USZ Universitäts Spital Zürich

Xenotransplantation

Kidney and heart from GalSafe pigs to humans: Will this finally solve the problem of organ shortage?

Technical Journal Club, 29.03.2022, USZ, Dr. med. Eva Breuer

The Guardian

Surgeons successfully test pig kidney transplant in human patient

Researchers in US say trial on dead person is a 'significant step' toward animal-to-human organ transplants

A surgical team at the hospital in New York examines a pig kidney attached to the body of a deceased recipient for any signs of rejection. Photograph: Joe Carrotta/AP

Surgeons have attached a pig's kidney to a human and watched it begin to work, a small step in the decades-long quest to one day use animal organs for life-saving transplants.

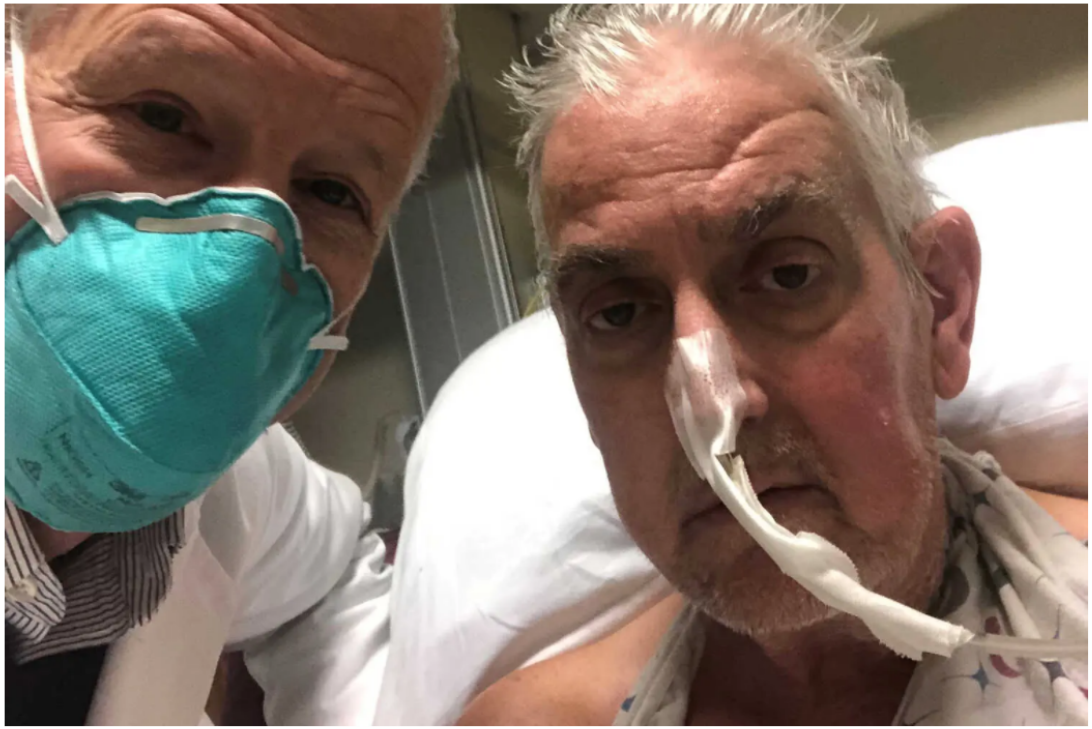
Pigs have been the most recent research focus to address the organ shortage, but a sugar in their cells, which is foreign to the human body, causes immediate organ rejection. The kidney for this experiment came from a gene-edited animal, engineered to eliminate that sugar and avoid an immune system attack.

Monday, January 10, 2022
Today's Paper

The New York Times

In a First, a Man Receives a Heart From a Genetically Altered Pig

- Surgeons at the University of Maryland School of Medicine performed the eight-hour procedure on a 57-year-old man with a life-threatening heart disease.
- Researchers hope the breakthrough may lead one day to new supplies of animal organs for transplant into human patients.



