Expert Opinion Special Feature: Patient Selection for Initial Clinical Trials of Pig Organ Transplantation

Richard N. Pierson III, MD,[1] James S. Allan, MD,[1] David K.C. Cooper, MD, DPhil,[1] David A. D'Alessandro, MD,[1] Jay A. Fishman, MD,[1] Tatsuo Kawai, MD,[1] Gregory D. Lewis, MD,[1] Joren C. Madsen, MD, DPhil,[1] James F. Markmann, MD, PhD,[1] and Leonardo V. Riella, MD[1]

Recent developments in xenotransplantation have included the creation of pigs with multiple gene edits aimed at protecting their organs from the primate innate immune response and the availability of several novel immunosuppressive agents that block the CD40/CD154 T-cell costimulation pathway. Combining these approaches, the survival of life-supporting kidneys in nonhuman primates has extended to more than a year,[1, 2] and pig hearts have been found to support life for over 6 mo in baboons[3-5] and for 2 mo in a human (Griffith et al)[6].

The benefit and risk associated with a pig organ xenograft will be uncertain until clinical experience accumulates. This uncertainty cannot be resolved by preclinical studies alone[7,8] or by further organ transplants in deceased human subjects in which follow-up will likely be restricted to only a few days.[9-11] Thus, in our estimation, pilot clinical experimentation in parallel with preclinical studies is scientifically justifiable. In this context, ethical precepts established to guide biomedical research and clinical experimentation mandate that initial trials of xenotransplantation for each organ be designed on the basis of the best available evidence from preclinical studies and enroll patients lacking timely access to an allograft or who are expected to be served poorly by currently available therapeutic alternatives.

Because recent experimental success in the gene-edited pig-to-nonhuman primate model suggests that clinical trials of pig kidney and heart transplantation are warranted, this commentary will concentrate on these organs.

An initial clinical trial in kidney transplantation provides the option of return to dialysis should the xenograft fail or intractable infection necessitate discontinuation of immunosuppressive therapies and graft excision.[12] Such long-term life-supporting "rescue" therapies are not expected to be available to patients with a pig heart xenograft.[13] Nevertheless, transplantation of a pig heart in patients for whom no alternative therapy is available is ethically defensible; indeed, the transplant in Mr Bennett has illustrated its therapeutic potential and should yield valuable new insights regarding remaining barriers (Griffith et al[6]).

Allowing for situationally justifiable exceptions, the criteria used for the selection of patients for initial xenotransplantation trials should closely resemble those used to determine candidacy for allotransplantation. Medical or surgical comorbidities that are associated with significantly shorter survival (eg certain cancers, frailty, malnutrition, uncontrolled infection)[14] and severe extracardiac vascular disease that would technically limit the ability to perform the surgery safely should remain contraindications for xenotransplantation. As for other new experimental therapies (eg, allotransplantation in HIV-infected recipients), local expertise must be available to provide the clinical and scientific support required to optimize the investigation of the immunologic phenomena and to track infectious disease risks even beyond the survival of the xenograft.[14-17] Knowledge gained from clinical experience will inform improvements to clinical protocols and prioritize questions to be investigated in preclinical models. Current guidance regarding storage of blood and tissue samples,[14-17] although burdensome to patients, close contacts, and investigators, should be largely similar to those already practiced in association with allotransplantation in North America and Europe.

Across all organs, recipient sensitization to alloantigens presents a formidable barrier, both concerning access to a cross-match–negative donor-recipient pair, high waitlist mortality, and inferior outcomes after transplant. Although paired kidney exchanges provide access to a compatible allograft for some sensitized candidates with a living donor, many remain disadvantaged, particularly

Received 31 March 2022. Revision received 18 April 2022.

Accepted 22 April 2022.

[1] Center for Transplantation Sciences, Department of Surgery, Massachusetts General Hospital and Harvard Medical School, Boston, MA.

D.K.C.C., J.F.M., and J.A.F. are consultants to eGenesis Bio, Cambridge, MA. The other authors declare no conflicts of interest.

