Society for Heart Failure and Transplantation

SOCIETY FOR HEART FAILURE AND TRANSPLANTATION'S

THE REVIVAL

Promoting Academics to Improve Clinical Outcomes.

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EDITOR'S NOTE

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Dear Colleagues,

Greetings from the Editor's desk. The June 2022 issue of The Revival delves into the exciting novel realm of Cardiac Xenotransplantation. Our guest author is Dr Dhruva Sharma Associate Professor of CTVS at SMS Hospital, Jaipur. We invited Dr Kumud Dhital to be the Guest Editor to review the article and throw his valuable insight on the subject. Dr Bartley Griffiths in January 2022 created history by performing cardiac xenotransplantation from a genetically modified pig. The main advantages of a porcine model is the similarity with respect to organ size and function, reduced risk of zoonotic infections and ability to produce immunologically and microbiologically safer xeno colonies. The major limitations are overcoming the immunological barrier. CRISPR/Cas (clustered regularly interspaced short palindromic repeats linked to Cas nuclease) technology is a genome editing tool and has transfigured xenotransplantation. With further advancements in genetic engineering and immunosuppression, we hope the long-term results of cardiac xenotransplantation will improve and set the stage for making this modality available in regions where there is a paucity of donor hearts.

On behalf of our editorial team, I thank Dr Dhital and Dr Dhruva for this 'crisp' article. I wish our dear Readers a Happy Reading!

- Dr Manoj Durairaj
Editor "The Revival"

SUB EDITOR

Dr Talha Meeran

MBBS, MD, FACC, Consultant Cardiologist, Dept of Advanced Cardiac Sciences and Cardiac Transplant, Sir HN Reliance Foundation Hospital, Mumbai.

Dear Colleagues,

Limited availability of cadaveric donors is a major hurdle in the advancement of cardiac transplantation around the globe. Performing cardiac xenotransplantation utilizing a genetically engineered primate or pig organ that is readily available would be ideal in a utopian state. However there is much more to unravel in this enigmatic field before it becomes standard practice. The recent short term success of the cardiac xenotransplantation procedure at University of Maryland has again brought the transplant world focus on xenotransplantation. Dr Dhruva Sharma has succinctly reviewed this exciting topic in this month's REVIVAL backed by our guest editor, Dr Kumud Dhital.

Sincerely,
Dr Talha Meeran
Sub Editor "The Revival"

PRESIDENTIAL MESSAGE

Prof. (Dr) V. Nandakumar

Director & Chief, Division of Cardio Vascular/Thoracic Surgery & Cardiac Transplantation, Metromed International Cardiac Centre, Calicut, Kerala.

Dear Colleagues,

Greetings from the Society for Heart Failure and Transplantation.

Here is the June issue of 'The Revival'. In this, Dr. Dhruva Mukesh Sharma has clearly outlined the major issues related to the use of pig's heart for xenotransplantation. As we have shortage of organs for heart transplantation and the number of patients in the waiting list are on the increase, Xenotransplantation is a good option to go closer to the goal of adequate organ supply. With genetic engineering, it has been made possible to delete 4 porcine genes and insert six human genes. This led to the longest survival of the first xenotransplanted heart for two months. Further research will hopefully lead us to find solution for long term survival of these grafts which will be a big boon to the patients with end stage heart disease.

We are fortunate to have Dr. Kumud Dhittal as guest editor, who has pioneered DCD heart transplant and has extensive experience in extracorporeal organ perfusion.

Best wishes Best wishes,

- Prof. (Dr) V. Nandakumar
President

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Special thanks to Guest Author Dr. Dhruva Sharma and Guest Editor Dr. Kumud Kumar Dhital for authoring this month's article.

Designed by Maithili Kulkarni

CARDIAC XENOTRANSPLANTATION

GUEST AUTHOR

Dr. Dhruva Mukesh Sharma

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Dr Dhruva Sharma is currently working as an Associate Professor in the Department of Cardiothoracic and Vascular Surgery, Sawai Man Singh Medical College and Hospital, Jaipur,serves on Editorial Board of World Journal of Transplantation. He has pursued one and a half year Fellowship training in Advanced Heart and Lung Transplantation and Mechanical Circulatory Support under the esteemed guidance of Dr. K. R. Balakrishnan and team at Chennai. He has played an important role in team that performed first ever Heart Transplant in Government Sector Medical College in Rajasthan at SMS Medical College and Hospital, Jaipur. He has authored and co-authored various review and research articles on Heart and Lung Transplantation in reputed national and international journals. He has a vision to develop State of the Art a successful and viable Heart and Lung Transplantation and Mechanical Circulatory Programme at SMS Medical College and Hospital Jaipur for the benefit of general patient population.