Dr. Bartley Griffith, left, and David Bennett, who received a genetically modified pig's heart. University of Maryland School of Medicine

the future?

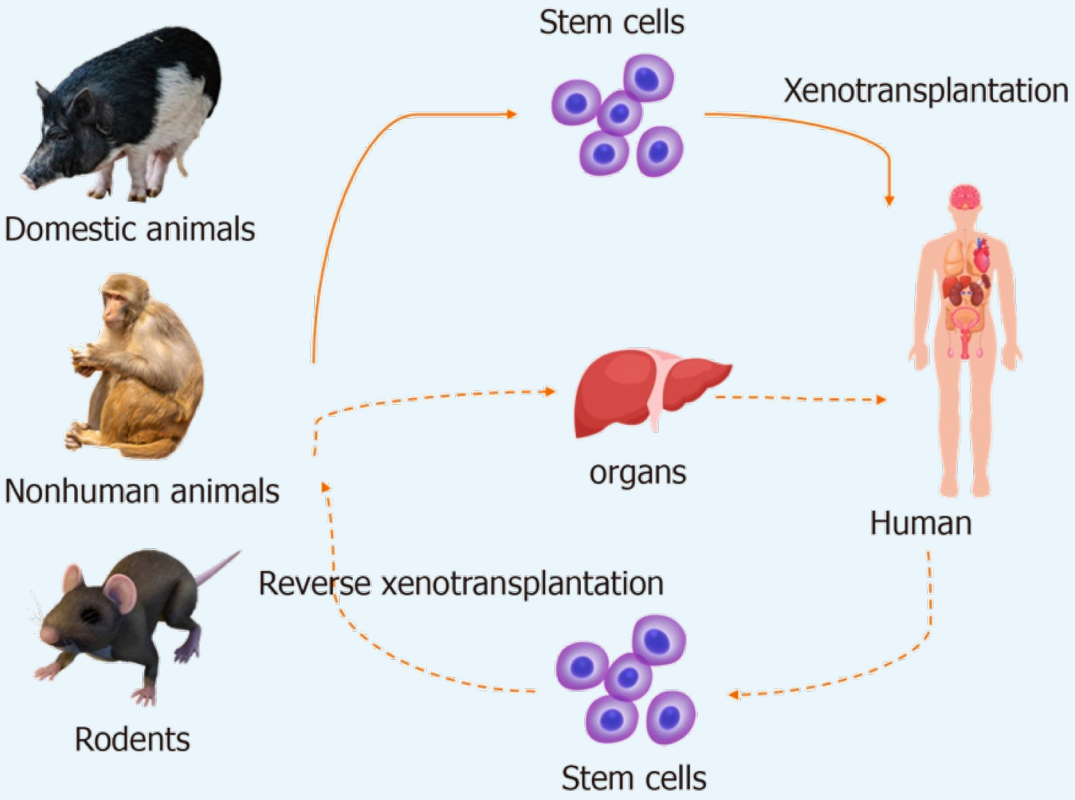
Agenda

1. Xenotransplantation: a brief history
2. Galactose-α1,3-galactose → GalSafe
3. Pig-to-primate xenografts
4. The first genetically modified pig kidney transplanted
5. The «first» heart xenotransplantation
6. Future applications?

Xenotransplantation

What is Xenotransplantation?