D.K.C.C., J.F.M., and R.N.P. receive xeno-related grant support from the National Institutes of Health (U19 AI090959, U01 AI146248, and UO1 AI153612) and the Department of Defense (W81XWH2010559; D.K.C.C.). D.K.C.C. and R.N.P. have previously received xeno-related research funding from Revivicor, a subsidiary of United Therapeutics and Lung Biotechnology PBC. For work in xenotransplantation, J.F.M., J.C.M., T.K., and R.N.P. have received research support from eGenesis, and T.K., D.K.C.C., and R.N.P. have received research support from Tonix.

All authors contributed substantially to the conception and design of the article. R.N.P. drafted the work, which was then critically revised by each other author, with each author contributing intellectually important content. Each author gave final approval of the revised, submitted version. All authors accept accountability for all aspects of the article.

Correspondence: Richard N. Pierson III, MD, Division of Cardiac Surgery, Department of Surgery, Cox 662, 55 Fruit St, Boston, MA 02114. (rpierson@mgh.harvard.edu).

ISSN: 0041-1337/20/1069-1720

DOI: 10.1097/TP.0000000000004197

<zdoi;10.1097/TP.0000000000004197>

those with very high panel-reactive anti-HLA antibodies, those with blood types B or O, and those who live in regions of the world with long waiting times, such as the Northeastern United States.[18,19] Thus, some HLA-sensitized patients with a negative cross-match against the organ-source pig could be considered for an initial xenograft clinical trial, provided they do not exhibit any cross-reactivity to swine leukocyte antigens from the organ-source pig.

Similarly, enrolling subjects under emergency circumstances that do not allow a thorough assessment of patient suitability should be avoided. Current or recently treated advanced malignancy or an active infection may cause life-limiting complications under immunosuppressive therapy. Adequate psychosocial support, a robust process of preprocedure education, and thorough informed consent for both the patient and his/her caregivers are important to encourage compliance with posttransplant treatment, monitoring, and public safety protocols. Adherence to such basic principles will enhance public support for xenotransplantation. Finally, in our estimation, studies should be designed to generate data that will improve the safety of subsequent xenograft procedures.

In the United States, patients on the waitlist have an approximate 45% likelihood of being removed from the waitlist within 5 y (Figure 1), either because of death or because of worsening health condition to the point that they would no longer be considered a suitable candidate for transplantation, for example, from the development of new comorbidities.[18,19] The likelihood of receiving a kidney within 5 y of listing varies in the United States between <20% and almost 80%, depending on the region in which the patient is listed; in some regions, patients with blood groups B or O may wait well beyond 5 y.[19] In Europe, similar variance exists in wait times and access to organs based on residency and blood type.

We suggest, therefore, that patients in the good physiological condition who are in their 60s or 70s, but who are likely to remain on the waitlist for >5 y might welcome a pig kidney graft to avoid the restrictions imposed by long-term dialysis while they remain on the waitlist for a deceased human kidney.[18,23] Therefore, the xenograft may

