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COMMENTARY

Pig-to-human kidney transplantation using brain-dead donors as recipients: One giant leap, or only one small step for transplantkind?

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Abstract

Pig kidney xenotransplantation is increasingly regarded as a realistic solution to the current shortage of human organ donors for patients with end-stage organ failure. Recently, the news of three pig-to-human transplantation cases has awakened public interest. Notably, the case by the Alabama team reported detailed and important findings for the xenotransplantation field. Using a genetically modified pig, two porcine kidneys were transplanted into a brain-dead recipient. They applied several approaches established in the preclinical NHP study, including gene-edited pig kidney graft and preoperative laboratory inspection such as crossmatching and infection screening. The pig-to-human kidney xenotransplantation had no unexpected events during surgery or evidence of hyperacute rejection. Unfortunately, the grafts did not work appropriately, and the study had to be terminated due to the decompensation of the recipient. While this study demonstrated the outstanding achievement in this research area, it also revealed remaining gaps to move xenotransplantation to the clinic. While brain-dead human recipients could reinforce the compatibility achievements of gene-edited pigs in NHP, their pro-inflammatory and pro-coagulant environment, in combination with short-duration of experiments will limit the assessment of kidney function, infection and rejection risk post-transplant, in particular antibody-mediated rejection. The use of successful immunosuppressive protocols of non-human primates xenotransplant experiments including anti-CD154 antibody will be critical to maximize the success in the first in-human trials.

KEYWORDS
immunosuppression, rejection, xenotransplantation

1 | INTRODUCTION

Despite constant efforts to increase the donor pool, over 96 000 patients with end-stage kidney disease are waitlisted in the US. To address this organ shortage, the use of other organ sources, especially pigs, has been investigated for a long time. With the progress of gene-editing technology,[1–4] two significant barriers to xenotransplantation have been overcome, including the removal of porcine endogenous retroviruses and of pig carbohydrates, against which humans have natural antibodies.[5–8] In September 2021, the first public news about a pig-to-human kidney transplantation in NY was received with great excitement despite the lack of details about the outcomes. Sequentially, the team from University of Maryland reported the first pig-to-human heart transplant case in a heart

TABLE 1 Genetic modifications of 10-GE pigs

Genes	Knock-in/ knock-out	Function
hCD46	Knock-in	Complement inhibitor
hDAF	Knock-in	Complement inhibitor
hTBM	Knock-in	Anti-coagulant
hEPCR	Knock-in	Anti-coagulant
hCD47	Knock-in	Immune regulation
hHO1	Knock-in	Immune regulation
pGGTA1	Knock-out	Pig carbohydrate antigen (Gal)
pβ4GalNT2	Knock-out	Pig carbohydrate antigen (DSa)
pCMAH	Knock-out	Pig carbohydrate antigen (Neu5Gc)
Pig growth hormone receptor	Knock-out	Growth hormone receptor

transplant candidate and University of Alabama reported another kidney xenotransplant case in a human decedent. Unfortunately, although all these cases should be regarded as an outstanding achievement, further detailed clinical information are still pending from most of these cases, except the pig-to-human kidney transplantation that has been reported by the team of University of Alabama.[9] Here, we discuss what we have learned from this case report from a clinical point of view.

2 | WHAT IS ACHIEVED IN THIS STUDY?

Locke's team presented five goals for her study, including demonstrating the feasibility of porcine genetic modification in the human setting, the documentation of prospective crossmatching assays, the absence of life-threatening intraoperative complications and infections, and establishing the appropriate practices for the future clinical trial. In the pig-to-NHP kidney transplant model, over 1 year of graft function has been achieved in recent studies using genetically engineered pig kidney graft.[6–8] The current achievements of pig-to-human organ transplantation in preclinical models are well described in other articles.[10,11]