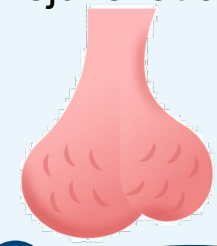
- Xenotransplantation is the transplantation of organs, tissues or cells from one species to another



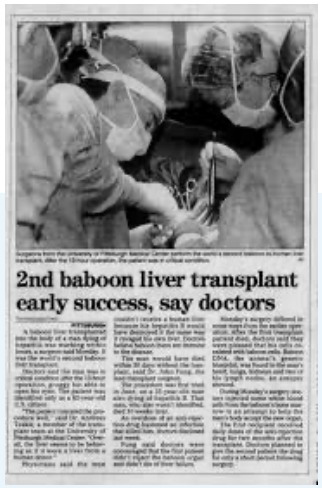
The history of Xenotransplantation

17th century
dog skull bone

1920
testicles for „sexual rejuvenation“

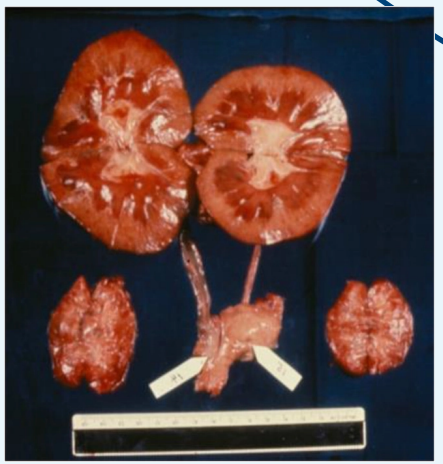


1964
chimpanzee heart
heart too small

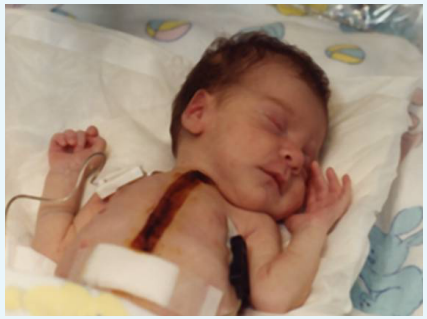


1992
Baboon livers
one graft survived 2 months

19th century
frog skin grafts