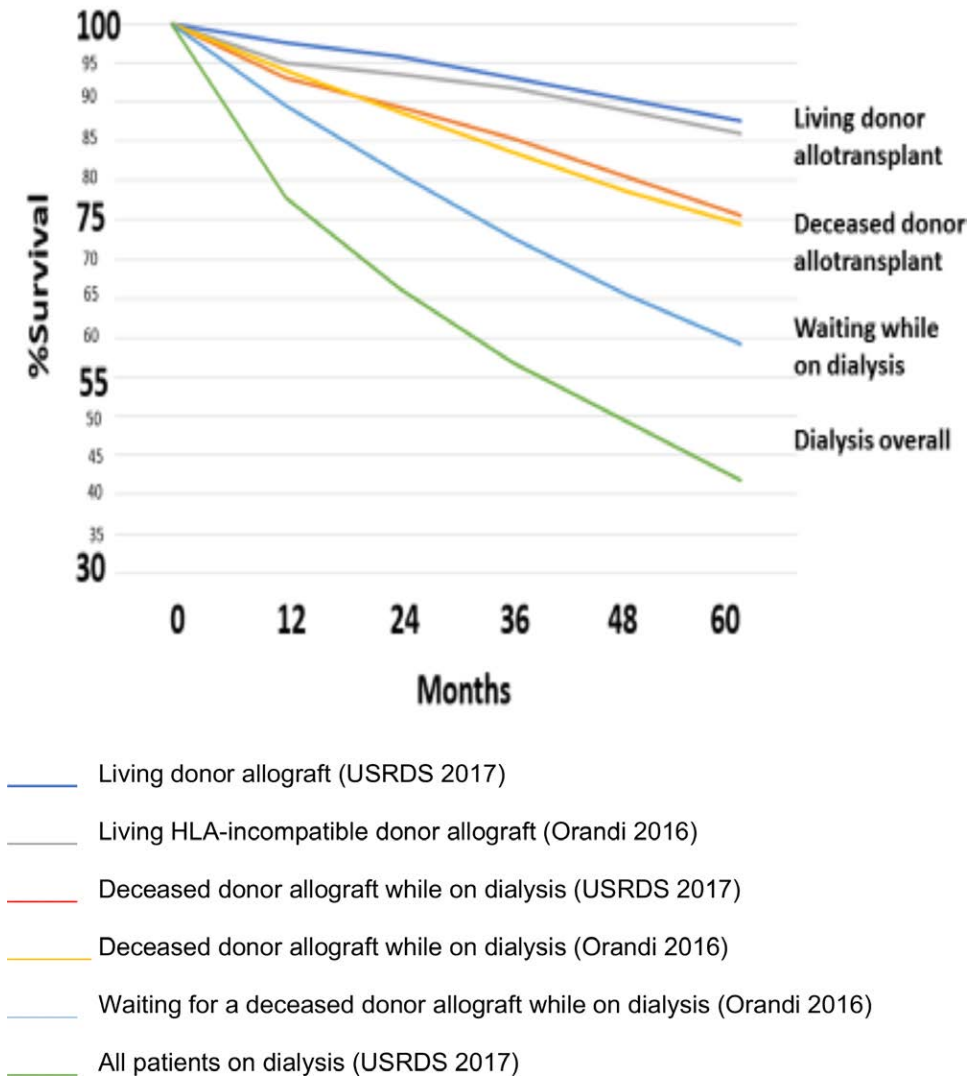

FIGURE 1. Percentage survival of ESRD patients by treatment modality in 2010 (reproduced with permission from Cooper et al[20]), and based on data from 2 sources (i) USRDS,[21] and (ii) Orandi BJ et al.[22] ESRD, end-stage renal disease.

serve as a bridge while providing important data to inform the design of destination therapy trials.

Other indications for pig kidney transplantation might include (1) a complete loss of vascular access for dialysis (although, in the event of graft failure, such a patient would not be able to rely on the backup of a return to dialysis), (2) high panel-reactive HLA antibodies that significantly limit compatible donor options (see comments above), and (3) rapidly recurrent kidney diseases, such as hemolytic uremic syndrome or focal sclerosing glomerulosclerosis in which a previous graft loss because of focal sclerosing glomerulosclerosis raises the risk of immediate recurrence to >80% on retransplantation.[23,24] However, these processes may confound the interpretation of clinical outcomes after xenotransplantation if the pig xenograft proves susceptible to the underlying disease. In contrast, if xenografts prove resistant to ≥1 of these disease mechanisms, then available allografts could be deployed to better long-term effect in other patient populations.

If a pig kidney transplant is even modestly successful under the above-mentioned conditions, then the availability of further gene-edited pig kidneys will enable the patient to receive a second or subsequent xenograft if deemed worthwhile, particularly if elicited immune mechanisms are not the principal driver of the initial xenograft failure.

Because the heart is a life-supporting organ, pig cardiac xenografts should be used initially as a "bridge-to-transplant" in patients for whom a subsequent allograft offers a viable "exit strategy," but for whom mechanical circulatory support options are limited or associated with predictably poor outcomes. Thus far, the longest reported survival of a baboon with a life-supporting pig orthotopic heart transplant is <9 mo[3-5]; based on Mr Bennett's case, long-term graft function is by no means assured (Griffith et al[6]). Graft and patient survival of months duration could provide short- to medium-term bridging for patients otherwise acceptable for allotransplantation as long as cardiac xenograft dysfunction proves predictable. Current data indicate that sensitization to pig antigens will *not* elicit anti-HLA antibodies and thus will likely not be detrimental to the outcome of a subsequent allograft.[25]

