Update on Xenogeneic Transplantation as Treatment for End Stage Organ Failure

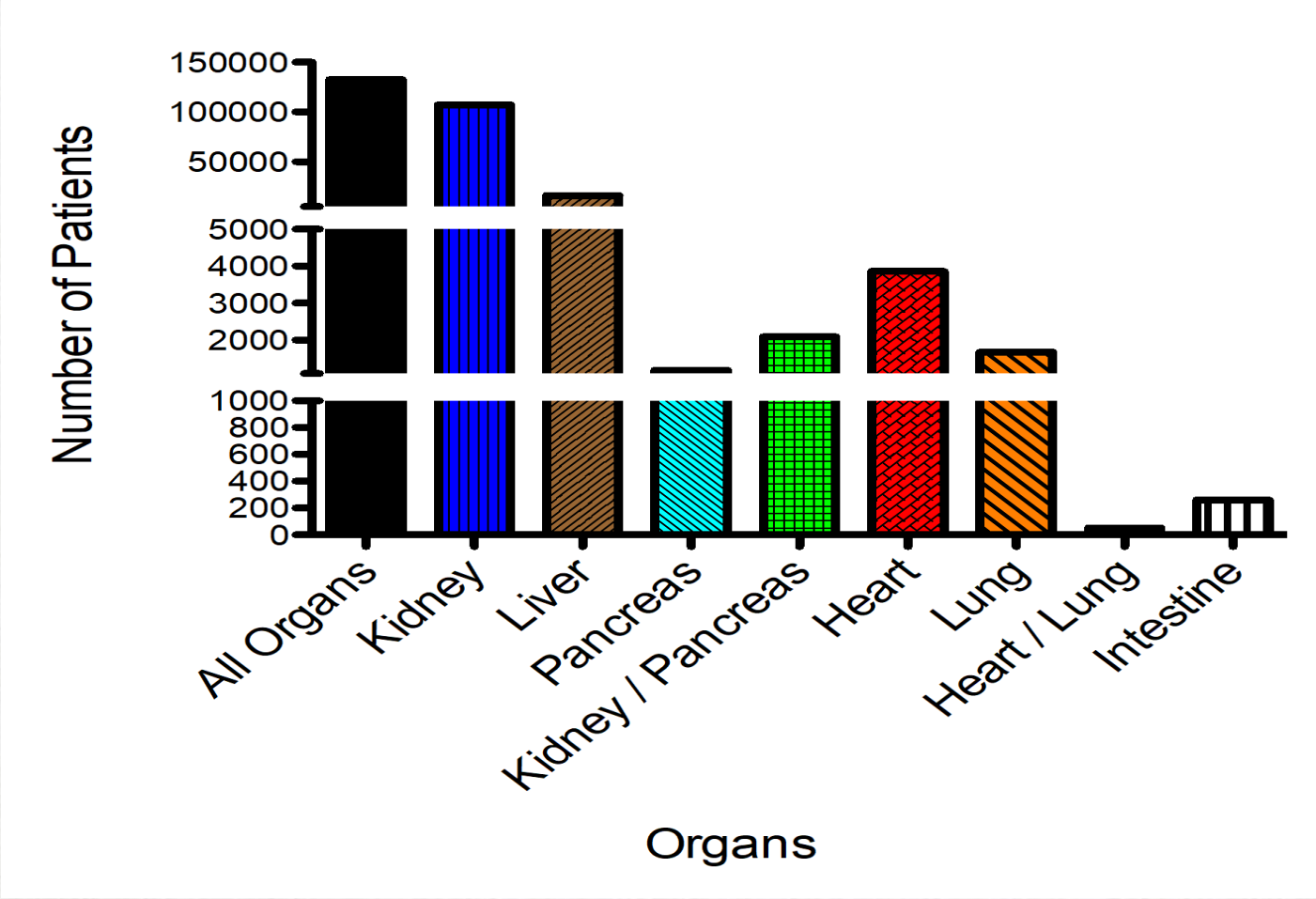
9 September 2022

LUNG
BIOTECHNOLOGY

INTRODUCTION

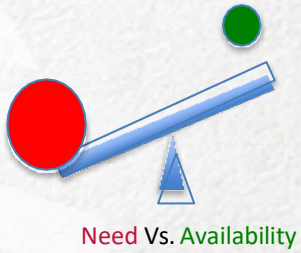
- Genetic engineering
- Immunosuppression
- Pre- Clinical experiments in Xenogeneic Kidney Transplantbation with Tolerance
- Pre- Clinical experiments in Xenogeneic Heart Transplantation
- Recent events
  - Decedent Xeno transplants (NYU, UAB)
  - First in Human, porcine cardiac xenotransplant, University of Maryland
- Summary and look forward

Candidates Awaiting Donor Organs for Transplantation



Heart Transplants/ year, EU ~ 2,100
Heart Transplants /year, US ~ 3,800

Kidney Transplants/ year, EU ~ 17,000
Kidney Transplants/ year, US ~ 24,500

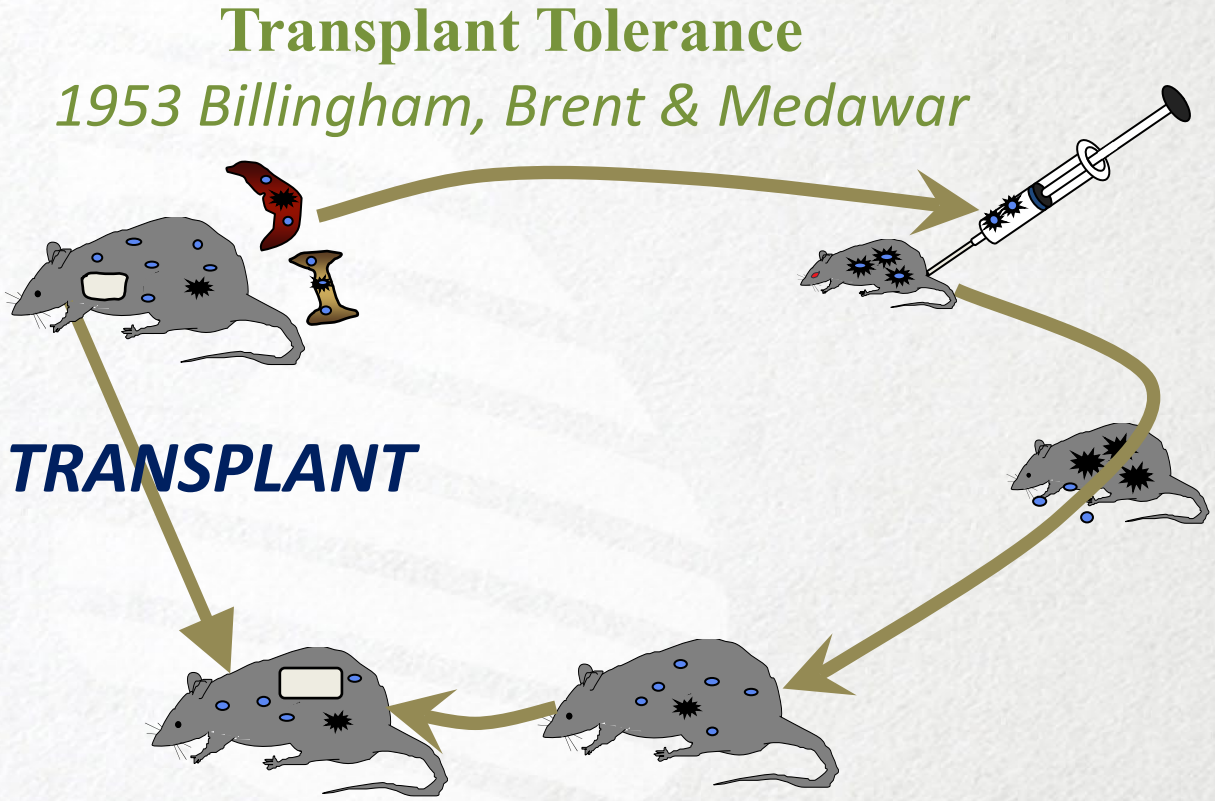


Need Vs. Availability

Overcoming Barriers to Xenogeneic Transplantation

- Genetic Engineering
- Immunosuppression

- Tolerance
  - The most efficient approach to overcome the multifaceted immunologic attack against xenografts?



α1,3Gal Knockout Pig; GalSafe™

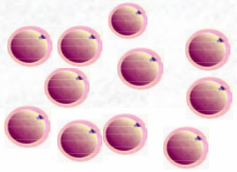
2002 – a1,3-galactosyltransferase KO in cloned pigs

- Dai Y, Nature Biotechnology. 2002.
- Liangxue L et al. 2002. Science. 295:1089
- Simonds DK, et al. 2004. PNAS. 101:7335

Revivicor, Inc, Blacksburg, VA, USA

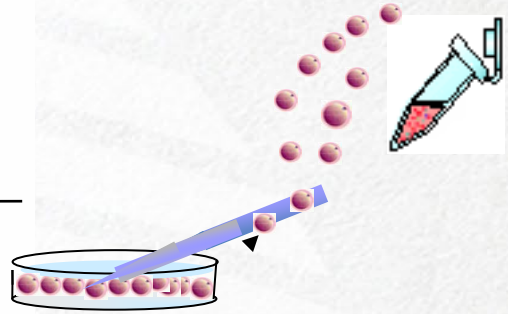
- 1995-2003, US Division of PPL Therapeutics
- 2000 World's 1st Cloned pigs
- 2011 United Therapeutics Corporation – Focus on Xenotransplantation
- GalSafe™ [Genotype: Gal gene knockout; Phenotype: Gal- null]
  - Stable breeding over multiple generations w/ normal, growth, fertility, health, etc.
  - Characterization of pig/safety/regulatory
  - Preclinical efficacy studies - ongoing
    - Kidney, heart, lung, Islet, skin transplantation
- 2020 GalSafe™ NADA completed – 1st FDA approval for human food & therapeutics

Nuclear Transfer



Collection of oocytes from donor ovaries retrieved from USDA approved abattoir (shipped by FedEx from Tennessee)

Microscopic aspiration of all genomic DNA: polar body and metaphase plate



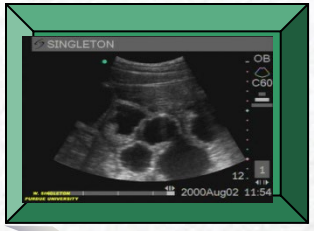
•**Genetically modified cells with DNA designed w/specific characteristics: ie. Gal knockout**

