The 2017 IXA Presidential Lecture:

Recent Developments in Xenotransplantation

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Running head: Recent developments in xenotransplantation

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Abbreviations used

GTKO	α1,3-galactosyltransferase gene-knockout
hEPCR	human endothelial protein C receptor
hTBM	human thrombomodulin
IXA	International Xenotransplantation Association
NHP	non-human primate
PERV	porcine endogenous retrovirus

Introduction

This is a brief personal viewpoint of some of the important developments and themes in xenotransplantation during my 2 years (2015-17) as President of the International Xenotransplantation Association (IXA), with a few more recent references included. It is not intended as an exhaustive review of all that has happened in the field over that period.

Improved Xenograft Survival in Preclinical Non-Human Primate (NHP) Models

The resurgence of interest, activity and funding in xenotransplantation (1) is intricately linked to significant advances in xenograft survival times in preclinical pig-to-NHP models (Table 1). Notably, maximum survival of life-supporting kidney xenografts increased from <100 days before 2014 to 499 days in 2017; of islet xenografts, from <400 days before 2015 to 950 days in 2016; of heterotopic heart xenografts, from 380 days in 2014 to 945 days in 2016; and of corneal xenografts, from >398 days in 2014 to >933 days in 2015. Survival of the two most 'difficult' organs, liver and lung, continues to lag behind, although it is fair to say that some progress is being made.

Genetic modification of the donor pig has played an important role in extending xenograft survival. Elimination of the major xenoantigen αGal by deletion of the *GGTA1* gene (GTKO), plus transgenic expression of one or more human complement regulatory proteins (hCD46, hCD55, hCD59), seems to be critical to protect solid organ xenografts. Although the data are not yet conclusive, it has become apparent recently that the benefit of additional transgenes may differ for different xenograft types. The human anticoagulant/anti-inflammatory protein thrombomodulin (hTBM) provides a good example of this. The longest survival of cardiac xenografts was achieved with GTKO/hCD46 hearts expressing hTBM (2). However, hTBM does not appear to be essential for long-term renal xenograft survival (3) and has little impact on the performance of porcine lungs perfused *ex vivo* with human blood, a model in which expression of human endothelial protein C receptor (hEPCR) was protective (4).

Progress Towards Clinically Applicable Immunosuppression

The most successful immunosuppressive protocols for xenotransplantation generally include some form of T cell costimulation blockade, by interfering with (i) the CD28/B7 interaction using CTLA4-Ig (belatacept), and/or (ii) the CD40/CD154 interaction using anti-CD40 or anti-CD154 monoclonal antibodies. Belatacept is approved for clinical solid organ transplantation, and anti-CD40 is the subject of phase 2 clinical trials in renal transplantation (5). 'High dose' anti-CD40 was a key component of the protocol used to achieve long-term cardiac xenograft survival (2), although some evidence suggests that anti-CD154 may be more effective than anti-CD40, at least in the preclinical islet model (6). However, anti-CD154 in its current form cannot be used clinically, as clinical trials were halted due to the unexpected development of thromboembolic events in some patients. This problem may be solved by the generation of an 'Fc-silent' version of the antibody, which has shown promising safety and efficacy in an NHP renal allograft model (7).

Progress Towards Minimizing Microbiological Risks

Careful husbandry and rigorous screening are critical to exclude pathogenic microorganisms from the donor pig herd (8). Interestingly, in the case of islets, the product may be 'clean' even if the herd is 'dirty'. Crossan et al isolated islets from pigs which had tested positive for one or more of five potentially zoonotic viruses in serum or peripheral blood mononuclear cells (9). All of the islet preparations were found to be negative for all viruses tested. Porcine endogenous retrovirus (PERV) remains a potential concern because of its vertical mode of transmission in the host animal. There is as yet no evidence that PERV is pathogenic in humans. PERV transmission was not detected in a long-term follow-up of burns patients treated with living pig skin (10), nor in recipients of microencapsulated porcine islets (11). Furthermore, PERV is sensitive to clinical anti-retroviral agents (12). Nevertheless, some have argued that elimination of PERV from the porcine genome is essential to guard against the possibility of PERV transmission in the clinical setting (13), although this view is controversial (14).

The Impact of CRISPR/Cas9

CRISPR is a remarkable technology that enables precise and efficient engineering of mammalian genomes. In the few short years since its first application in xenotransplantation, it has provided the means to simultaneously knock out multiple porcine genes that contribute to the xenoimmune response (15), to knock protective transgenes into detrimental loci (16), and even to eradicate all copies of PERV from the porcine genome (17). This is likely to be just the beginning. Previously, the lengthy period required to generate and breed multi-modified donor pigs was a significant brake on progress. Now, with the acceleration provided by CRISPR, there is a plethora of new pigs available, and the bottleneck has become the rigorous testing of these pigs in NHP models.

