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The Road to the First FDA-Approved Genetically Engineered Pig Heart Transplantation into Human

Avneesh K. Singh[1], **Bartley P. Griffith**[1], **Corbin E. Goerlich**[1], **David Ayares**[2], **Muhammad M. Mohiuddin**[1]

[1]University of Maryland School of Medicine, Baltimore, MD

[2]Revivicor Inc, Blacksburg, VA

Abstract

We have been testing genetically engineered (GE) pig hearts and optimizing immunosuppression (IS) in non-human primates (NHPs) since 2005. We demonstrate how we translated this preclinical investigation into an Food and Drug Administration (FDA)-approved clinical cardiac xenotransplantation. First, genetically engineered (GE) pig hearts were transplanted into the abdomen of NHP along with IS, which included anti-CD20 and anti-CD40-based co-stimulation blockade antibodies. We reported 945 days of survival of three gene GE pig hearts in NHPs. Building on this proof-of-concept, we tested 3 to 10 gene-modified GE pig hearts (in order to improve the immunocompatability of the xenograft further) in a life-supporting orthotopic model, but had limited success due to perioperative cardiac xenograft dysfunction (PCXD). With novel non-ischemic continuous perfusion preservation (NICP), using the XVIVO© Heart solution (XHS), life-supporting survival was extended to 9 months. We approached the FDA under an application for “Expanded Access” (EA), to transplant a GE pig heart in a patient with end-stage non-ischemic cardiomyopathy. He was without other therapeutic options and dependent on VA-ECMO. A team of FDA reviewers reviewed our preclinical research experience and data and allowed us to proceed. This clinical cardiac xenotransplantation was performed, and the patient survived for sixty days, demonstrating the translational preclinical investigation of cardiac xenotransplantation from bench to bedside. The ultimate etiology of graft failure is currently a topic of investigation and lessons learned will progress the field forward.

On January 7th, 2022, a 57-year-old male on veno-arterial extracorporeal membrane oxygenation (VA-ECMO) with end-stage non-ischemic cardiomyopathy (NICCM) received a genetically modified pig heart under expanded access (EA) authorization granted by the United States (U.S.) Food and Drug Administration (FDA) (also known as “compassionate use”).

Our research at the cardiac xenotransplantation program at the National Institutes of Health, leading to this moment, accelerated in 2005. Until this time, cardiac xenograft survival was limited to 179 days in the heterotopic (abdominal) position using α1,3-galactosyl transferase gene-knockout (GTKO) pigs and anti-CD154 (ABI793) co-stimulation blockade (CoS-B)[1],

Corresponding author: Muhammad M Mohiuddin, Professor of Surgery, Director, Program in Cardiac Xenotransplantation, University of Maryland School of Medicine, 10 S. Pine Street, MSTF 434B, Baltimore, MD 21201, mmohiuddin@som.umaryland.edu.

along with other immunosuppressive (IS) drugs, which included T cell depletion by ATG, MMF and complement inhibition by cobra venom factor. B-cell depletion with anti-CD20 (Rituximab) antibody (Ab) was added to T-cell and complement-depleting induction to curb antibody responses to xenografts after transplantation. We demonstrated 236 days of survival of GE pig heart (i.e., GTKO with additional expression of human complement regulatory protein CD46 (hCD46)) in non-human primates (NHPs, i.e., baboon) using anti-CD154 (5C8) based co-stimulation blockade immunosuppression[2]. Anti-CD154 Ab was found to be associated with arterial thromboembolic (TE) phenomena[3,4]. But, CD40/CD40L pathway was deemed essential for rejection-free survival, so an alternative co-stimulation blockade agent was required to prevent these complications.

The NIH Nonhuman Primate Reagent Resource Center provided two monoclonal antibodies that targeted CD40, instead of its pro-thrombogenic ligand CD154. These antibodies included a mouse anti-human CD40 Ab (3A8) and recombinant mouse non-human primate chimera raised against macaque CD40 (2C10R4). These blocked baboon B-cell activation in vitro and inhibited Ab production in vivo[5]. Chimeric 2C10R4 anti-CD40 antibody was found to be superior in prolonging cardiac xenograft survival in NHPs, compared to other anti-CD40 agents[5]. In 2016, we demonstrated rejection-free survival of cardiac xenografts (i.e., GTKO.hCD46.hTBM) up to 945 days using 50mg/kg dosing for 2C10R4 along with previously used immunosuppression[2,6]. The longest surviving abdominal cardiac xenograft in NHPs was rejected only after the withdrawal of anti-CD40 Ab[6]. This proof-of-principle approach demonstrated successful long-term survival of heterotopic cardiac xenotransplantation in a pig-to-baboon model.