- Quiescent to immune system
- Reduce immunosuppression
- Enhance healing

Add back GE cells; Electrical fusion

Putative embryos

~ 150 putative embryos transferred to unmodified estrus synchronized surrogates



Gene Modifications

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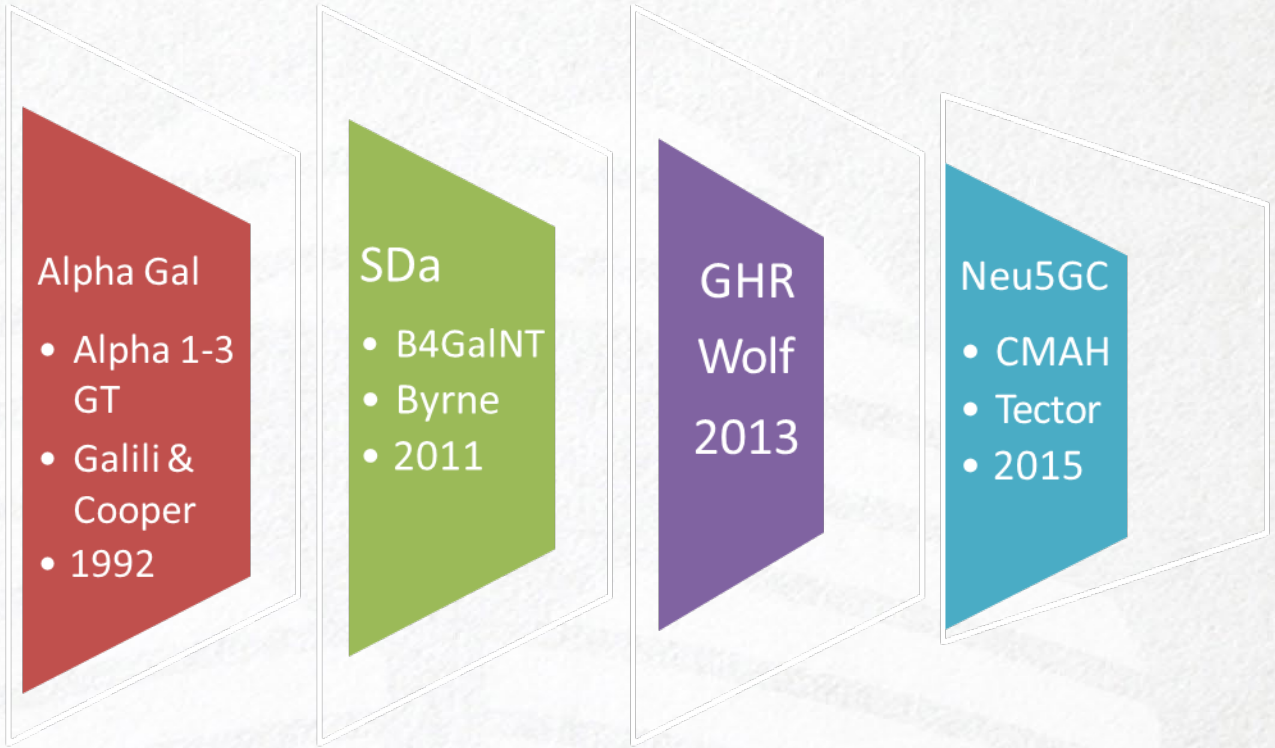
Progressive gene knockouts



Progressive addition of Human Transgenes

Xenogeneic Transplantation; Revivicor Gene Modifications

#	Gene Modifications Tested	GE (n)
1	GTKO	1
2	GTKO.hCD46	2
3	GTKO.hCD46.hTBM	3
4	GTKO.B4KO.hCD46.hHLAE	4
5	GTKO.B4KO.hCD46.hCD47	4
6	GTKO.CD46.CD47.EPCR	4
7	GTKO.CMAHKO.hCD46.hCD47.hTFPI	5
8	GTKO.CMAHKO.hCD46.hEPCR.hCD55	5
9	GTKO.CMAHKO.hTBM.hCD46.hCD47.HO1	6
10	GTKO.CD46.TBM.CD47.EPCR.HO1	6
11	GTKO.CMAHKO.hTBM.hEPCR.hCD46.hCD55.hHO1	7
12	GTKO.B4KO.GHRKO.hTBM.hEPCR.hCD46.hCD47	7
13	GTKO.B4KO.hTM.hEPCR.hCD46.hCD47.hHO1.hVWF	8
14	GTKO.B4KO.hCD46.hCD55.hTBM.hEPCR.hCD47.hHO1	8
15	GTKO.B4KO.CMAH.hCD46.hCD55.hTBM.hEPCR.hCD47.hHO1	9
16	GTKO.B4KO.CMAHKO.GHRKO.hCD46.hCD55.hTBM.hEPCR.hCD47.HO1	10

Xenogeneic Donor Organs for Transplantation

Current UT/ Revivicor Focus

Gal-TKO; (GalSafe™) Pig

‘10-GE’; 4 KO + 6 human transgenes

Pre- Clinical Xenogeneic Kidney Transplantation using Genetically Engineered Porcine Donors into Baboons

David H. Sachs, MD
Megan Sykes, MD, PhD
Kazuhiko Yamada, MD, PhD

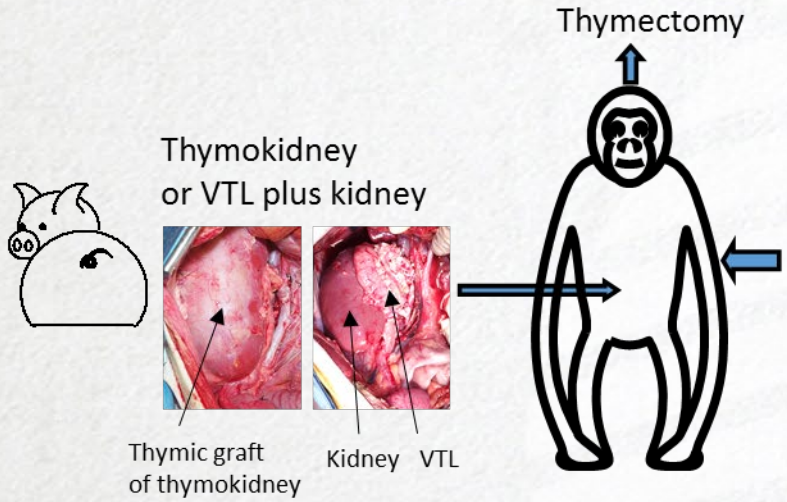
Induction of Transplantation Tolerance

Method	Rodents	NHP
Enhancement	+	-
DST	+	-
Peptides	+	-
Anti-MHC mAbs	+	-
Cyclosporin	+	-
ALS/ ATG	+	-
Anti-CD4	+	-
Anti-CD25	+	-
TLI	+	-
Anti-CD3 toxin	+	-
Co-stimulatory blockade	+	-
Chimerism	+	+

Two approaches for chimerism based transplant tolerance

Tolerance by Thymus Transplantation

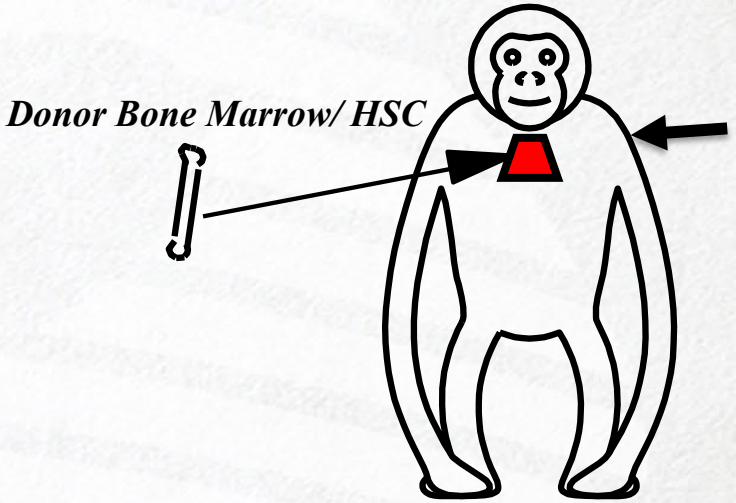
Pig GalT-KO Thymokidney or VTL plus Kidney to Baboons



Pre-op
T cell depletion
Rituximab

Pre-op (tapering)
Costimulatory blockade
MMF/Rapamycin

Tolerance by Mixed Chimerism



Pre- op
T- cell depletion
Rituximab
CTLA4-Ig w/ tapering
MMF/ rapamycin

CONTRASTING TOLERANCE STRATEGIES

	Thymic Transplantation	SC Transplantation (Mixed Chimerism)
Tolerance mechanism	T-cell only	Multilineage: B-, T, and NK-cells
Procedure	One stage: Simultaneous TK/VTL + Xeno Organ	Two Stage: 60d interval between SC + XenoOrgan
Donor	Single	Likely multiple
Immunosuppression	Taper; may need minimal ongoing	Wean→off
Target Organs	Kidney (*TBD: Heart*)	Lung (Innate tolerance essential)
R+D need	Kidney: Ready for pre-IND study; eIND	Additional GE

Inducing T and B Cell Tolerance to Xenografts

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Thymic transplantation - Tolerizes T cells

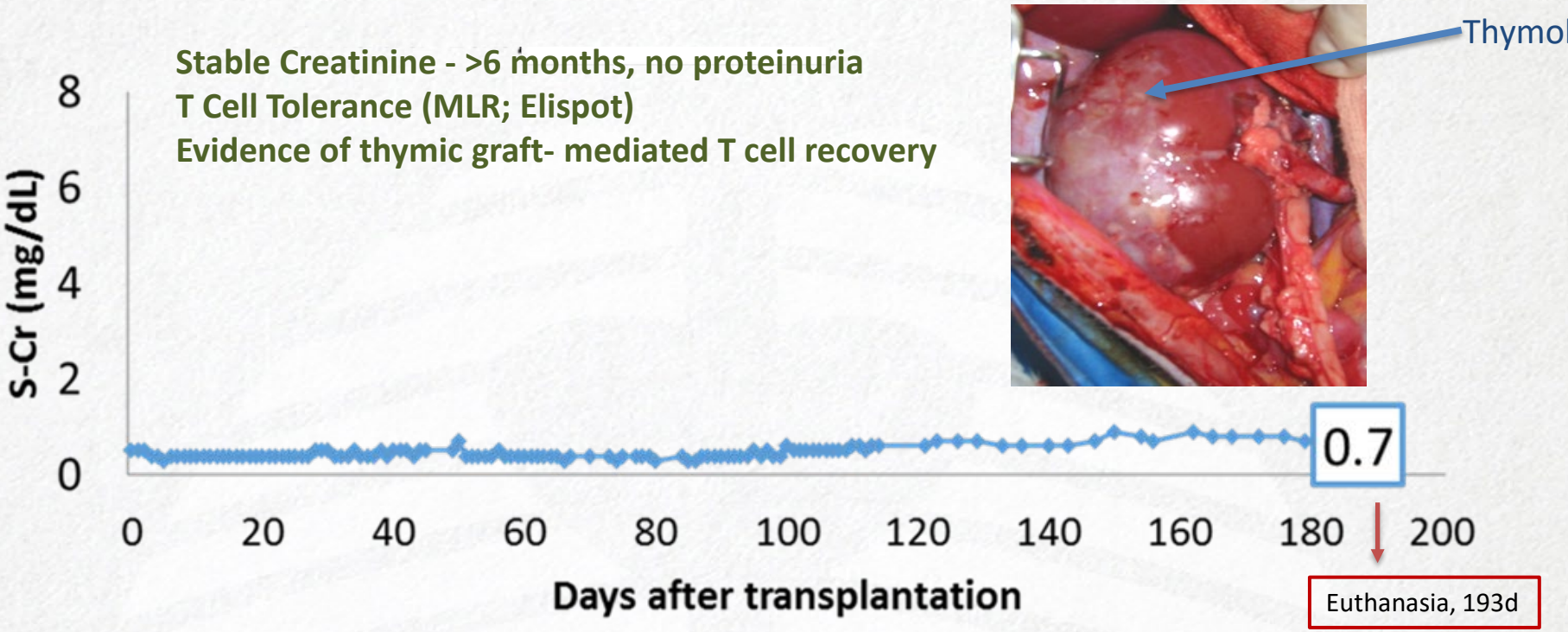
Mixed chimerism - Tolerizes T, B cells, NK cells