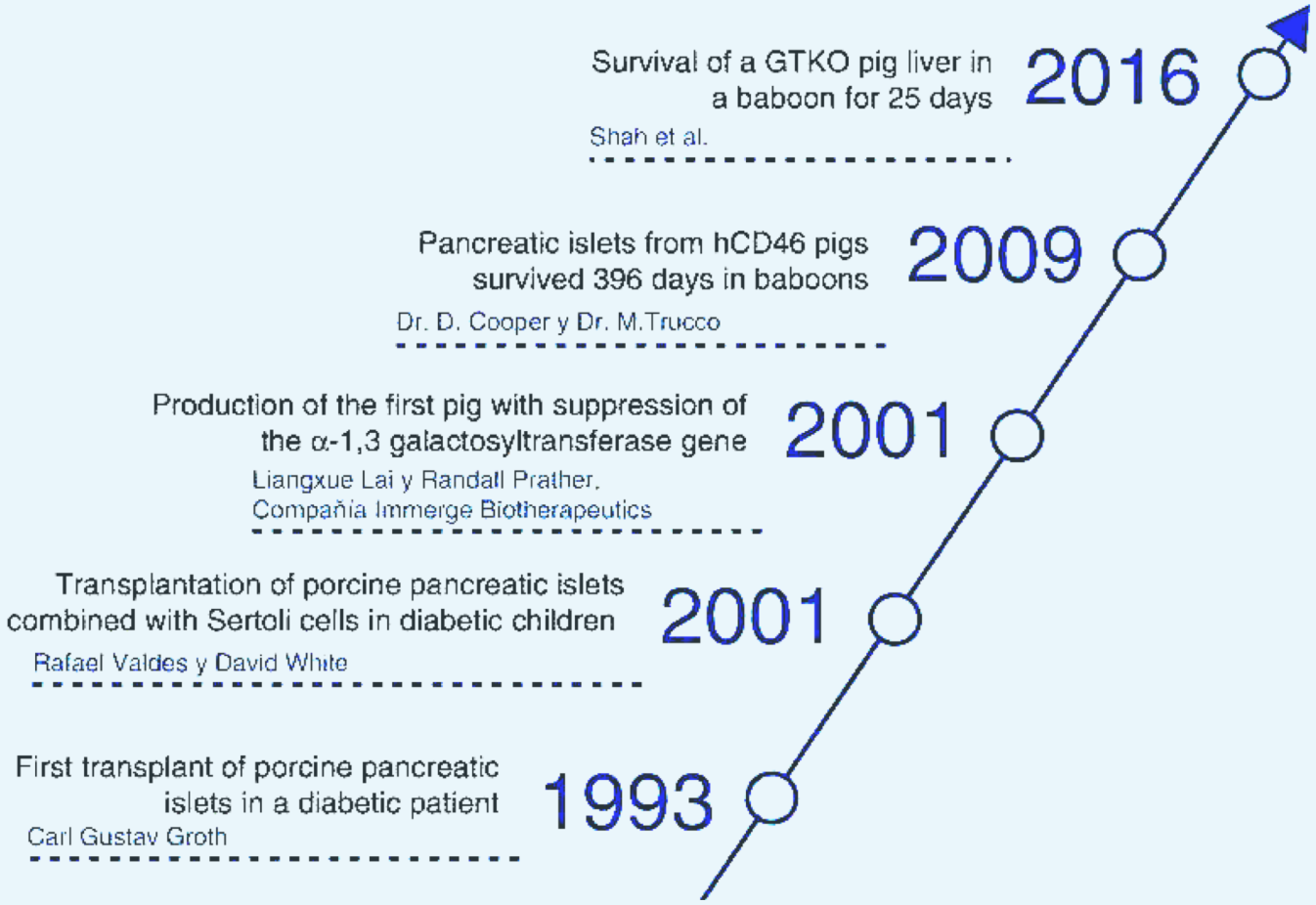


1963-65
chimpanzee kidneys
one graft survived 9 months

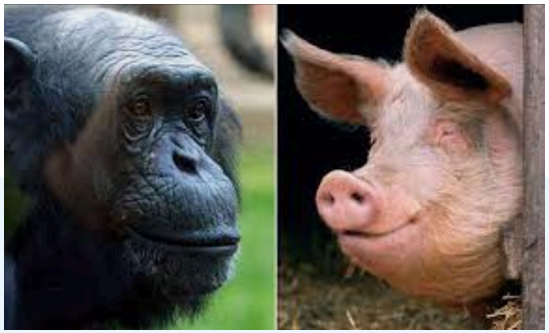


1984
„Baby Fae“ with baboon heart
died after 20 days

The history of Xenotransplantation



Nonhuman primates (NHP) vs. pigs

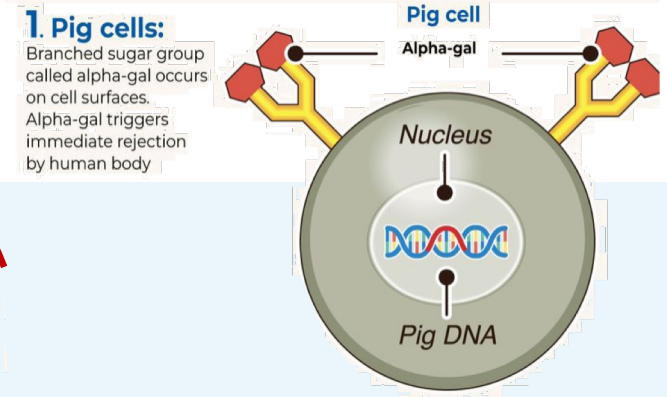


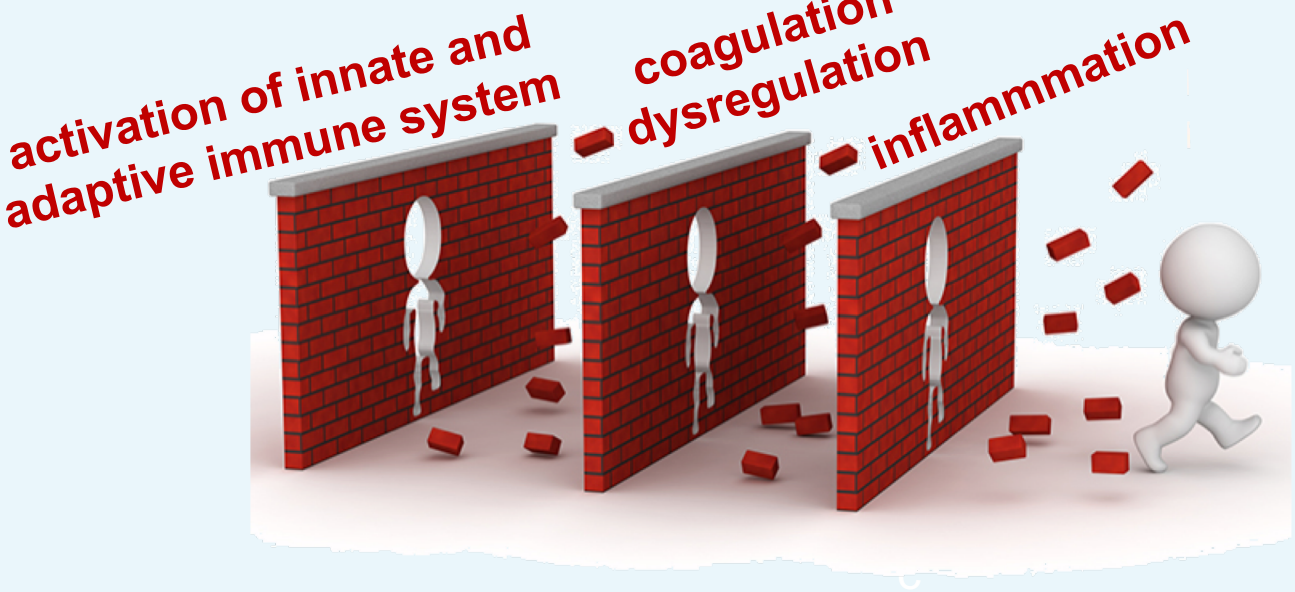
- Primates share most of our genetic make up
- Primates are not available in numbers

- Pigs have **good breeding potential**