GUEST EDITOR

Dr. Kumud Kumar Dhital

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Having qualified in Medicine from Oxford University, Dr Dhital obtained his Cardiothoracic Surgical training at Guy's & St Thomas Hospitals in London with Fellowships at the John Radcliffe Hospital in Oxford and Specialist Transplant Fellowship at Papworth Hospital, Cambridge, UK.

Following his surgical training, Dr Dhital was a Consultant Cardiothoracic & Transplant Surgeon and Assistant Professor of Surgery for the University of Pittsburgh Medical Centre (USA) to establish a heart and lung transplant service at its Mediterranean Institute of Advanced Therapies (IsMeTT) in Palermo, Italy. He then returned to the UK as a Consultant Cardiothoracic and Transplant Surgeon, and Director of Lung Transplantation at the Royal Papworth Hospital, Cambridge before moving to Australia in 2009. He spent a decade at St Vincent's Hospital, Sydney as Consultant Cardiothoracic & Transplant Surgeon in Sydney, with a busy public, private and Research portfolios. He then became the Director of Cardiothoracic Surgery & Transplantation and Program Director of Heart & Lung services at the Alfred Hospital in Melbourne, as well as Professor of Cardiothoracic Surgery and Transplantation at Monash University.

Dr. Dhital provides a comprehensive practice in cardiac and thoracic surgery with a special focus on surgical strategies for heart & lung failure including transplantation and implantation of mechanical circulatory support. He also has a niche expertise in pulmonary endarterectomy for patients with chronic thrombo-embolic pulmonary hypertension. He has practiced at Senior Consultant level in the UK, Italy, Australia and more recently in India since 2020.

Dr. Dhital is globally renowned for being the surgical pioneer of DCD heart transplantation from distantly procured organs with the use of machine perfusion to reanimate, preserve and transport the donor heart. In 2014, he was the principal surgeon and led the teams for both the initial organ retrieval and the DCD heart transplant at St Vincent's Hospital in Sydney, Australia. He was also performing DCD lung transplants using standard lung preservation in Australia. Dr Dhital has significant experience with machine perfusion of donor lungs, and was the Principal Investigator in Australia for the international INSPIRE trial which validated the safety and positive outcomes from machine preservation of donor lungs for clinical lung transplantation.

Dr. Dhital's main research interest is focused on improving the quality and number of available donor organs for heart & lung transplantation and includes extensive experience of utilising extracorporeal organ perfusion devices for preservation and reconditioning of donor hearts and lungs.

Introduction

For eligible patients with end-stage heart disease, heart transplantation remains the gold standard treatment for alleviation of symptoms, for better prognosis, and quality of life. There is a vital deficit of donor organs for patients who are wait- listed for organ transplantation. Xenotransplantation, the replacement of human cells, tissues or organs from another species, is gaining more momentum in the clinical arena, and could eventually provide an unlimited supply of organs for transplantation. [1]

History

Although xenotransplantation of tissues into humans goes back to the 19th century, such heterologous transplantation of solid organs was not attempted in man until the 1960's. The first cardiac xenotransplant, utilising a chimpanzee heart, was performed by Dr. Hardy at the University of Mississippi in 1964 with the recipient only surviving for 2 hours. Further limited attempts were made with primate xenografts by Christian Barnard's group at the Groote Schuur Hospital in Cape Town, and by Leonard Bailey at the Loma Linda University in California, all of them without good outcomes. [1-2] Almost 4 decades later, and on the background of rigorous research in non-human primates, cardiac xenotransplantation has recently been catapulted back in being finally achievable. Dr Bartley Griffiths and his team at the University of Maryland, Baltimore, performed an adult cardiac xenotransplant in January 2022 with the graft from a genetically modified pig with recipient survival to 2 months. [2-3] The precise cause of death, details of any preceding infective or rejection episodes, and final post-mortem reports are still awaited.

Barriers to xenotransplantation

The major limiting factor for xenotransplantation is the immunological barrier. Due to phylogenetic distance between the species, such genetically diverse animals cannot be used without modification to preserve human life. [4] To mitigate this incompatibility, advanced genetic engineering technologies including CRISPR–Cas9 genome editing, have been used to genetically modify pigs towards better graft and recipient survival.[5] Disparity in organ size, increased chances of infections, ethical limitation on the use of primates, and the difficulty of breeding non- human species to clinical requirements, are some of the barriers to xenotransplantation.