Major obstacles to moving xenotransplantation have been focused on the risks of hyperacute rejection and thrombosis. Hyperacute rejection occurs within minutes and leads to lethal damage to the graft, by which pre-existing anti-pig antibody binding and following complement complex formation trigger this catastrophic cascade.[12] To avoid this, genetically modified pigs were used as donors.[13] The pig used in this study had 10 genetic modifications (10-GE pigs, Table 1, Figure 1). These genetic modifications were expected to avoid hyperacute and acute-phase graft injury. Some human genomes were inserted to avoid complement formation (hDAF, hCD46),[14–17] inhibit coagulation factor activation (hTBM, hEPCR),[18–22] and modulate the immune response (hCD47, hHO1).[22–24] In addition to human gene insertion, some pig-derived genes were deleted to avoid antibody-mediated hyperacute graft injury (pGGTA1, pβ4GalNT2, pCMAH)[25–28] and the graft overgrowth after implantation (pig growth hormone receptor, which is discussed later).[29] In combination, these genetic modifications were expected to prevent hyperacute antibody-mediated rejection and overactivation of the complement pathway that could lead to microvascular thrombosis. Indeed, the authors mentioned that after implantation of 10-GE pig kidney, there was no evidence of these conditions except for a transient presence of thrombotic microangiopathy (TMA) on the glomeruli that resolved at day 3. In addition, pretransplant histocompatibility testing was also performed to detect any preformed antibody against the donor pig kidney, followed by prospective flow cytometer crossmatches. Selection of the most compatible donor pig-recipient pair has been shown to be essential to minimize complications and improve graft survival.[30,31]

Infection agents of the donor pig were tested before transplantation. Comprehensive screening for pathogens of donor pig is required to avoid adverse outcomes that are derived from pig-to-human transmission after transplantation.[32] The authors screened the pathogens and almost all tests were negative, except for porcine endogenous retrovirus (PERV) A and B. PERV is one of the pathogens in which risks for humans are unclear.[33–35] Porcine-derived products, indicative of the transmission of the infectious agent, were not detectable in human blood after reperfusion, at least within 3 days though longer follow up would be required to ensure safety. In summary, the recent approach that aimed to avoid hyperacute graft rejection and ensure short-term recipient safety proved appropriate from this study.

3 | WHAT IS NOT ACHIEVED IN THIS STUDY?

Although the authors have shown that the previous approach for the NHP xenotransplantation model can be feasible for human models, we have to mention that there remain some critical points to be elucidated.

First, the transplanted kidney seemed not to work after implantation. Although the right kidney graft produced urine initially following the surgery, its actual glomerular filtration rate was minimal, and the amount of urine flow decreased over time. Moreover, the left kidney graft did not work following transplant. Reviewing the histology, the most significant finding was the tubular injury, while glomeruli were only transiently affected by the presence of TMA. TMA etiology is likely multifactorial, including complement activation, hypercoagulable state of brain-dead recipient, and the ischemia-reperfusion injury.[36] The systemic instability of brain-dead recipients can induce an uncontrolled hyperinflammatory state and endothelial damage. The recipient exhibited multiorgan dysfunction such as liver failure and coagulation/fibrinolysis disorder before transplantation, and these dysfunctions worsened after transplantation. That could promote endothelial activation, inflammation, and coagulopathy,[37–40] which can trigger the onset of TMA. Whether tubular necrosis was ultimately the cause of primary graft dysfunction is unclear,[41] and only a longer experiment documenting recovery would really confirm it as the dominant etiology.

Second, as the team had to terminate the study within 3 days in part due to physiologic instability of the recipient, it was not possible to