- Adequate **size** of adult organs
- Significantly **lower cost** of maintenance
- Distant relation of the immune system to human
- Considerable knowledge of tissue typing
- **Experience with genetic engineering** in pig
- Low risk of transfer of **infection**
- Pigs are already slaughtered every day for meat

Galactose-α1,3-galactose → GalSafe®

Galactose-α1,3-galactose [Gal]





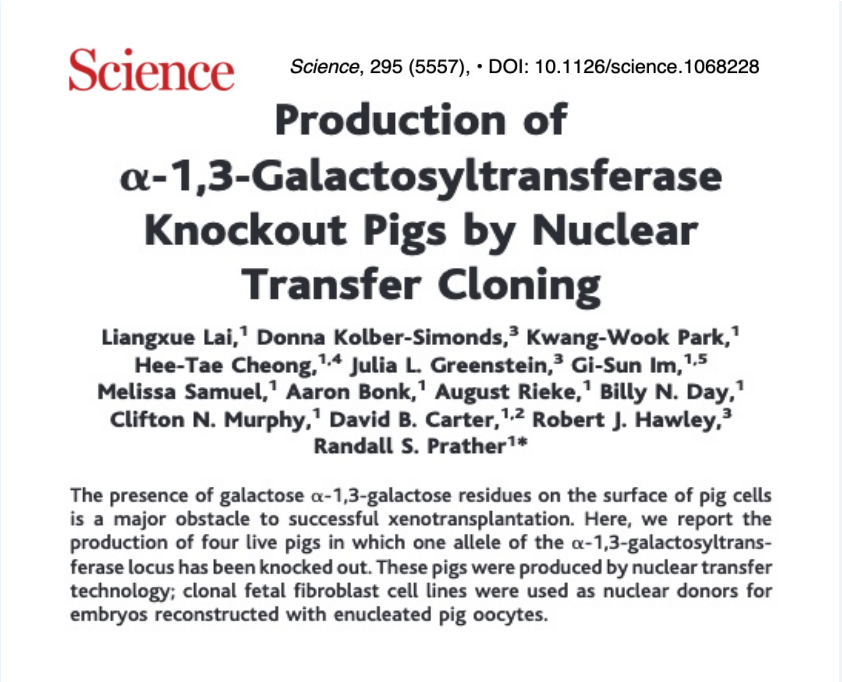
→ Most related to the presence of natural anti-pig antibodies