Advantages of a porcine model

Main advantages of selecting the pig as a preferred model for xenotransplant are of: greater physiological similarity to humans in respect of organ size and function; there being a short maturation period; of reduced risk of zoonotic infections; and the ability to produce immunologically and microbiologically safer porcine xeno-colonies under strictly controlled conditions to scale because of large litter size.

Limitations of cardiac xenotransplantation

- Presence of natural preformed antibodies resulting in hyperacute and acute humoral xenograft rejection
- Innate and adaptive immune responses leading to cellular xenograft rejection
- Dysregulation of coagulation system by complement pathway leading to vascular thrombosis
- Zoonotic viral transmission [porcine endogenous retrovirus (PERV), porcine cercovirus, (PCV)]
- Continued growth and hypertrophy of the xenograft post transplantation.
- Ethical issues

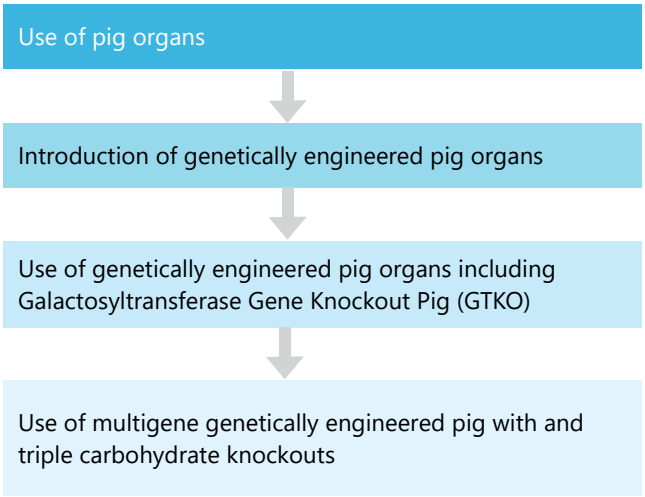
Measures adapted to overcome challenges to xenotransplantation

- Deletion/knock-out of 3 porcine genes that encode for enzymes which add carbohydrate antigens to porcine cell surfaces which in turn activate the human immune system responsible for hyperacute rejection [α1-3 galactosyltransferase (GGTA1), cytidine monophosphate-N-acetylneuraminic acid hydroxylase (CMAH) and -1,4-N-acetyl- galactosaminyltransferase 2 (B4GALNT2)]. [7]
- Deletion/knock-out of the porcine Growth Hormone Receptor gene to prevent the unchecked growth and hypertrophy of the donor heart. [5-7]
- Insertion/Knock-in of six human genes into the porcine genome to permit immune acceptance. Two of these were anticoagulant genes, human Thrombomodulin (TBM) and Endothelial Cell Protein C Receptor (EPCR) were inserted to prevent thrombotic microangiopathy. Two complement inhibitor genes, Cluster of Differentiation 46 (CD46) and Decay Accelerating Factor

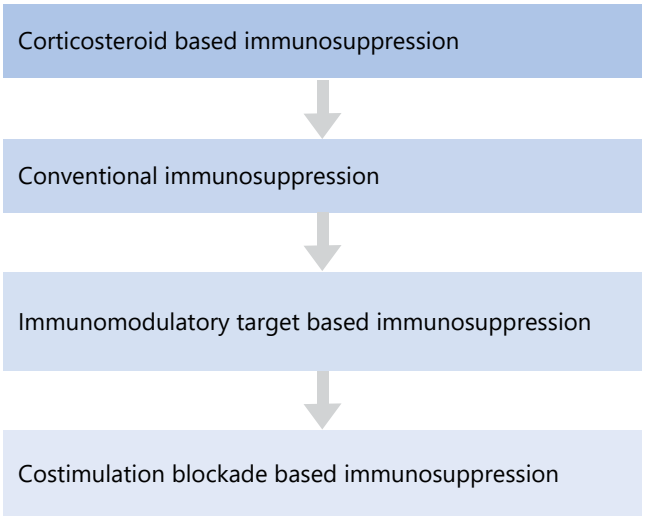
(DAF or CD59) were inserted to suppress antibody-mediated responses. Two anti-inflammatory genes, human hemeoxygenase 1 (HO 1) and CD47 were also knocked-in to the porcine genome. [7]

- Use of newer immunosuppressive drug regimens including the inhibition of costimulation pathway to more specifically modulate T cell signalling.
- By preventing transmission of viral infection (PERV inactivated pigs using CRISPR- Cas gene editing) [5-7]