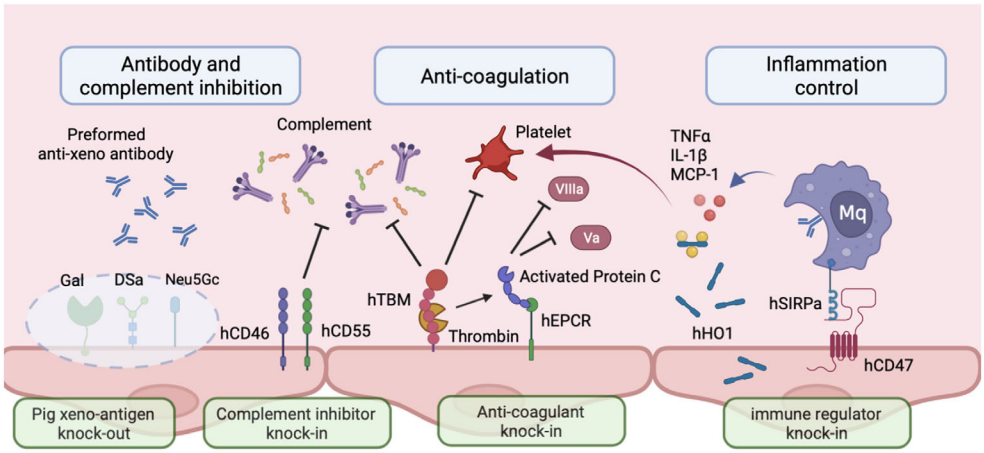

FIGURE 1 Illustration of the genetic modifications of 10-GE pigs and their potential impacto on the xeno-immune response

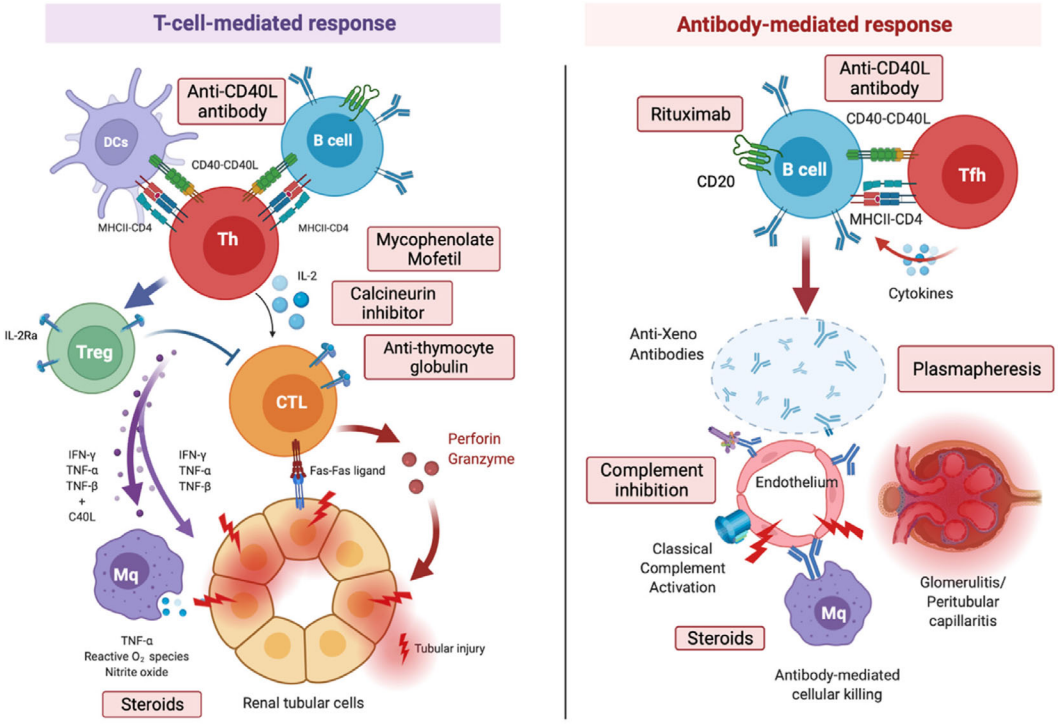

FIGURE 2 Immunosuppressive drugs proposed to be used in first clinical human xenotransplant trials (based on non-human primate xenotransplant experiments). DCs, dendritic cells; Th, helper T cells; Treg, regulatory T cells; CTL, cytotoxic T cells; Mq, macrophages; IL-2, interleukin 2; IFN, interferon; TNF, tumor necrosis factor.