Outlook

After an initial flurry of investment and activity in the 1990s, enthusiasm for xenotransplantation cooled, mainly due to fears about safety (notably PERV) but also to a stagnation in progress. Over the last few years, several developments have re-kindled interest around the world. These advances include reports from the Mohiuddin group of the survival of pig cardiac xenografts in baboons for more than 1 year (18), and then for more than 2 years (2). More recently, the elimination of PERV from cultured pig cells (19) and ultimately from pigs (17) spawned numerous articles in both the popular and the scientific press, including the prestigious journal *Science* (20). Although the reporting in mainstream media occasionally suggests an incomplete understanding of the science, I believe that this publicity has generally been positive for the field, because it familiarizes the public with our work and overturns the previously pessimistic outlook of the broader transplant community. In summary, therefore, I feel privileged to have served as President of the IXA during this exciting period, and I remain very optimistic that “pigs will fly” (Fig. 1) sooner rather than later.

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References

1. Cowan PJ, Tector AJ. The Resurgence of Xenotransplantation. Am J Transplant. 2017 Oct;17(10):2531-6. PubMed PMID: 28397351.
2. Mohiuddin MM, Singh AK, Corcoran PC, Thomas Iii ML, Clark T, Lewis BG, et al. Chimeric 2C10R4 anti-CD40 antibody therapy is critical for long-term survival of GTKO.hCD46.hTBM pig-to-primate cardiac xenograft. Nature communications. 2016;7:11138. PubMed PMID: 27045379. Pubmed Central PMCID: 4822024.
3. Kim S, Higginbotham LB, Mathews DV, Breeden C, Dong Y, Stephenson A, et al. CD4 depletion is necessary and sufficient for long-term pig-to-nonhuman primate renal xenotransplant survival. Xenotransplantation. 2017;24:e12328.
4. Harris DG, Quinn KJ, French BM, Schwartz E, Kang E, Dahi S, et al. Meta-analysis of the independent and cumulative effects of multiple genetic modifications on pig lung xenograft performance during ex vivo perfusion with human blood. Xenotransplantation. 2015 Mar-Apr;22(2):102-11. PubMed PMID: 25470239. Pubmed Central PMCID: 4390422.

5. Malvezzi P, Jouve T, Rostaing L. Costimulation blockade in kidney transplantation: an update. Transplantation. 2016;100:2315-23.

6. Shin JS, Kim JM, Min BH, Yoon IH, Kim HJ, Kim JS, et al. Pre-clinical results in pig-to-non-human primate islet xenotransplantation using anti-CD40 antibody (2C10R4)-based immunosuppression. Xenotransplantation. 2018;25.

7. Kim SC, Wakwe W, Higginbotham LB, Mathews DV, Breeden CP, Stephenson AC, et al. Fc-silent anti-CD154 domain antibody effectively prevents nonhuman primate renal allograft rejection. Am J Transplant. 2017;17(5):1182-92.

8. Gazda LS, Collins J, Lovatt A, Holdcraft RW, Morin MJ, Galbraith D, et al. A comprehensive microbiological safety approach for agarose encapsulated porcine islets intended for clinical trials. Xenotransplantation. 2016 Nov;23(6):444-63. PubMed PMID: 27862363.

9. Crossan C, O'Hara Z, Mourad N, Gianello P, Scobie L. Examining the potential for porcine-derived islet cells to harbour viral pathogens. Xenotransplantation. 2017.

10. Scobie L, Padler-Karavani V, Le Bas-Bernardet S, Crossan C, Blaha J, Matouskova M, 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(6):2907-15.

11. Morozov VA, Wynyard S, Matsumoto S, Abalovich A, Denner J, Elliott R. No PERV transmission during a clinical trial of pig islet cell transplantation. Virus research. 2017 Jan 02;227:34-40. PubMed PMID: 27677465.

12. Argaw T, Colon-Moran W, Wilson C. Susceptibility of porcine endogenous retrovirus to anti-retroviral inhibitors. Xenotransplantation. 2016 Mar;23(2):151-8. PubMed PMID: 27028725. Pubmed Central PMCID: 4873160.

13. Guell M, Niu D, Kan Y, George H, Wang T, Lee IH, et al. PERV inactivation is necessary to guarantee absence of pig-to-patient PERVs transmission in xenotransplantation. Xenotransplantation. 2017;24(6):e12366.