Thymus Transplantation: T cell Tolerance via 2 Mechanisms

- Donor (Pig) thymic epithelium and APCs delete developing T cells recognizing porcine antigens

- Donor (Pig) thymic epithelium positively selects regulatory T cells (Tregs) that suppress anti-pig responses (deals with residual host T cells)

GE Pig to Baboon Thymokidney Transplantation



- Histologically and functionally normal kidney function
- New cells, coming from the donor (pig) thymus, bearing donor (pig) cellular markers were identified in the recipient (baboon) circulation – another measure of chimerism
- In vitro, donor specific unresponsiveness – reflecting immunologic tolerance (MLR & Elispot assays)
- Euthanasia @ 193d, due to kidney growth w/ abdominal compartment syndrome, & renal txp ischemia (histology)

Tanabe, Sachs, Yamada et al. *Am J Transplant.* 2017

Immunosuppression, XenoKidney

Thymic Tolerance

1. Thymectomy prior to transplant
2. Splenectomy at time of transplant
3. Medications:

Pre Tx:	Rabbit ATG
	Rituximab
	MMF, beginning pre tx, then post tx tapering every 7-14 days
	[consider D/C MMF @ 60d (monitoring parameters/ requirements TBD)]
Post Tx:	Anti-CD40 mab (dosing & frequency TBD)
	Rituximab
	Belatacept, once weekly (GTKO donor kidney)
	Rapamycin, beginning POD 21 (upon wound healing) to minimize organ growth

4. Target ongoing reduction, POD 90 if stable with immune testing (e.g. MLR/ Elispot/ emerging anti pig Ab levels, etc.)

XenoKidney Tolerance; Revivicor, Donor Pigs, 2018 – 2019 (n=18)

Summary

- Tolerance induced by thymic chimerism can prevent rejection of pig kidneys in baboons
- Absent infection &/or technical complications, durable xenokidney survival (154-187d) w/ varied gene edits (n= 1- 9) has been observed
- Xenograft organ growth limited long-term survival of GE pig kidneys in baboons with normal renal fxn to the 6-9 mo range
- Baboon recipients of GTKO Pig kidneys have suffered nephrotic range proteinuria, associated with glomerular podocyte effacement; the proteinuria was effectively
  - Delayed by rituximab administration, which was found to prevent podocyte disruption, and
  - Controlled with weekly administration of CTLA4-Ig (Belatacept) starting on POD #2

American Journal of Transplantation

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ORIGINAL ARTICLE

First clinical- grade porcine kidney xenotransplant using a human decedent model

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The NEW ENGLAND JOURNAL of MEDICINE

Original Article

Results of Two Cases of Pig-to-Human Kidney Xenotransplantation

Robert A. Montgomery, M.D., D.Phil., Jeffrey M. Stern, M.D., Bonnie E. Lonze, M.D., Ph.D., Vasishta S. Tatapudi, M.D., Massimo Mangiola, Ph.D., Ming Wu, M.D., Elaina Weldon, M.S.N., A.C.N.P.-B.C., Nikki Lawson, R.N., Cecilia Deterville, M.S., Rebecca A. Dieter, Pharm.D., B.C.P.S., Brigitte Sullivan, M.B.A., Gabriella Boulton, B.A., Brendan Parent, J.D., Greta Piper, M.D., Philip Sommer, M.D., Samantha Cawthon, B.S., Erin Duggan, M.D., David Ayares, Ph.D., Amy Dandro, M.S., Ana Fazio-Kroll, Ph.D., Maria Kokkinaki, Ph.D., Lars Burdorf, M.D., Ph.D., Marc Lorber, M.D., Jef D. Boeke, Ph.D., Harvey Pass, M.D., Brendan Keating, Ph.D., Adam Griesemer, M.D., Nicole M. Ali, M.D., Sapna A. Mehta, M.D., and Zoe A. Stewart, M.D., Ph.D.

n engl j med 386;20 nejm.org May 19, 2022

Cardiac Xenotransplantation

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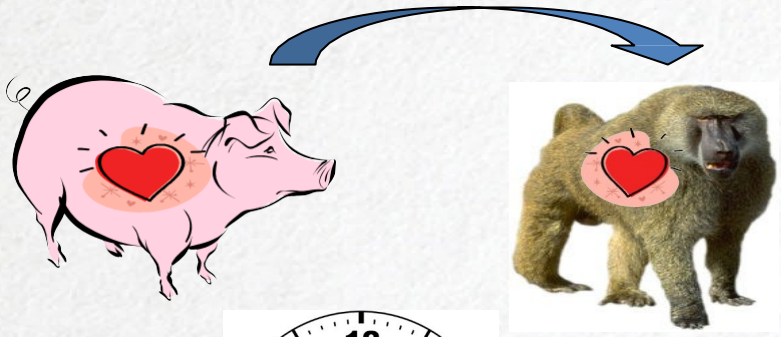
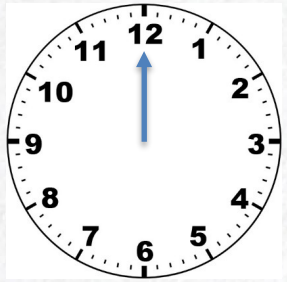
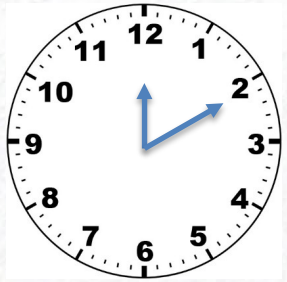
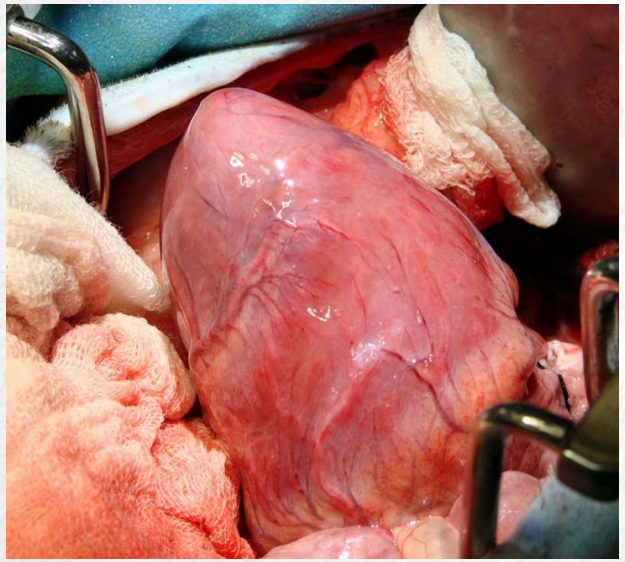
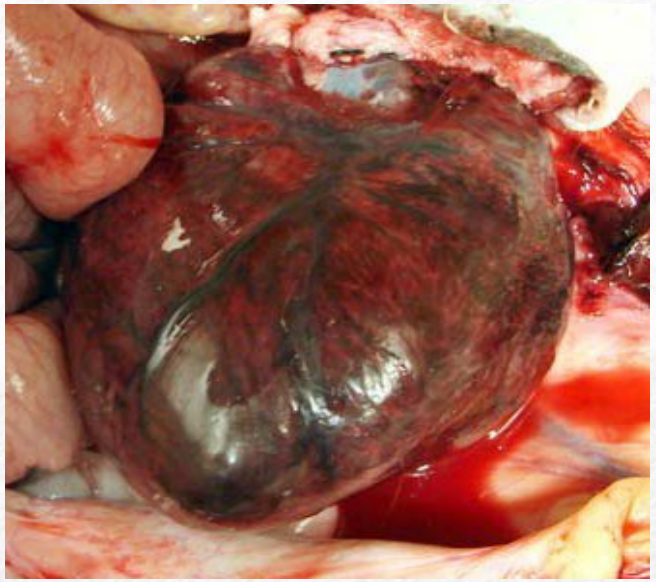
Rejection

