical trial has been raised [62].

There will always be those who object to the use of animals, but the fact that in the USA alone more than 100 million pigs are slaughtered each year for food reduces the concern for using pigs for xenotransplantation. Organ-source pigs will be housed under ideal conditions and will be euthanized under anesthesia after the surgical removal of the organs. This will be much more humane than the methods of killing pigs in industrial farming facilities, and will ensure that the detrimental effects of brain death are not present in the organs.

REGULATION OF CLINICAL XENOTRANSPLANTATION

The national regulatory authorities in a few countries have published guidelines for those contemplating clinical trials of xenotransplantation. Many of these relate to the potential infectious risks, e.g., related to PERVs, and include the requirement that tissues and body fluids from the organ-source pig will need to be archived for many years, as will tissues and body fluids from the human recipient.

FUTURE DEVELOPMENT OF CLINICAL PIG KIDNEY XENOTRANSPLANTATION.

Organs, tissues, and cells from genetically-modified pigs have massive clinical therapeutic potential. We envisage further gene-editing to protect the organ from the human adaptive immune response, thus enabling exogenous immunosuppressive therapy to be significantly reduced or, indeed, ultimately unnecessary. For example, pigs have already been produced in which expression of SLA class 1 has been deleted [63] or SLA class II has been downregulated [64], or in which PD-L1 [65,66] has been expressed.

The ultimate goal of both allotransplantation and xenotransplantation is the induction of immunologic tolerance, in which the recipient no longer attempts to reject the graft. Although efforts in this respect in xenotransplantation have to date been unsuccessful, in view of the potential offered by genetic engineering of the pig, it would seem it is more likely to be achieved in xenotransplantation than in allotransplantation.

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Conflict of interest statement

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Author contributions

Data were collected by LW, QW and DKCC; the article was prepared by LW, QW and DKCC, and revised and approved by all authors.

Ethical consideration

Not applicable.

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