Relative or absolute contraindications to bridging with mechanical circulatory support include restrictive or hypertrophic cardiomyopathy, mechanical or dysfunctional bioprosthetic heart valves, severe biventricular failure, inability to tolerate requisite chronic anticoagulation with mechanical support, and congenital heart defects with failing single-ventricle physiology. Each of these patient populations is subject to unpredictable deterioration and might benefit from timely access to a pig heart as a bridge to allotransplantation. Access to total artificial heart support is restricted to patients with body surface area of >1.8 m^2, and generally, unsatisfactory results with total artificial heart or biventricular device options create an opportunity to evaluate pig hearts as a bridging alternative. "Destination therapy" could be offered to patients for whom a heart allograft is unlikely to be successful for immunologic reasons, such as the presence of high panel-reactive antibody titers or rapidly recurrent cardiac allograft vasculopathy in a second or subsequent allograft.[12,13,23,26,27] As with pig kidney transplantation, a failing pig heart might be replaced as long as the initial xenograft failure is not caused by elicited immune mechanisms, which would likely prove catastrophic for the subsequent xenograft.

Two baboon liver xenograft recipients[28] and 1 lung xenograft recipient[29] have survived for over 3 wk, but survival beyond a few days remains exceptional. Systemic inflammation, thrombocytopenia, anemia, and inconsistent life-supporting organ function are prominent in both models. In our estimation, these results do not yet justify clinical translation, and further nonhuman primate studies are needed to understand and mitigate the organ-specific causes of liver and lung xenograft failure. However, once *consistent* survival of even a few weeks is achieved in a preclinical model, patients experiencing fulminant hepatic failure may be supported by repeated ex vivo pig liver perfusions or by the insertion of an auxiliary pig liver until the native liver recovers (eg, after acetaminophen toxicity) or an allograft becomes available.[30] Patients with irreversible pulmonary failure who may require a longer period of support and who are unlikely to quickly identify a suitable allograft might choose bridging by an experimental xenograft rather than by extracorporeal membrane oxygenator support.[29]

Patients requiring dual-organ replacement (heart-kidney, heart-liver, liver-kidney) should be considered only after safety and effectiveness of each individual organ xenograft have been established.

The recent approval for a clinical pig heart transplant at the University of Maryland at Baltimore by the US Food and Drug Administration on compassionate grounds indicates that, under special circumstances, (1) grafts from pigs with multiple genetic manipulations are acceptable to regulatory authorities, (2) cloned source animals may be acceptable, (3) inactivation of porcine endogenous retroviruses, although possibly advantageous, is not essential, and (4) novel agents that block the CD40/CD154 pathway may be used for immunosuppression before full regulatory approval.[13,31]

With careful selection of patients for initial clinical trials, it is likely that patients will ultimately benefit from xenotransplantation while advancing the field beyond what is possible in pig-to-nonhuman primate models.

There is nothing more powerful than an idea whose time has come.

—Victor Hugo (1802–1885)

REFERENCES

1. 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;274:473–480.
2. Ma D, Hirose T, Lassiter G, et al. Kidney transplantation from triple-knockout pigs expressing multiple human proteins in cynomolgus macaques. *Am J Transplant*. 2022;22:46–57.
3. Längin M, Mayr T, Reichart B, et al. Consistent success in life-supporting porcine cardiac xenotransplantation. *Nature.* 2018;564:430–433.
4. Cleveland DC, Jagdale A, Carlo WF, et al. The genetically engineered heart as a bridge to allotransplantation in infants: just around the corner? *Ann Thorac Surg*. 2022;114:536–544.
5. Goerlich CE, Griffith B, Hanna P, et al. The growth of xenotransplanted hearts can be reduced with growth hormone receptor knockout pig donors. *J Thorac Cardiovasc Surg*. [Epub ahead of print. September 4, 2021]. doi:10.1016/j.jtcvs.2021.07.051
6. Griffith BP, Goerlich CE, Singh AK, et al. Genetically modified porcine-to-human cardiac xenotransplantation. *N Engl J Med*. 2022;387:35–44.