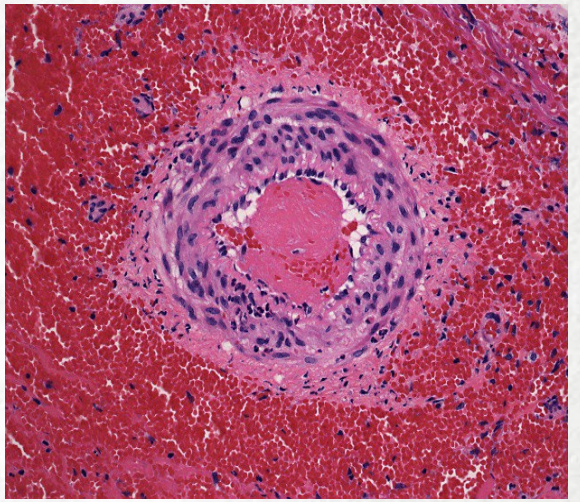


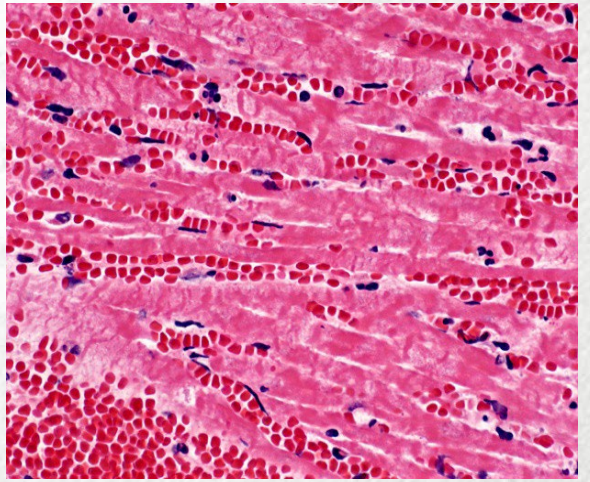




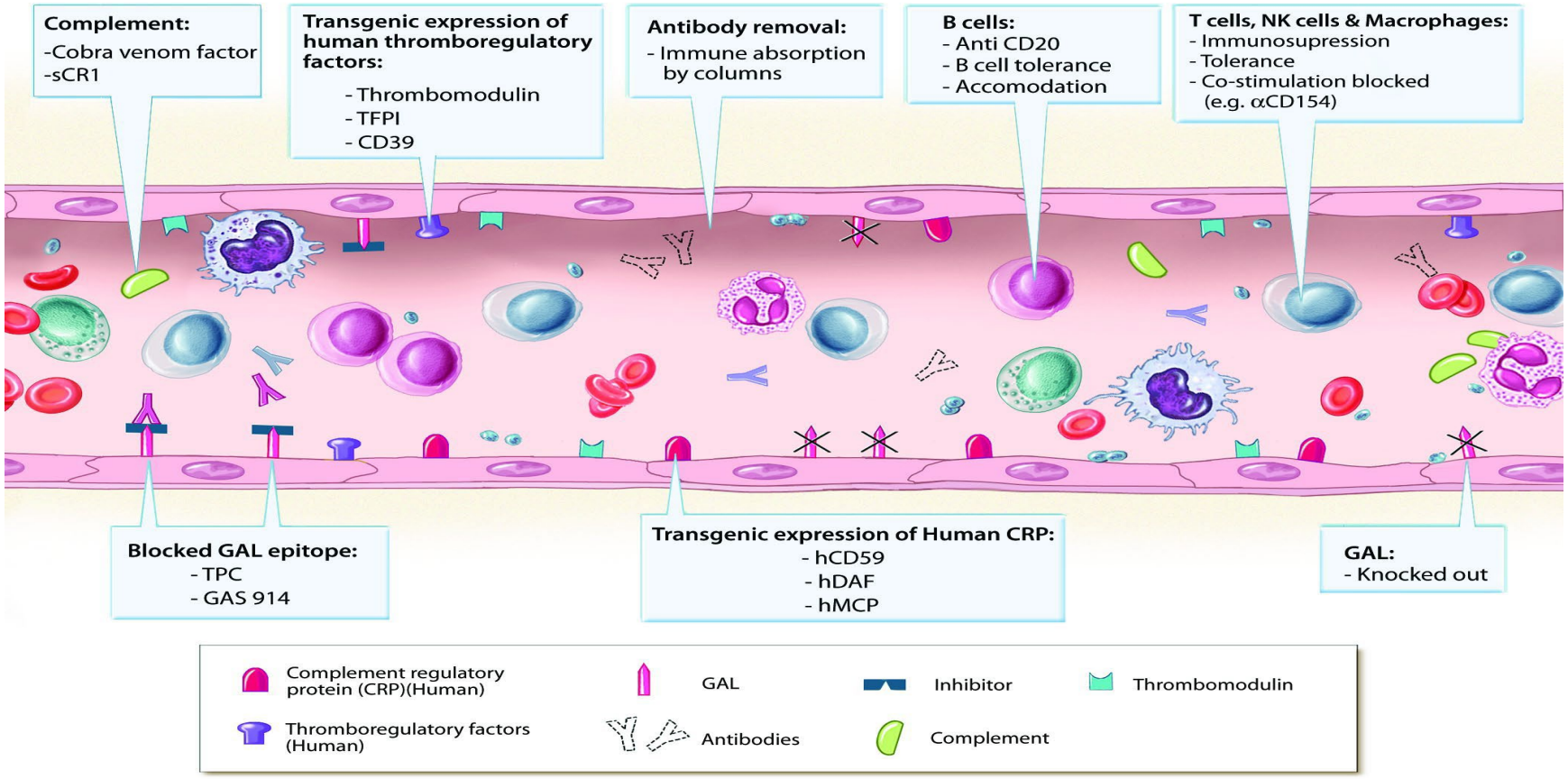






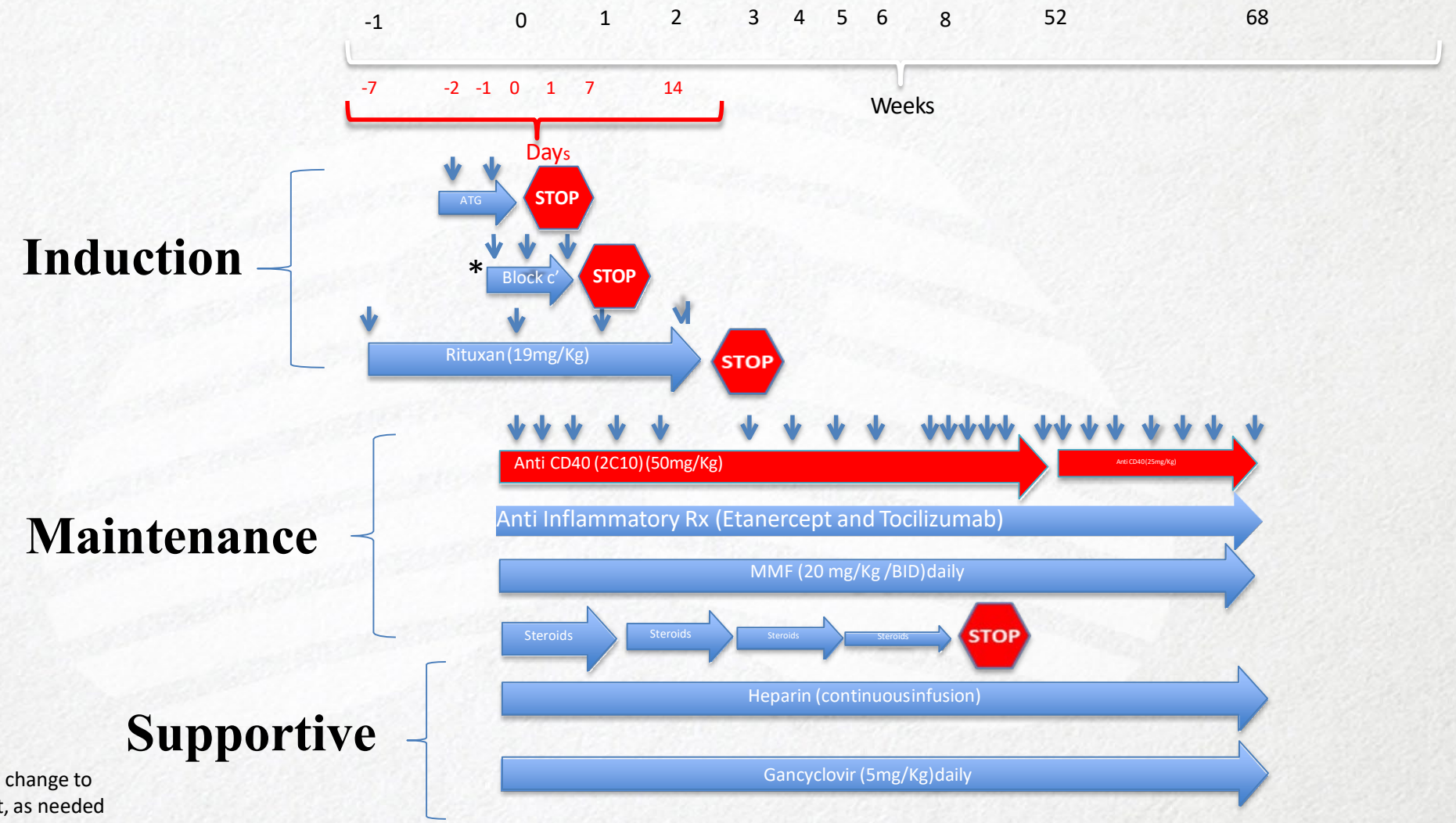


Current Methods to Prevent Xenograft Rejection



Mohiuddin, M. M. Clinical xenotransplantation of organs: why aren't we there yet? *PLoS Med.* **4**, e75 (2007).

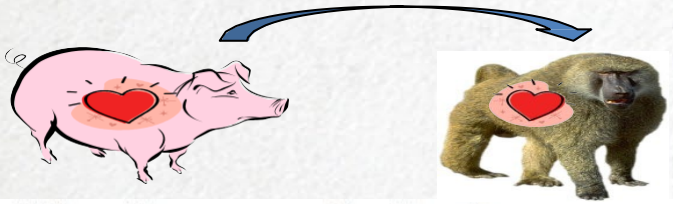
Mohiuddin Immunosuppression Regimen

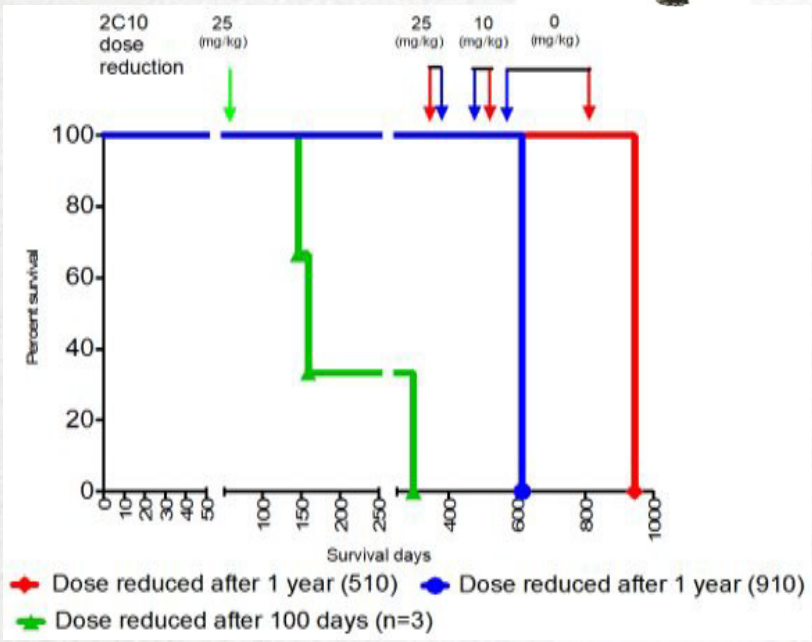


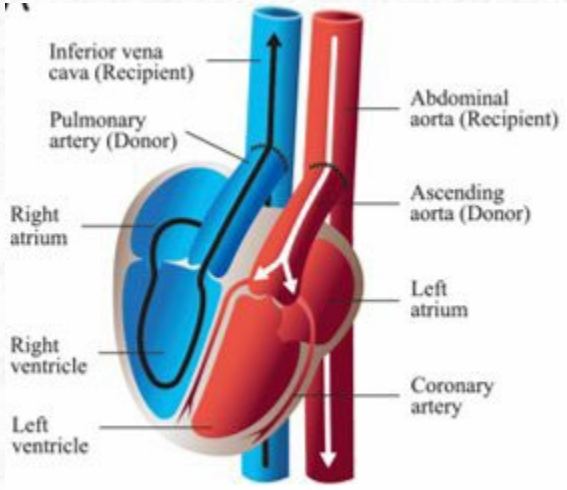
- c' inhibition initially used CVF; change to C1 esterase inhibitor, Berinert, as needed

