Published in final edited form as:
Am J Transplant. 2014 February ; 14(2): 488–489. doi:10.1111/ajt.12562.

One-Year Heterotopic Cardiac Xenograft Survival In A Pig To Baboon Model

Muhammad M Mohiuddin, MD[1,4], **Avneesh K Singh, PhD**[1], **Philip C. Corcoran, MD**[1], **Robert F. Hoyt, DVM**[2], **Marvin L Thomas III, DVM**[3], **Billeta GT Lewis, MS**[3], **Michael Eckhaus, DVM**[3], **Keith A. Reimann, PhD**[4], **Nikolai Klymiuk**[5], **Eckhard Wolf, PhD**[5], **David Ayares, PhD**[6], and **Keith A. Horvath, MD**[1]

[1]Cardiothoracic Surgery Research Program, NHLBI, NIH, Bethesda, MD, USA

[2]Animal Surgery Research, NHLBI, NIH, Bethesda, MD, USA

[3]Division of Veterinary Resources, ORS, NIH, Bethesda, MD, USA

[4]MassBiologics, University of Massachusetts, Boston, MA

[5]Institute of Molecular Animal Breeding and Biotechnology, Ludwig-Maximilians-Universität München

[6]Revivicor Inc., Blacksburg, VA, USA

To the Editor

We have now demonstrated that the heterotopic pig cardiac xenograft survival in a baboon can exceed one year by utilizing porcine hearts with customized genetics (alpha galactosyl transferase gene knock out (GTKO) to eliminate alpha Gal antibody mediated rejection, transgenic expression of human complement regulatory protein (hCD46) to inhibit complement activation and human thrombomodulin molecules (TBM) to prevent coagulation) (Revivicor, Inc. Blacksburg, VA) and an immunomodulatory treatment regimen consisting of co-stimulation blockade by a primatized anti CD40 antibody (Clone 2C10R4) (50 mg/Kg / weekly), anti CD20 antibody (19mg/Kg on d -14,-7,0 and 7), ATG (5mg/Kg on day -2 and -1), MMF (20mg/Kg BID) and steroids (2mg/Kg tapered of in 4-6 weeks). Graft survival of all 5 animals in this group is shown in Table 1.

This is to our knowledge the first demonstration of long-term vascular xenograft survival beyond one year in any large animal xenotransplantation model. All previous reported graft survivals were at least 4 months less (1, 2). Antibodies, both preformed and elicited against various xenoantigens, that mediate graft rejection (3) and thrombotic microangiopathy or consumptive coagulopathy due to platelet activation (4), have been the main obstacles to successful xenograft survival. In this study both these mechanisms were efficiently

[4]Address correspondence to: Muhammad M Mohiuddin, MD, CSRP/NHLBI/NIH, Building 10, Rm B1D47G, 10 Center Drive, MSC 1550, Bethesda, MD 20892, mohiuddinm@mail.nih.gov.

Disclosure: The authors of this manuscript have conflicts of interest to disclose as described by the *American Journal of Transplantation*. Dr. David Ayares is the Executive Vice President and Chief Scientific Officer of Revivicor Inc. Dr Keith Reimann holds equity in Primatope Therapeutics who has licensed the 2C10 antibody.

controlled in all 5 baboons by altering the genes of the donor pig and recipient treatment with a regimen that includes anti CD40 antibody.

It is hard to comment definitively on the advantage of the genetic modification of pigs or the use of anti CD40 antibody but the combination has clearly played a significant role in prolonging graft survival. All hemodynamic and coagulation parameters remained within the normal range in all the animals in this group. This was especially true of the platelet counts, control of which, historically, had been a key issue in this model. Prevention of thrombocytopenia by an initially low and thereafter tapering dose (20 mg/Kg) of anti CD40 antibody (clone 2C10R4) has also been demonstrated in our laboratory (Mohiuddin et al, xenotransplantation, in press) but all GTKO. hCD46 grafts (n=6) in that study rejected within 149 days. Thus, it seems the addition of the hTBM transgene had a further beneficial effect. As shown in the table, only one graft out of 5 in this experimental group ceased function and stopped contracting after surviving for 146 days. This baboon suffered from a prolonged period of infection which was resistant to all available antibiotics. On necropsy, CMV inclusion bodies were discovered indicating a probable CMV infection. The histology of this rejected heart showed mostly necrotic cardiac myocytes with fibrosis. As of the date of publication, all of the remaining 4 graft recipient baboons are still healthy with strong xenograft contractile function (graft scores are shown in Table 1). Due to the use of anti CD20 antibody, no B cells were detected in these baboons for the first 60 days. Both non Gal IgM and IgG antibodies remained at pretransplant levels throughout all experiments, indicating that the antibody response against xenoantigen was adequately controlled.

In addition to the above manipulations in genetics and immunosuppression regimens, in our opinion, another key reason for improved graft survival is our ability to identify and intervene at the earliest sign of any complication due to continuous telemetric and video monitoring of the baboon recipient.

Survival of a heterotopic cardiac xenograft for more than one year is a significant milestone in the field of xenotransplantation. To advance the field further, the next logical step should be to test the pig genetics used in this experiment along with the optimal immunomodulation protocol utilized in an orthotopic cardiac xenograft model, to investigate the life sustaining capability of this pig xenograft. We hope that this result will drive further activity and innovation in the field to make clinical xenotransplantation a reality.

Acknowledgments

We would like to acknowledge DVR, ASR and flow cytometry core staff for their help in surgery, animal care and FACS analyses, Ms. Carol Phelps, Mr. Todd Vaught and Ms. Suyapa Ball (of Revivicor Inc.) for transgenic pig production and Ms. Patricia Jackson for her Administrative help.

References

1. Kuwaki K, Tseng YL, Dor FJ, Shimizu A, Houser SL, Sanderson TM, et al. Heart transplantation in baboons using alpha1,3-galactosyltransferase gene-knockout pigs as donors: initial experience. NatMed. 2005; 11(1):29–31.
2. Mohiuddin MM, Corcoran PC, Singh AK, Azimzadeh A, Hoyt RF Jr, Thomas ML, et al. B-cell depletion extends the survival of GTKO.hCD46Tg pig heart xenografts in baboons for up to 8 months. Am J Transplant. 2012; 12(3):763–771. [PubMed: 22070772]

3. Tazelaar HD, Byrne GW, McGregor CG. Comparison of Gal and non-Gal-mediated cardiac xenograft rejection. Transplantation. 2011; 91(9):968–975. [PubMed: 21403591]

4. Lin CC, Cooper DK, Dorling A. Coagulation dysregulation as a barrier to xenotransplantation in the primate. TransplImmunol. 2009; 21(2):75–80.

Table 1

Graft survival

No	Baboon ID	Graft survival (days)[a]	Graft Score*	LVP mmHg**	Complications	Graft / Recipient status
1	15009	146	0	<10	CMV infection	EXP / Euthanized
2	510	380	++++	> 80@	Klebsiella infection on day 300/ Treated	Contracting/ Alive
3	910	91	++++	>70	None	Contracting/ Alive
4	110	84	++++	>70	None	Contracting/ Alive
5	210	77	+++	>60	None	Contracting/ Alive

[a] All grafts and baboons remain viable/surviving except for baboon# 15009 where the graft rejected at 146 days.

* Range: Strong contractility (++++) to Rejection (0)

** Strong contractility LVP > 60 mmHg and Rejection LVP< 10 mmHg

@ Telemetry battery ran out of charge at day 300.