Advancement in donor genetic engineering capabilities



Advancement in immunosuppression



CRISPR/Cas technology

CRISPR/Cas (clustered regularly interspaced short palindromic repeats linked to Cas nuclease) technology has transfigured xenotransplantation. Out of 40 different known families of CRISPR and three main types (Type I, II and III), CRISPR/ cas type II system has found application in genetic engineering as it requires only one Cas protein to function i.e. Cas9. It is a very elementary and methodical genome editing tool. [4]

Few limitations of CRISPR/ Cas technology are:

- The genesis of DNA breaks outside the target locus.
- Higher risk of developing off- target mutations.
- Dependence on a three-nucleotide-long PAM sequence (specifically NGG, where N is an arbitrary base nucleotide followed by two guanosine nucleotides).
- One of the considerable limitations in large animal models is the development of genetic mosaicism [7]

Survival up to 945 days was seen following xenotransplantation of heterotopic hearts in the baboon from multimodified pigs incorporating knock-out of porcine GGTA1 and knock-in of human CD46 and TBM, combined with anti-CD40 antibody-based immunosuppression. [8]

The outcome was favourable with orthotopic cardiac xenotransplantation when performed using the same genetic modifications and immunosuppression regimen, with maximum survival of only 195 days. [9]

CONCLUSION:

Xenotransplantation might help in solving the crisis of organ shortage. Survival of xenotransplant has increased significantly with advancement in the genetic engineering technologies. The durability and reproducibility will be decided by large randomized clinical trials for which the scene has been set by the recent porcine xenotransplant by Dr Griffith's team at the University of Maryland School of Medicine. David Bennet, the 57 year-old recipient was not a candidate for either a conventional cardiac transplant or for ventricular assist device therapy. Despite surviving only 2 months, the xenotransplant procedure he underwent has set a significant milestone in the evolution of thoracic transplantation. Although a myriad of social, cultural, religious, legal and ethical hurdles remain before xenotransplantation becomes widely accepted as standard of care, the principle barrier remains that of clinical readiness. Whilst we await the final deliberations on the exact sequence of event leading to the demise of this recipient, the possibility that it may have been initiated by the presence of porcine cytomegalovirus in the donor heart will prompt necessary caution in the anticipated wider roll-out of cardiac xenotransplantation. [10]

References:

1. Cooper DKC, Gaston R, Eckhoff D, et al. Xenotransplantation-the current status and prospects. Br Med Bull. 2018;125(1):5-14.
2. Berney T, Naesens M, Schneeberger S. Xenotransplantion: Defeating the "Shumway Curse" An Interview With Drs. Bartley Griffith, Jayme Locke, Robert Montgomery, and Bruno Reichart. Transpl Int. 2022;35:10439.
3. Hein, R, Sake, HJ, Pokoyski, C, et al. Triple (GGTA1, CMAH, B2M) modified pigs expressing an SLA class Ilow phenotype—Effects on immune status and susceptibility to human immune responses. Am J Transplant. 2020; 20: 988– 998.
4. Ryczek N, Hryhorowicz M, Zeyland J, Lipiński D, Słomski R. CRISPR/Cas Technology in Pig-to-Human Xenotransplantation Research. Int J Mol Sci. 2021;22(6):3196.
5. Lu T, Yang B, Wang R, Qin C. Xenotransplantation: Current Status in Preclinical Research. Front Immunol. 2020;10:3060.
6. French, BM, Sendil, S, Pierson, RN, Azimzadeh, AM. The role of sialic acids in the immune recognition of xenografts. Xenotransplantation. 2017;e12345. https://doi.org/10.1111/xen.12345
7. Fu Y., Foden J.A., Khayter C., Maeder M.L., Reyon D., Joung J.K., Sander J.D. High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells. Nat. Biotechnol. 2013;31:822–826.
8. Mohiuddin M.M., Singh A.K., Corcoran P.C., Thomas M.L., Clark T., Lewis B.G., Hoyt R.F., Eckhaus M., Pierson R.N., Belli A.J., et al. Chimeric 2C10R4 anti-CD40 antibody therapy is critical for long-term survival of GTKO. hCD46.hTBM pig-to-primate cardiac xenograft. Nat. Commun. 2016;7:11138.
9. Längin M., Mayr T., Reichart B., Michel S., Buchholz S., Guethoff S., Dashkevich A., Baehr A., Egerer S., Bauer A., et al. Consistent success in life-supporting porcine cardiac xenotransplantation. Nature. 2018;564:430–433.
10. Sykes M, Sachs DH. Transplanting organs from pigs to humans. Sci Immunol. 2019;4(41):eaau6298.

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