Proof of Principle

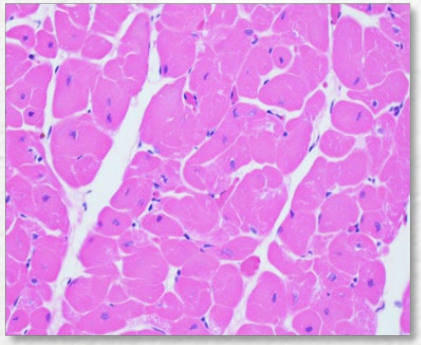
Xenograft Rejection CAN be avoided





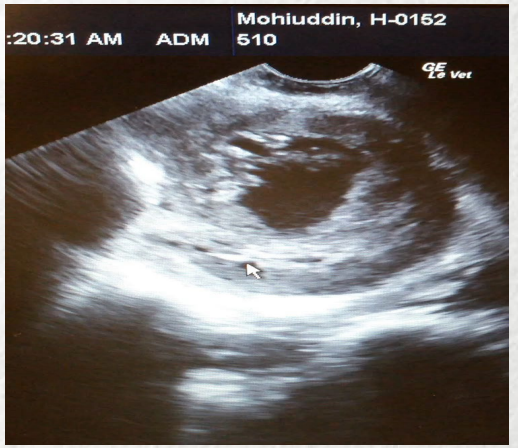


- Consistent graft survival over 1 year
- Longest survival for 945 days
- Minimum IS is required to maintain survival
- Rejection on withdrawal of anti CD40
- Antibody rejection of removal o anti CD40



Normal histology before removal of anti CD40 antibody treatment

Normal echo before removal of anti CD40 antibody treatment



Mohiuddin et al, Nature Communications. **volume 7**, Article number: 11138 (2016)

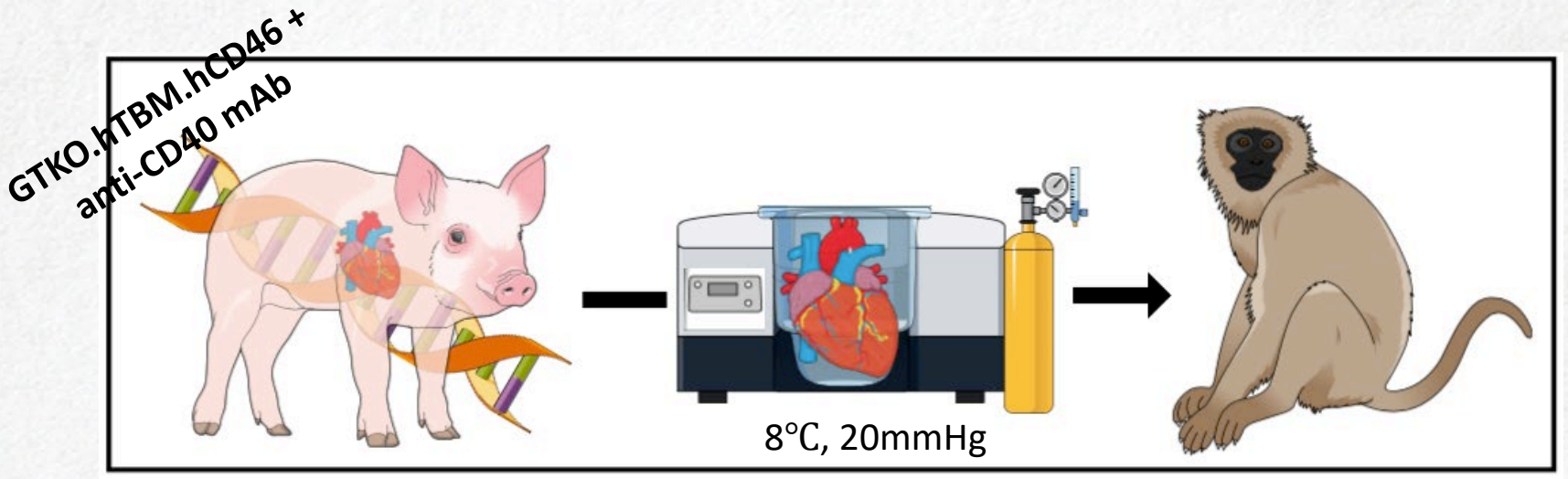
Transition to Orthotopic Porcine to Baboon Heart Transplant Model

Initial UMB Orthotopic XenoHeart Experience, 2017 – 2020

- Orthotopic XenoHeart transplantation with consistent technical success
- GE porcine donor organs with varied gene edits (n = 2-7)
- Standard Mohiuddin Immunosuppression as developed in heterotopic experience
- Unlike the heterotopic experience consistent early mortality, presumably linked to post-bypass inflammatory responses
- No histologic or functional evidence of rejection
- Characterized by **P**erioperative **C**ardiac **X**enograft **D**ysfunction (*PCXD)

*Byrne & McGregor. 2012. PCXD. Curr Opin Organ Transpl

Overcoming PCXD



Perfusate:

Table 3. The perfusion medium used for 24-hour heart preservation.

Na^+	136 mmol/L
K^+	23 mmol/L
Ca^{2+}	1.3 mmol/L
Mg^{2+}	8.0 mmol/L
Cl^-	142 mmol/L
HCO_3^-	25 mmol/L
PO_4^{2-}	1.3 mmol/L
D-Glucose	6.3 mmol/L
Albumin	75 g/L
Cocaine	6 pmol/L
Noradrenaline	6 pmol/L
Adrenaline	6 pmol/L
T3	3 pmol/L
T4	2 pmol/L
Cortisol	420 pmol/L
Insulin	8 U/L
Imipenen	20 mg/L
Erythrocytes (Hct)[a]	15%
96% O_2 + 5% CO_2[b]	0.2 L/min

[a]When all drugs and erythrocytes have been added and mixed and the PCO_2 has stabilized, pH is adjusted to 7.40 by means of sodium bicarbonate.
[b]Administered through the oxygenator.

Electrolytes (Hyperkalemic, Hyperosmolar)

Noradrenaline, Adrenaline, T3/T4, Cocaine, Cortisol

Erythrocytes, oxygen exchange

Langin et al. 2018. Nature.
https://doi.org/10.1038/s41586-018-0765-z

UMB Orthotopic XenoHeart Results: XVIVO Heart Preservation System 2020-2021

- Introduction of extracorporeal non- ischemic continuous perfusion (*Steen; **Langin)
  - XVIVO Heart Preservation System (XVIVO Perfusion, Gothenburg, Sweden)
  - GE pigs (Revivicor, Blacksburg, VA, USA) with varied numbers of gene edits (7-10)
  - Standard Mohiuddin Immunosuppression regimen
- No PCXD was observed
- Extended survival up to 264 days among 12 sequential orthotopic transplants
  - 6 of 12 >90 days
  - 3 of 12 >180 days

Received: 21 January 2022 | Revised: 2 March 2022 | Accepted: 10 March 2022

DOI: 10.1111/xen.12744

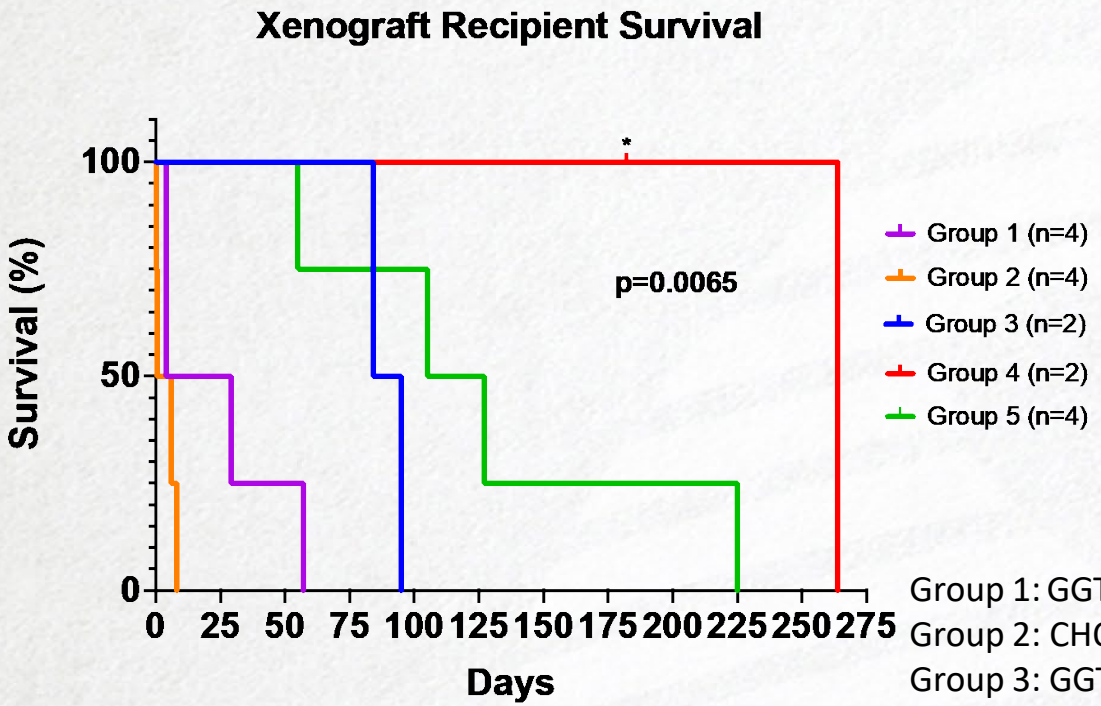
Xenotransplantation WILEY

ORIGINAL ARTICLE

Progressive genetic modifications of porcine cardiac xenografts extend survival to 9 months

Muhammad M. Mohiuddin[1] | Corbin E. Goerlich[1,2,*] | Avneesh K. Singh[1,*] | Tianshu Zhang[1] | Ivan Tatarov[1] | Billeta Lewis[1] | Faith Sentz[1] | Alena Hershfeld[1] | Gheorghe Braileanu[1] | Patrick Odonkor[3] | Erik Strauss[3] | Brittney Williams[3] | Allen Burke[4] | Jamie Hittman[4] | Adnan Bhutta[5] | Ali Tabatabai[6] | Anuj Gupta[7] | Todd Vaught[8] | Lori Sorrells[8] | Kasinath Kuravi[8] | Amy Dandro[8] | Will Eyestone[8] | David J. Kaczorowski[1] | David Ayares[8] | Bartley P. Griffith[1]

Consistent long-term cardiac xenograft survival w/progressive elimination of immunogenic pig antigens and expression of human transgenes



- All recipients easily weaned off CPB
- Improved survival with progressive genetic modifications (no need for temsirolimus)
- Xenografts without thromboregulatory proteins (TRP) performed poorly, regardless of the addition of complement regulatory proteins (CRP)
- Xenografts with multiple KO, TRP and CRP performed markedly better than “3- gene”

Group 1: GGTA1KO.hTBM.hCD46
Group 2: CHO KO without thromboregulatory proteins +/- hCD46 and hDAF
Group 3: GGTA1KO.B4GalNT2KO.**CMAHKO**.hTBM.hEPCR.hCD46.hDAF.hCD47.HO1
Group 4: GGTA1KO.B4GalNT2KO.**GHRKO**.hTBM.hEPCR.hCD46.hCD47
Group 5: GGTA1KO.B4GalNT2KO.**CMAHKO.GHRKO**. hTBM.hEPCR.hCD46.hDAF.hCD47.HO1

Mohiuddin, M., Goerlich, C., Singh, A., *et al. Progressive Genetic Modifications with Growth Hormone Receptor Knockout Extends Cardiac Xenograft Survival to 9 Months.* Xenotransplantation. (Under Review). 2021

The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

Genetically Modified Porcine-to-Human Cardiac Xenotransplantation

Bartley P. Griffith, M.D., Corbin E. Goerlich, M.D., Ph.D., Avneesh K. Singh, Ph.D., Martine Rothblatt, Ph.D., Christine L. Lau, M.D., Aakash Shah, M.D., Marc Lorber, M.D., Alison Grazioli, M.D., Kapil K. Saharia, M.D., Susie N. Hong, M.D., Susan M. Joseph, M.D., David Ayares, Ph.D., and Muhammad M. Mohiuddin, M.D.