7. Yamamoto T, Hara H, Iwase H, et al. The final obstacle to successful pre-clinical xenotransplantation? *Xenotransplantation.* 2020;27:e12596.
8. Cooper DKC. Advancing xenotransplantation to the clinic: how relevant is the pig-to-nonhuman primate kidney transplantation model today? *Transplantation*. 2022;106:1717–1719.
9. Porrett PM, Orandi BJ, Kumar V, et al. First clinical-grade porcine kidney xenotransplant using a human decedent model. *Am J Transplant.* 2022;22:1037–1053.
10. Montgomery RA, Stern JM, Lonze BE, et al. Results of two cases of pig-to-human kidney xenotransplantation. *New Engl J Med*. 2022;386:1889–1898.
11. Cooper DKC. Genetically-engineered pig kidney transplantation in a brain-dead human subject. *Xenotransplantation*. 2021;28:e12718.
12. Cooper DKC, Cleveland DC. The first clinical trial—kidney or heart? *Xenotransplantation.* 2021;28:e12644.
13. Pierson RN 3rd, Fishman JA, Lewis GD, et al. Progress toward cardiac xenotransplantation. *Circulation.* 2020;142:1389–1398.
14. Fishman JA. Infectious disease risks in xenotransplantation. *Am J Transplant.* 2018;18:1857–1864.
15. First World Health Organization global consultation on regulatory requirements for xenotransplantation clinical trials: Changsha, China, 19–21 November 2008. The Changsha Communique. *Xenotransplantation*. 2008;16:61–63.
16. World Health Organization. Second WHO global consultation on regulatory requirements for xenotransplantation clinical trials. Geneva Consultation. Published 2011. Available at https://apps.who.int/iris/handle/10665/341817. Accessed June 10, 2022.
17. 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.
18. Jagdale A, Kumar V, Anderson DJ, et al. Suggested patient selection criteria for initial clinical trials of pig kidney xenotransplantation in the United States. *Transplantation.* 2021;105:1904–1908.
19. Hart A, Lentine KL, Smith JM, et al. OPTN/SRTR 2019 annual data report: kidney. *Am J Transplant*. 2021;21(suppl 2):21–137.
20. Cooper DKC, Hara H, Iwase H, et al. Clinical pig kidney xenotransplantation: how close are we? *J Am Soc Nephrol.* 2020;31:12–21.
21. USRDS. Annual data report 2017. Available at https://usrds.org/previous-adrs/2010-2019/. Accessed June 10, 2022.
22. Orandi BJ, Luo X, Massie AB, et al. Survival benefit with kidney transplants from HLA-incompatible live donors. *New Engl J Med* 2016;374:940–950.
23. Cooper DKC, Wijkstrom M, Hariharan S, et al. Selection of patients for initial clinical trials of solid organ xenotransplantation. *Transplantation.* 2017;101:1551–1558.
24. Uffing A, Pérez-Sáez MJ, Mazzali M, et al. Recurrence of FSGS after kidney transplantation in adults. *Clin J Am Soc Nephrol.* 2020;15:247–256.
25. Li Q, Hara H, Zhang Z, et al. Is sensitization to pig antigens detrimental to subsequent allotransplantation? *Xenotransplantation*. 2018;25:e12393.
26. Pierson RN 3rd, Burdorf L, Madsen JC, et al. Pig-to-human heart transplantation: who goes first? *Am J Transplant.* 2020;20:2669–2674.
27. Chaban R, Cooper DKC, Pierson RN III. Pig heart and lung xenotransplantation: present status. *J Heart Lung Transplant*. 2022;41:1014–1022.
28. Shah JA, Patel MS, Elias N, et al. Prolonged survival following pig-to-primate liver xenotransplantation utilizing exogenous coagulation factors and costimulation blockade. *Am J Transplant.* 2017;17:2178–2185.
29. Burdorf L, Laird CT, Harris DG, et al. Pig-to-baboon lung xenotransplantation: extended survival with targeted genetic modifications and pharmacologic treatments. *Am J Transplant.* 2022;22:28–45.
30. Lamm V, Ekser B, Vagefi PA, et al. Bridging to allotransplantation-is pig liver xenotransplantation the best option? *Transplantation.* 2022;106:26–36.
31. Cooper DKC. Initial reflections on the world's first clinical genetically-engineered pig heart transplant. *Xenotransplantation*. 2022:29:e12737.