assess the xeno-antigen specific immune response that may be generated within weeks or months of transplantation.[42] Indeed, recent meta-analyses indicated that the rate of antibody-mediated rejection is significantly higher in xeno- compared to allo-transplantation in NHP (42.4% vs. 5.8%).[43] In regards to the ideal immunosuppression, anti-CD154 antibodies have been shown to be crucial for the long-term survival of xenografts in nonhuman primates and previous NHP studies indicate that conventional immunosuppression currently used in clinical practice is not enough to prevent xenograft rejection and should likely be avoided in first in-human trials[17,44] (Figure 2). Several clinical studies demonstrate the superiority of costimulation blockade of the CD40-CD154 pathway compared to calcineurin-inhibitor-based immunosuppression.[7,8,30,45,46] There are a few anti-CD154 antibodies being studied in humans now and this will permit the use of an immunosuppressive protocol as close to the NHP successful experiments as possible so that any rejection risk could be minimized in the first xenotransplant human trials.

Third, concern about the risk of infection, both of the common pathogens for humans and novel pathogens from pigs into humans, could not be entirely excluded in such an extremely short observation period. Long-term observation to accurately evaluate the risk of porcine-derived pathogens infection is needed, especially for PERV, which can infect human cells in an in vitro study,[33,47] although in vivo transmission of PERV to humans had not been reported yet. Moreover, genetic modifications and absence of HLA expression on the graft have the potential to change its susceptibility to pathogens,[48,49] and it also requires a long-term follow-up to evaluate the potential vulnerability against infection. Along with preventing the transmission of pig pathogens to humans, the development of new microbiological assays, which could detect unexpected or ill-defined antigenic elements[50] may also be required.

How large transplanted pig organs will grow is also an important concern to avoid post-transplant enlargements that may trigger a compartment syndrome, in particular in organs such as the heart. To avoid this, the growth hormone receptor knock-out pig has been established.[29] However, it needs a longer observation period to determine whether the growth hormone receptor-deficient organ shows appropriate development and maturation or keeps its organ functioning as a normal organ after xenotransplantation.

4 | IS A BRAIN-DEAD RECIPIENT AN APPROPRIATE STEP TOWARD HUMAN TRIALS?

This study raises new concerns about whether brain-dead recipients can be used as an appropriate intermediate step toward human trials and if it reflects living human physiology properly. Although it can serve as a test for the safety of the very high-risk or unestablished procedures, there are some limitations to the use of brain-dead recipients. As discussed above, it is challenging to keep their homeostasis for a long time. Unfortunately, the authors had to terminate the study in a very short period due to the recipient's decompensation. Multiorgan dysfunctions such as abnormal liver function, anemia and thrombocytopenia, and increased coagulation/fibrinolysis were already observed at the pretransplant phase, and a surgical procedure involving bilateral nephrectomies and a kidney transplant could only worsen their instability. The uncontrolled hyperinflammatory status induced by surgical stress could damage the implanted organ and distort the expected results. Therefore, despite the reassuring findings of the short-term brain-dead recipient experiment, further advancements in xenotransplantation will need longer follow-up periods of 6–12 months to assess the xeno-specific immune response and other physiological compatibilities of the pig kidney and human body using living, physiologically stable recipients. Of course, while the transition to clinical trials should be considered with maximum carefulness and global consensus,[51] carefully selected dialysis candidates on the waiting list with poor outcome if remaining on dialysis for over 5 years should be the next proposed step in humans.[52]

5 | CONCLUSION

While this milestone study showed promising achievement in kidney xenotransplantation, it also pointed out some of the limitations of the decedent model and reinforced the opinion that a phase 1 clinical trial in humans is now warranted in order to permit an extended period of observation, longer-term graft function, the efficacy of immunosuppression protocol against acute and chronic rejection, and infectious monitoring. The study of xenotransplantation is now about to turn the corner, but we are also approaching another corner, and how long the corner could pass depends on the collaborative effort across investigators, medical societies, industry, and patient groups.

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CONFLICT OF INTEREST

The authors have no financial conflicts of interest to declare concerning this communication.

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