SUMMARY

A 57-year-old man with nonischemic cardiomyopathy who was dependent on venoarterial extracorporeal membrane oxygenation (ECMO), and was not a candidate for standard therapeutics, including a traditional allograft, received a heart from a genetically modified pig source animal that had 10 individual gene edits. Immunosuppression was based on CD40 blockade. The patient was weaned from ECMO, and the xenograft functioned normally without apparent rejection. Sudden diastolic thickening and failure of the xenograft occurred on day 49 after trans-plantation, and life support was withdrawn on day 60. On autopsy, the xenograft was found to be edematous, having nearly doubled in weight. Histologic examination revealed scattered myocyte necrosis, interstitial edema, and red-cell extravasation, without evidence of microvascular thrombosis, findings that were not consistent with typical rejection. Studies are under way to identify the mechanisms responsible for these changes. (Funded by the University of Maryland Medical Center and School of Medicine.)

From the Department of Surgery (B.P.G., C.E.G., A.K.S., C.L.L., A.S., M.M.M.), the Program in Trauma, R. Adams Cowley Shock Trauma Center, Department of Medicine (A.G.), the Institute of Human Virology, Division of Infectious Diseases (K.K.S.), and the Department of Medi- cine, Division of Cardiology (S.N.H., S.M.J.), University of Maryland School of Medicine, Baltimore, and United Thera- peutics, Silver Spring (M.R., M.L.) — both in Maryland; and Revivicor, Blacksburg, VA (D.A.). Dr. Griffith can be contacted at bgriffith@som.umaryland.edu or at the Department of Surgery, University of Maryland School of Medicine, 110 S. Paca St., 7th Floor, Baltimore, MD 21201. Dr. Mohi- uddin can be contacted at mmohiuddin@ som.umaryland.edu or at the Depart- ment of Surgery, University of Maryland School of Medicine, 10 S. Pine St., MSTF 434B, Baltimore, MD 21201.

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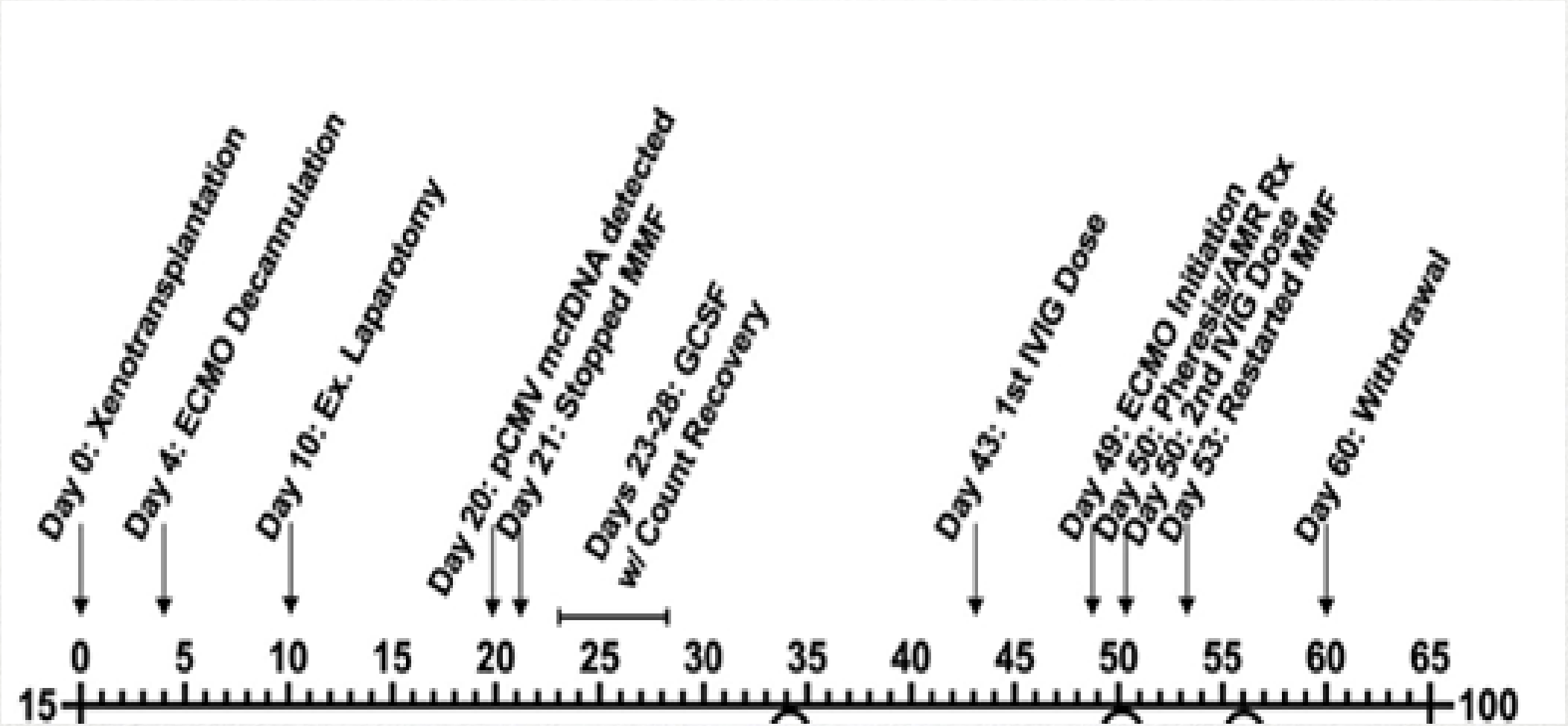
The Patient

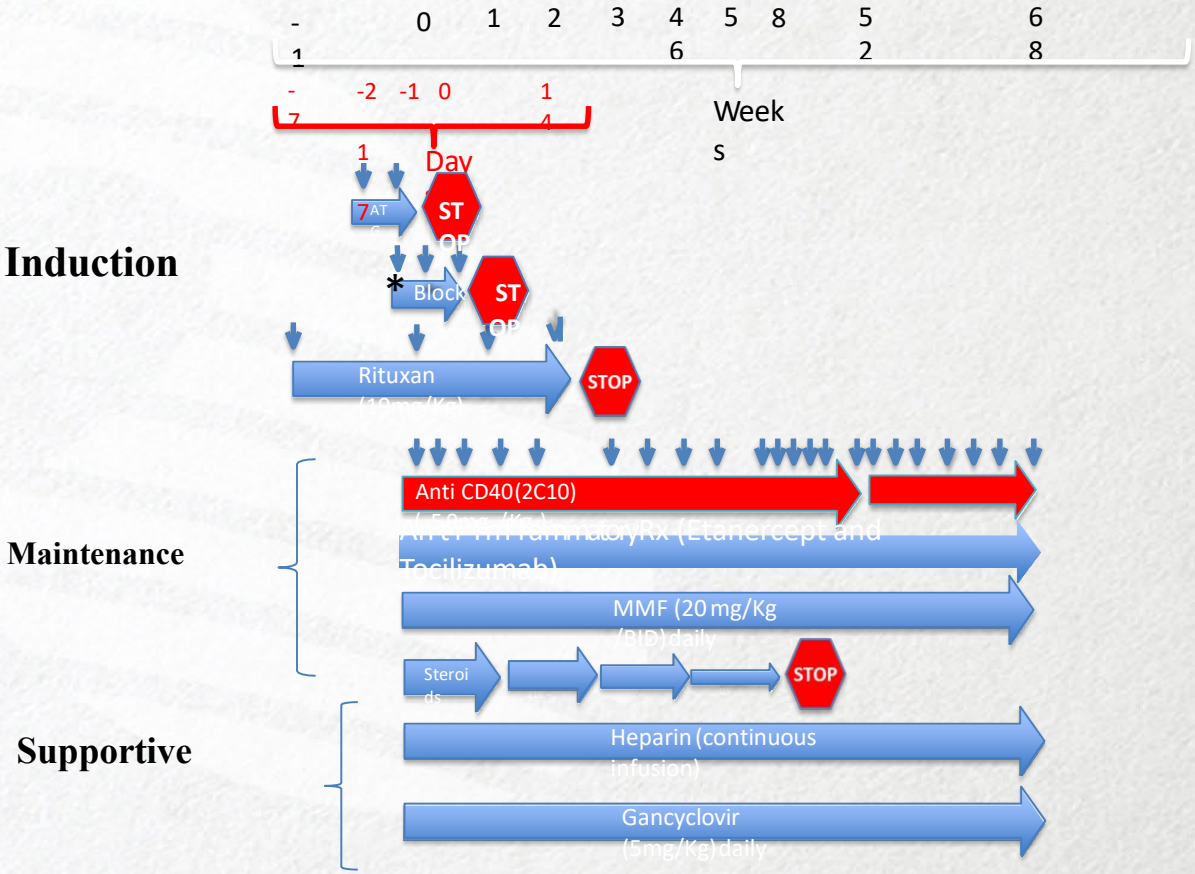
- 67- year old with non-Ischemic Cardiomyopathy
- History of mitral valve repair
- Multiple inotropic support
- Intra aortic balloon pump
- Ventricular arrythmias leading to arrest
- Biventricular failure with EF of 11%
- Sarcopenic
- Inability to ambulate for 2 months
- NON-COMPLIANT; therefore denied listing for allotransplantation
- VA ECMO support for 40 days