The team moved to the University of Maryland School of Medicine (UMSOM) in 2016 to test clinically translational, orthotopically positioned, genetically engineered (GE) pig hearts in the baboon. Initial long-term survival beyond 48 hours was the exception due to perioperative cardiac xenograft dysfunction (PCXD)[7]. This phenomenon occurred without any evidence of rejection. We tested three different cardiac preservation methods before implantation (i.e., crystalloid cardioplegia and blood cardioplegia followed by static ice storage and non-ischemic continuous perfusion preservation (NICP) using the XVIVO© Heart solution (XHS))[8]. We learned that PCXD could be mitigated by non-ischemic perfusion of the donor pig heart. The avoidance of PCXD permitted us the opportunity to examine a number of “multi-gene” cardiac xenografts (iterative genetic additions which included three carbohydrate antigen knockouts (GTKO, B4GaNTlKO, CMAHKO) along with human transgenes for anti-inflammation (CD47 & HO-1), thromboregulation (hTBM & hEPCR), and complement regulation (hCD46 & DAF)[9]. A growth hormone receptor knockout (GHRKO) construct was added to address the donor organ overgrowth noted in pig kidney xenotransplants and also suggested by the German xenoheart group[10]. This allowed us to respond to post-transplantation xenograft growth without using other immunosuppressants or adjuncts[11]. This achieved consistent life-supporting preclinical graft survival beyond six months (longest survival of 9 months)[9,11]. Our donor organ procurement occurred in an adjoining operating room and was refined to minimize ischemic insults to the heart through optimizing surgical technique and ischemia times[12]. We developed a rigorous standardized postoperative care which included telemetry, echocardiographic examination, xenograft-derived cell-free DNA (xd-cfDNA) assays, endomyocardial biopsy,

and protocolized care[13–15]. This care included protocolized resuscitation, prophylactic treatment of arrhythmias with amiodarone and lidocaine, and minimal use of inotropic agents. A continuous multi-disciplinary approach to recipient care was also employed, which engaged clinical and research staff. This allowed for a swift transition from the preclinical life-supporting model of cardiac xenotransplantation to clinical translation, as the protocols and research/clinical team had already been established.

Our iterative improvements with the outcomes in NHPs suggested a first-in-man study [9,11]. We believed that should a patient present to us with severe heart failure not treatable by continuing medical therapy, standard allotransplantation, or mechanical assist devices, and we would propose cardiac xenotransplantation from a GE pig. The primary qualifying criteria for FDA approval via an expanded use Investigational New Drug Application (eIND) would be that the experimental transplant would be associated with no worse outcome than continuing failing medical treatment.

A patient with refractory end-stage heart failure was transferred to the University of Maryland Medical Center (UMMC) in November of 2021. An application for expanded access authorization with the US FDA using a “multi-gene” pig heart with ten gene edits for human transplantation was submitted on December 20th, 2021, along with the use of a novel NICP system (XVIVO Heart Box, Göteborg, Sweden) and a humanized anti-CD40 co-stimulation blockade agent. Genetics of donor pig was GTKO, B4GaNTlKO, CMAHKO, GHRKO, hCD46, hDAF, hTBM, hEPCR, hCD47 & hHO-1 (table 1)[9,11]. The FDA approved our request on December 31st, 2021, after reviewing our preclinical data of multi-gene pig heart transplantation into NHPs. After receiving approval from the ethics committee, our local institutional review board (IRB), and the team of 4 psychiatrists (one external), the patient signed consent with the concordance of his family. UMMC provided institutional approval for the transplant, and first-of-its-kind cardiac xenotransplantation was performed on January 7th, 2022. A patient receiving GE pig heart with multi-gene modification survived for 60 days[16]. During this period, he was undergoing rehabilitation, alert, communicating with nursing staff, physicians, and family members, and selflessly stated, “I want to live, and if I don’t, you’ll learn something.” Ten gene modified cardiac xenograft function continues to be excellent on transthoracic echocardiogram (TTE) and no signs of antibody or acute cellular rejection was observed in endomyocardial biopsy performed on day 34. Anti CD40 antibody based costimulation blockade IS (Mohiuddin Immunomodulatory Regimen) was used, but due to patients’ severe thrombocytopenia and mild leukopenia, slight adjustments were made in the IS. Stringent zoonosis surveillance plan was used to monitor the porcine Viruses (PERV A/B and C, PCV and pCMV). No evidence of PERV A/B/C and PCV3 in the patient’s PBMCs was detected by PCR. However, very low titer of microbial cell-free DNA (mcf-DNA) for pCMV was detected during zoonosis surveillance by KT® assay (Karius, Inc), which is not validated for porcine virus detection yet (only research applications). However, presence of pCMV was tested by more sensitive PCR retrospectively in the donor spleen collected at the time of transplantation and only after 45 cycle amplification was pCMV DNA detected. pCMV-DNA was also detected in the explanted cardiac xenograft by similar methods. Detection of pCMV-DNA of pCMV by PCR could be due to pCMV- DNAemia and it has been reported earlier in preclinical model xenotransplantation[17]. However, CMV has been suggested