14. Scobie L, Denner J, Schuurman HJ. Inactivation of porcine endogenous retrovirus in pigs using CRISPR-Cas9, editorial commentary. Xenotransplantation. 2017;24(6):e12363.

15. Martens GR, Reyes LM, Butler JR, Ladowski JM, Estrada JL, Sidner RA, et al. Humoral Reactivity of Renal Transplant-Waitlisted Patients to Cells from GGTA1/CMAH/B4GalNT2, and SLA Class I Knockout Pigs. Transplantation. 2017 Jan 21. PubMed PMID: 28114170.

16. Nottle MB, Salvaris EJ, Fisicaro N, McIlfatrick S, Vassiliev I, Hawthorne WJ, et al. Targeted insertion of an anti-CD2 monoclonal antibody transgene into the GGTA1 locus in pigs using FokI-dCas9. Scientific reports. 2017 Aug 16;7(1):8383. PubMed PMID: 28814758. Pubmed Central PMCID: 5559588.

17. Niu D, Wei HJ, Lin L, George H, Wang T, Lee IH, et al. Inactivation of porcine endogenous retrovirus in pigs using CRISPR-Cas9. Science. 2017 Sep 22;357(6357):1303-7. PubMed PMID: 28798043.

18. Mohiuddin MM, Singh AK, Corcoran PC, Hoyt RF, Thomas ML, 3rd, Lewis BG, et al. One-year heterotopic cardiac xenograft survival in a pig to baboon model. Am J Transplant. 2014 Feb;14(2):488-9. PubMed PMID: 24330419. Pubmed Central PMCID: 4184155.

19. Yang L, Guell M, Niu D, George H, Lesha E, Grishin D, et al. Genome-wide inactivation of porcine endogenous retroviruses (PERVs). Science. 2015 Nov 27;350(6264):1101-4. PubMed PMID: 26456528.

20. Servick K. Xenotransplant advances may prompt human trials. Science. 2017;357(6538):1338.

21. Shin JS, Min BH, Kim JM, Kim JS, Yoon IH, Kim HJ, et al. Failure of transplantation tolerance induction by autologous regulatory T cells in the pig-to-non-human primate islet xenotransplantation model. Xenotransplantation. 2016 Jul;23(4):300-9. PubMed PMID: 27387829.

22. Hawthorne W, Chew YV, Salvaris E, Hawkes J, Ayoubi A, Barlow H, et al. >12 month function of genetically modified porcine neonatal islet xenografts in baboons. Xenotransplantation. 2017;24(5):e12328.

23. Choi HJ, Lee JJ, Kim DH, Kim MK, Lee HJ, Ko AY, et al. Blockade of CD40-CD154 costimulatory pathway promotes long-term survival of full-thickness porcine corneal grafts in nonhuman primates: clinically applicable xenocorneal transplantation. Am J Transplant. 2015 Mar;15(3):628-41. PubMed PMID: 25676390.

24. Aron Badin R, Vadori M, Vanhove B, Nerriere-Daguin V, Naveilhan P, Neveu I, et al. Cell therapy for Parkinson's disease: A translational approach to assess the role of local and systemic immunosuppression. Am J Transplant. 2016 Jan 8. PubMed PMID: 26749114.

25. Shah JA, Patel MS, Elias N, Navarro-Alvarez N, Rosales I, Wilkinson RA, et al. Prolonged survival following pig-to-primate liver xenotransplantation utilizing exogenous coagulation factors and costimulation blockade. Am J Transplant. 2017;17:2178-85.

26. Kubicki N, Laird C, Burdorf L, Pierson RN, 3rd, Azimzadeh AM. Current status of pig lung xenotransplantation. Int J Surg. 2015 Nov;23(Pt B):247-54. PubMed PMID: 26278663.

Table 1: Maximum xenograft survival in NHP models (all published 2015-17).

Graft	Donor	Recipient	Maximum Survival	Reference
Islets	WT	Monkey	950 days	Shin et al (21)
Islets	GTKO/hCD55/hCD59/HT	Baboon	>675 days	Hawthorne et al (22)
Cornea	WT	Monkey	>933 days	Choi et al (23)
Neurons	CTLA4-Ig	Monkey	>548 days	Aron Badin et al (24)
Heart	GTKO/hCD46/hTBM	Baboon	945 days	Mohiuddin et al (2)
Kidney	GTKO/hCD55	Monkey	499 days	Kim et al (3)
Liver	GTKO	Baboon	29 days	Shah et al (25)
Lung	GTKO + ?*	Baboon	8 days	Kubicki et al (26)

* Additional transgenes not specified.

Figure Legends

Figure 1: Pigs will fly.

Figure 1.