The Clinical Course



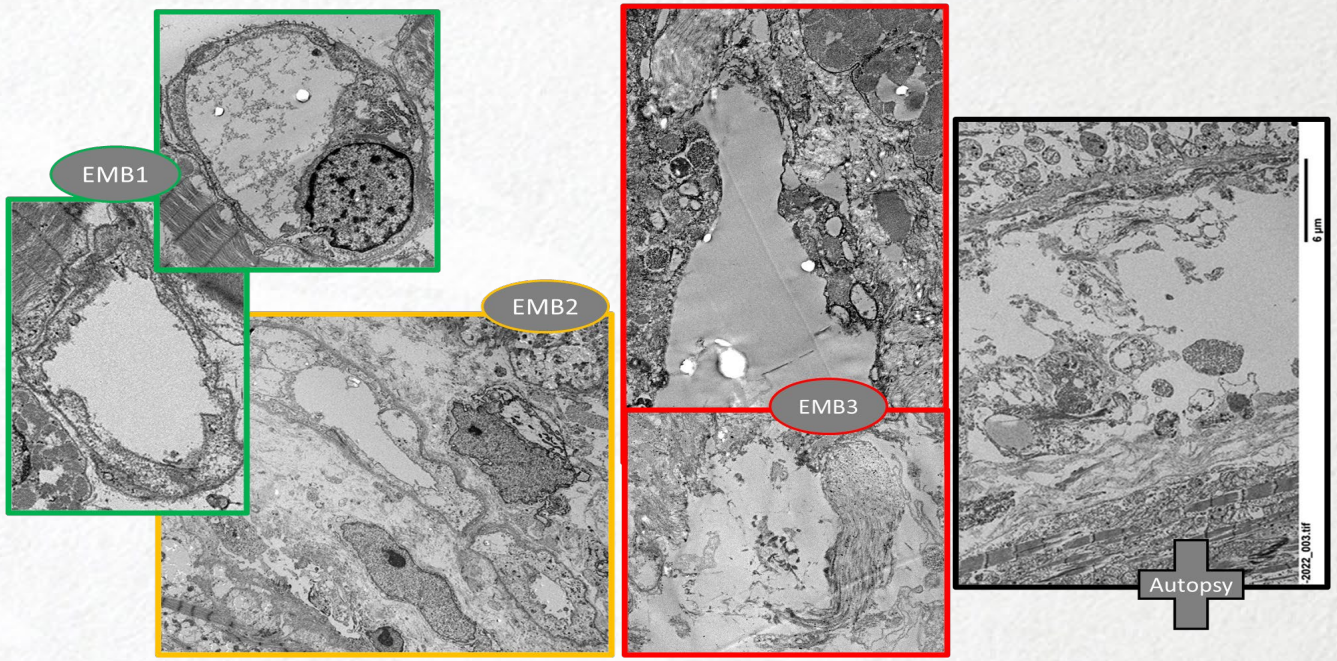
Immunosuppression

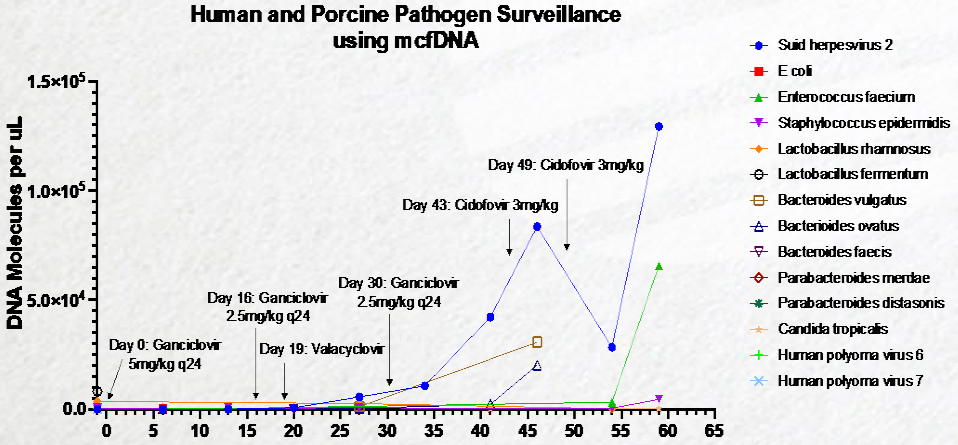
- Anti CD40 mAb, twice weekly titrated to therapeutic level
- Rituximab, day -1, day 8
- ATG 4 mg/kg total, completed day 7
- C1 esterase inhibitor day -1 and day 0
- MMF 500mg BID (HELD); replaced with low-dose Tacro
- Methylprednisone 125mg with rapid taper daily, to 30mg/ day

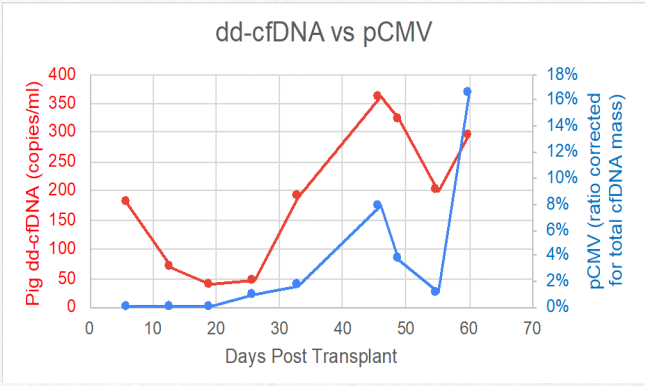


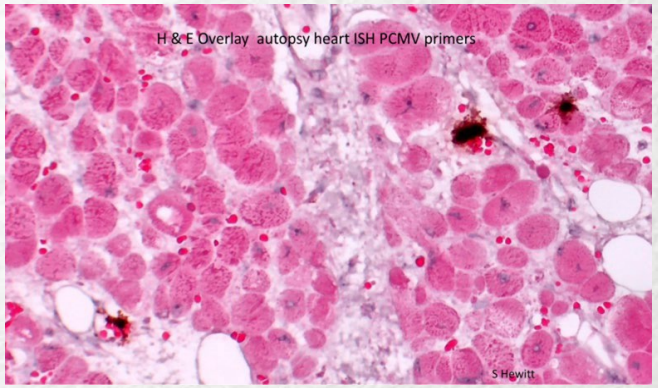
VIRAL SURVEILLANCE

- Donor pig spleen demonstrated 150 copies of pCMV DNA on PCR
- pCMV detected in explanted heart (low copies).
- **NO** Cytopathic changes
- **NO** Inclusions
- **NO** virus found on EM
- InSitu Hybridization results shows evidence of viral DNA but no cellular localization









Summary; What is Known

- Preexisting conditions , severe debilitated state, and repeated infections complicated recovery of the patient from surgery.
- Preexisting pancytopenia (Platelet <90K, WBC < 3) limited translation of IS protocol
- Preexisting condition like Sarcopenia, limited nutrition hampered recovery
- Repaired Dissection and circ arrest likely promoted ATN.
- No signs of xenograft rejection on biopsies. No infiltrates. Showed only interstitial edema.
- Interstitial edema and possibly fibrosis led to reduced diastolic function.

A Look Toward the Future; XenoTransplant Development at United Therapeutics

Revivicor GE Porcine Donors

- Xenoheart
- XenoKidney
- Xenogeneic ThymoKidney

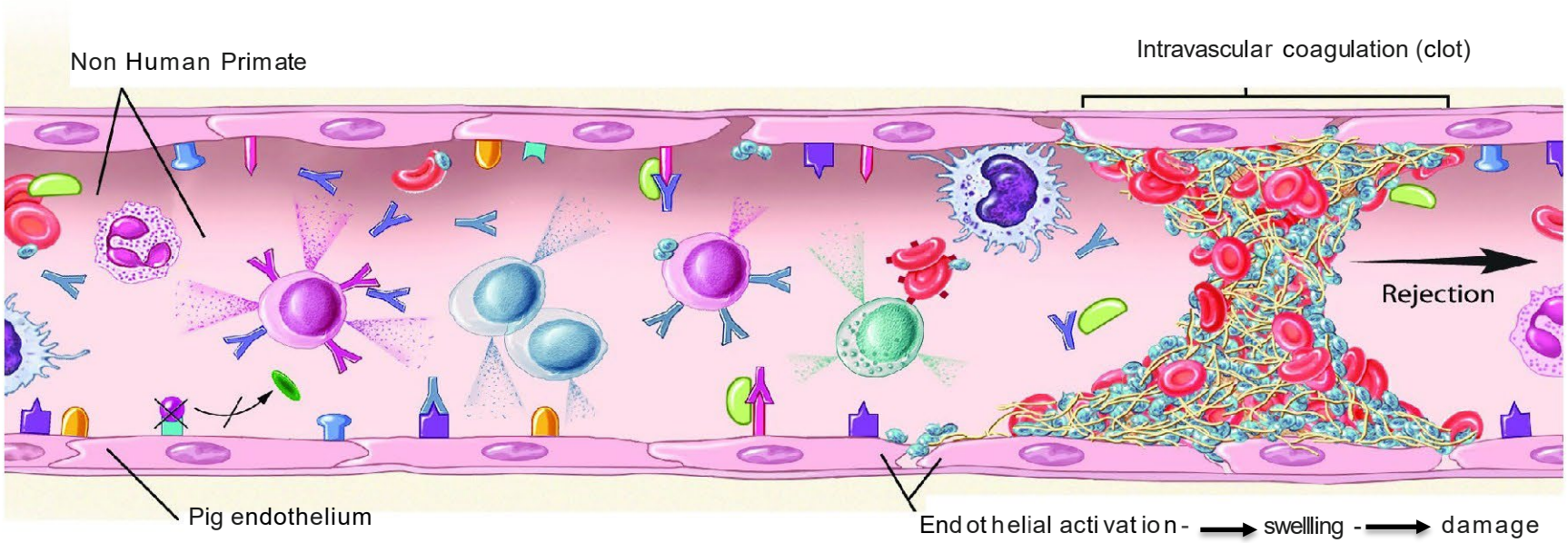
Back up Slides

Immunosuppression, XenoKidney

No Tolerance Induction Protocol

Pre Tx:	MMF (IV; 70 mg/kg/d), beginning day -7 Rabbit ATG, Day -3, then day -1 Rituximab, 10mg/kg, day -2 anti- CD40 mAb, 50 mg/kg, IV, day -1
Tx day 0:	MMF 70 mg/kg, IV anti-CD40 mAb, 50 mg/kg anti-IL6r mAb, 10 mg/kg Berinert,
Post Tx:	MMF (IV, 70 mg/kg) to day 60, then MMF, 100 mg/kg, p.o./d (if stable MMF blood levels) Anti-CD40 mAb, 50 mg/kg/d, IV, POD4, 7, 10, 14, then weekly Rapamycin, beginning POD 21 (upon wound healing) to minimize organ growth
Rescue Rx:	
(Elevated creatinine; Proteinuria)	Methylprednisolone (pulse & rapid taper; 10 mg/kg x 3 days;1 mg/kg x 3d, then .5 mg/kg x 3 d) Belatacept (10 mg/kg/wk until end of experiment)

Mechanisms of Xenograft Rejection



Incompatible complement regulatory proteins (pig)	NHP thrombin	Protein C	Lectin	Platelet
Incompatible thromboregulatory factors (pig)	GAL	Pig thrombomodulin	Tcell	Neutrophil
Preformed natural anibodies (XNA)	Anti GAL antibody	Complement	Bcell	Fibrin
Anti- Non- GAL antibodies	Non GAL	Red blood cells	NKcell	Macrophage

GE Pig Kidney to Baboon XenoTransplant + Thymic Tolerance (long survivors)