as species specific and there is no evidence that pCMV can infect human and NHP cells[18,19]. Patients received intravenous immunoglobulins (IVIg) for infectious concerns which coincided with a sharp increase of anti-pig IgG and to a lesser extent IgM antibody. Later the patient developed an acute biventricular thickening on the cardiac xenograft and diastolic failure which led to the compassionate withdrawal of further advanced supportive care on day sixty. Studies are underway to understand the cause of cardiac xenograft failure.

This FDA-approved application of xenotransplantation has uniquely demonstrated the ability to take a terminally ill patient from the point of certain death to a future of rehabilitation and recovery. The first scientific examination of this xenograft in a human recipient provides insight that the preclinical model of cardiac xenotransplantation has not provided. Continued scientific advancements may be warranted by utilizing this novel mechanism of regulatory approval in parallel with traditional means of clinical translation and preclinical research.

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Abbreviations

GE	Genetically engineer
eIND	Investigational New Drug Application
EPCR	Endothelial Cell Protein C Receptor
FDA	Food and Drug Administration
GHRKO	Growth Hormone Receptor Knockout
GTKO	1–3 alpha Galactosyltransferase Knockout
hCRP	human Complement Regulatory Protein
hDAF	human Decay Acceleration Factor
NHP	Non-human primates
NICCM	Non-ischemic cardiomyopathy
PCXD	Perioperative Cardiac Xenograft Dysfunction
TBM	Thrombomodulin
TKO	Triple knock out
TPC	alpha-Gal Polyethylene glycol polymer
VA-ECMO	Veno-Arterial Extracorporeal Membrane Oxygenation

xd-cfDNA	xenograft-derived cell free DNA
XHS	XVIVO© Heart solution

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Table 1:

Genetic Modifications of Source Animal for Human Transplantation

Xenogeneic Carbohydrate Knockout		
Galactose-α-1,3-galactose KO (GTKO)	Deletion of immunogenic Galactose-α-1,3-galactose (Gal) glycan through knockout of the synthetic enzyme alpha1,3-galactosyltransferase (GT)	Anti-Immunogenic
β1,4-N-acetylgalactosyltransferase KO (B4GalKO)	Deletion of immunogenic blood group SDa antigen through knockout of the synthetic enzyme (B4GalNT2)	
CMP-N-acetylneuraminic acid hydroxylase KO (CMAHKO)	Deletion of immunogenic glycan N-glycolylneuraminic acid (Neu5Gc) through knockout of the synthetic enzyme CMP-N-acetylneuraminic acid hydroxylase (CMAH)	
Growth Hormone Receptor Knockout (GHRKO)	Reduction of downstream insulin growth factor-1 (IGF-1) signaling	Reduce intrinsic graft growth
Human Transgene Expression		
CD46	Suppress human complement activity by mediating cleavage of C3b and C4b complement deposition	Complement Regulation
Decay Accelerating Factor (DAF)	Inhibits C3 and C5 convertase enzymes and downstream complement activation	
Endothelial Cell Protein C Receptor (EPCR)	Activates Protein C	Anti-Coagulation
Thrombomodulin (TBM)	Binds human thrombin, and activates Protein C via activated thrombin	
Hemeoxygenase-1 (HO-1)	Decreases oxidative products	Anti-Inflammatory
CD47	Interacts with macrophage signal regulatory protein (SIRP)α to prevent opsonization and phagocytosis of xenogeneic tissue	