Xeno – Organ(s)	Donor Pig/ GE	Tolerance Protocol	CD47/ distrib.	CTLA4I/ antiIL6r POD#	Proteinuria	Rejection	Survival	Complication/ reason for euthanasia
K + VT	Sachs / GTKO	Thymic	None	CTLA4 Ig POD 8 -	<1+	No	**193d**	Organ growth/ compartment syndrome
K + VT	Rev/ GalSafe	Thymic	None	CTLA4 Ig POD 2 -	<1+	No	**126d**	IVC thrombosis/ central catheter
K + VT	Rev / Galsafe	Thymic	None	CTLA4 Ig POD 2 -	<1+	No	**174d**	Organ growth/ compartment syndrome
K + VT	Rev/ 6GE	Thymic	Glom + tubular	CTLA4 Ig	<1+	No	**153d**	Organ growth/ compartment syndrome
K + VT	Sachs/ 3GE (47 lo)	Thymic	glom.	No	<1+	No	**128d**	Organ growth/ compartment syndrome
K + VT	Rev/ 9GE	Thymic	Diffuse/ tubular	antiIL6r POD 21–60	<1+	No	**154d**	Organ growth/ compartment syndrome
K + VT	Rev/ 8GE	Thymic	Diffuse/ tubular	antiIL6r POD21-42	<1+	No	**187d**	MMF precip with SVC mass
								-

Initial UMB Orthotopic XenoHeart Experience, 2017 – 2020

LUNG BIOTECHNOLOGY

Perioperative **C**ardiac **X**enograft **D**ysfunction (*PCXD)

#	Genetics	GE (n)	Survival
1	GTKO.hCD46	2	14 hours
2	GTKO.B4KO.hCD46.hHLAE	4	7 hours
3	GTKO.B4KO.hCD46.hTM.hEPCR.hCD47.hHO1.hVW	8	26 hours
4	GTKO.CD46.TBMlow.CD47low.EPCR.HO1	6	3 hours
5	GTKO.CD46.TBMlow.CD47low.EPCR.HO1	6	22 hours
6	GTKO.CMAHKO.hCD46.hCD47.hTFPI	5	5 hours
7	GTKO.hCD46.hTBM	3	6 hours
8	GTKO.hCD46.hTBM	3	4 days
9	GTKO.hCD46.hTBM	3	29 days
10	GTKO.CMAHKO.hCD46.hEPCR.hDAF	5	2.5 days
11	GTKO.CMAHKO.hCD46.hEPCR.hDAF.hTBM.hHO1	7	8 hours
12	GTKO.CMAHKO.hCD46.hEPCR.hDAF.hTBM.hHO1	7	30 hours
13	GTKO.hCD46.hTBM	3	10 hours
14	GTKO.hCD46.hTBM	3	46 hours
15	GTKO.hCD46.hTBM	3	3 hours
16	GTKO.hCD46.hTBM	3	57 hours
17	GTKO.hCD46.hTBM	3	4 hours
18	GTKO.hCD46.hTBM	3	1 hours
19	GTKO.B4KO.GHRKO.hTBM.hEPCR.hCD46.hCD47	7	2 hours
20	GTKO.B4KO.GHRKO.hTBM.hEPCR.hCD46.hCD47	7	2 hours

*Byrne & McGregor. 2012. Curr Opin Organ Transpl

UMB Orthotopic XenoHeart with XVIVO Heart Preservation System 2020 – 2021

N	Porcine Donor Genotype	GE (n)	pCMV	Survival (days p tx)	Clinical Course	Xenoheart Histology	Echo	CD40 Pathway Blockade
1	GTKO.B4KO.GHRKO.hTBM.hEPCR.hCD46.hCD47	7	Neg	264*	Resp. distress; mortality, infection	Chronic vasculopathy	EF 55%; LV mass +33%	2C10R4
2	GTKO.B4KO.GHRKO.hTBM.hEPCR.hCD46.hCD47	7	Neg	182	Euthanasia; severe gingivitis/ wt loss	Unremarkable	EF 65% LV mass +33%	2C10R4
3	TKO (GTKO.B4KO.CMAH +/- no GHRKO) + CD46.DAF + TBM. EPCR.CD47.HO1	8	Neg	84	Diastolic heart failure	Antibody mediated rejection	EF 60% LV mass +181%	2C10R4
4	TKO (GTKO.B4KO.CMAH +/- no GHRKO) + CD46.DAF + TBM.EPCR.CD47.HO1	8	Neg	95	Diastolic heart failure	Inflammatory myocarditis	EF 65% LV mass +99.6%	2C10R4
5	QKO (GTKO.B4KO.CMAH$_+$ GHRKO) + CD46.DAF + TBM.EPCR.CD47.HO1	10	Neg	105*	Resp. distress; mortality, infection	Chronic vasculopathy; no rejection	EF 75% LV mass +53.5%	2C10R4
6	QKO (GTKO.B4KO.CMAH$_+$ GHRKO) + CD46.DAF + TBM.EPCR.CD47.HO1	10	Pos	55*	Resp. distress, mortality, infection	Acute cellular rejection	EF 50% LV mass +101%	2C10R4
7	QKO (GTKO.B4KO.CMAH$_+$ GHRKO) + CD46.DAF + TBM.EPCR.CD47.HO1	10	Pos	14*	Resp. distress; mortality, infection	No acute rejection; myodegeneration w/ fibrosis	-	2C10R4
8	QKO (GTKO.B4KO.CMAH$_+$ GHRKO) + CD46.DAF + TBM.EPCR.CD47.HO1	10	Pos	225	IV infect; ARF, gentamicin tox	Unremarkable	EF 75% LV mass +36%	2C10R4
9	QKO (GTKO.B4KO.CMAH$_+$ GHRKO) + CD46.DAF + TBM.EPCR.CD47.HO1	10	Pos	19	Tachypneia/ withdrawn	Interstitial hemorrhage; edema; endothelialitis	-	AT-1501
10	QKO (GTKO.B4KO.CMAH$_+$ GHRKO) + CD46.DAF + TBM.EPCR.CD47.HO1	10	Pos	34	Tachypneia/ withdrawn	Interstitial hemorrhage; edema; endothelialitis	-	AT-1501
11	QKO (GTKO.B4KO.CMAH$_+$ GHRKO) + CD46.DAF + TBM.EPCR.CD47.HO1	10	Pos	127	Diastolic HF; vFib, arrest; mortality	Subendothelial ischemia w/ vasculopathy	EF 75% LV mass +46%	2C10R4
12	QKO (GTKO.B4KO.CMAH$_+$ GHRKO) + CD46.DAF + TBM.EPCR.CD47.HO1	10	Neg	47	Diastolic HF	Lymphocytoplasmic inflammation; chronic vasculopathy	Ventricular thickening	KPL-404

* Animals were housed in separate cages within the same animal room; each animal developed acute, progressive dyspnea, leading to respiratory insufficiency and acute mortality.

UMB Orthotopic XenoHeart with XVIVO Heart Preservation System
2020 – 2021

- Orthotopic XenoHeart transplantation with consistent technical success (N=12 transplants)
- GE pigs (Revivicor, Blacksburg, VA, USA) with 7-10 gene edits
- Standard Mohiuddin Immunosuppression regimen
- Introduction of extracorporeal non-ischemic continuous perfusion (*Steen; **Langin)
  - XVIVO Heart Preservation System (XVIVO Perfusion, Gothenburg, Sweden)
- No PCXD was observed
- Extended survival up to 264 days among 12 sequential orthotopic transplants
  - 6 of 12 >90 days
  - 3 of 12 >180 days
- 8 of 12 xenohearts transplanted with 10 GE (product pig)
  - One 10 GE >180 days

* Steen S et al. 2016. Scand. Cardiovasc. J. 50:193

** Längin M, et al. 2021. Xenotransplantation. *28:e12636*

UMB Orthotopic XenoHeart with XVIVO Heart Preservation System 2020 – 2021

Txp Date	Porcine Donor Genotype	GE (n)	pCMV	Survival (days p tx)	Clinical Course	Xenoheart Histology	Echo	CD40 Pathway Blockade
6/16/20	GTKO.B4KO.GHRKO.hTBM.hEPCR.hCD46.hCD47	7	Neg	264*	Resp. distress; mortality, infection	Chronic vasculopathy	EF 55%; LV mass +33%	2C10R4
7/8/20	GTKO.B4KO.GHRKO.hTBM.hEPCR.hCD46.hCD47	7	Neg	182	Euthanasia; severe gingivitis/ wt loss	Unremarkable	EF 65% LV mass +33%	2C10R4
8/24/20	TKO (GTKO.B4KO.CMAH +/- no GHRKO) + CD46.DAF + TBM. EPCR.CD47.HO1	8	Neg	84	Diastolic heart failure	Antibody mediated rejection	EF 60% LV mass +181%	2C10R4
9/21/20	TKO (GTKO.B4KO.CMAH +/- no GHRKO) + CD46.DAF + TBM.EPCR.CD47.HO1	8	Neg	95	Diastolic heart failure	Inflammatory myocarditis	EF 65% LV mass +99.6%	2C10R4
12/7/20	QKO (GTKO.B4KO.CMAH$_+$ GHRKO) + CD46.DAF + TBM.EPCR.CD47.HO1	10	Neg	105*	Resp. distress; mortality, infection	Chronic vasculopathy; no rejection	EF 75% LV mass +53.5%	2C10R4
1/11/21	QKO (GTKO.B4KO.CMAH$_+$ GHRKO) + CD46.DAF + TBM.EPCR.CD47.HO1	10	Pos	55*	Resp. distress, mortality, infection	Acute cellular rejection	EF 50% LV mass +101%	2C10R4
2/23/21	QKO (GTKO.B4KO.CMAH$_+$ GHRKO) + CD46.DAF + TBM.EPCR.CD47.HO1	10	Pos	14*	Resp. distress; mortality, infection	No acute rejection; myodegeneration w/ fibrosis	-	2C10R4
3/8/21	QKO (GTKO.B4KO.CMAH$_+$ GHRKO) + CD46.DAF + TBM.EPCR.CD47.HO1	10	Pos	225	IV infect; ARF, gentamicin tox	Unremarkable	EF 75% LV mass +36%	2C10R4
3/31/21	QKO (GTKO.B4KO.CMAH$_+$ GHRKO) + CD46.DAF + TBM.EPCR.CD47.HO1	10	Pos	19	Tachypneia/ withdrawn	Interstitial hemorrhage; edema; endothelialitis	-	AT-1501
4/14/21	QKO (GTKO.B4KO.CMAH$_+$ GHRKO) + CD46.DAF + TBM.EPCR.CD47.HO1	10	Pos	34	Tachypneia/ withdrawn	Interstitial hemorrhage; edema; endothelialitis	-	AT-1501
8/4/21	QKO (GTKO.B4KO.CMAH$_+$ GHRKO) + CD46.DAF + TBM.EPCR.CD47.HO1	10	Pos	127	Diastolic HF; vFib, arrest; mortality	Subendothelial ischemia w/ vasculopathy	EF 75% LV mass +46%	2C10R4
12/8/21	QKO (GTKO.B4KO.CMAH$_+$ GHRKO) + CD46.DAF + TBM.EPCR.CD47.HO1	10	Neg	47	Diastolic HF	Lymphocytoplasmic inflammation; chronic vasculopathy	Ventricular thickening	KPL-404

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