→ Most important: Galactose-α1,3-galactose

- Humans and Old World monkeys have lost the galactosyltransferase activity during evolution

→ Activation of the complement cascade → hyperacute rejection

Galactose-α1,3-galactose [Gal]



Science

Science, 295 (5557), • DOI: 10.1126/science.1068228

Production of α-1,3-Galactosyltransferase Knockout Pigs by Nuclear Transfer Cloning

Liangxue Lai,[1] Donna Kolber-Simonds,[3] Kwang-Wook Park,[1] Hee-Tae Cheong,[1,4] Julia L. Greenstein,[3] Gi-Sun Im,[1,5] Melissa Samuel,[1] Aaron Bonk,[1] August Rieke,[1] Billy N. Day,[1] Clifton N. Murphy,[1] David B. Carter,[1,2] Robert J. Hawley,[3] Randall S. Prather[1]*

The presence of galactose α-1,3-galactose residues on the surface of pig cells is a major obstacle to successful xenotransplantation. Here, we report the production of four live pigs in which one allele of the α-1,3-galactosyltransferase locus has been knocked out. These pigs were produced by nuclear transfer technology; clonal fetal fibroblast cell lines were used as nuclear donors for embryos reconstructed with enucleated pig oocytes.

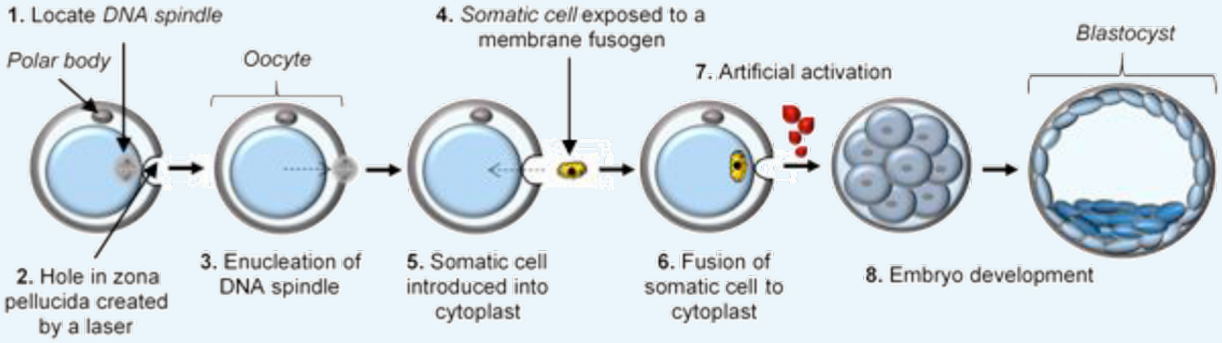
- Genetic knockout of the **α-1,3-galactosyltransferase (GGTA1)** locus
- Nuclear transfer technology
- Four live pigs with **one allele of GGTA1** knocked out

Galactose-α1,3-galactose [Gal]

Nuclear transfer technology

Nuclear donor
Clonal fetal fibroblast cell line

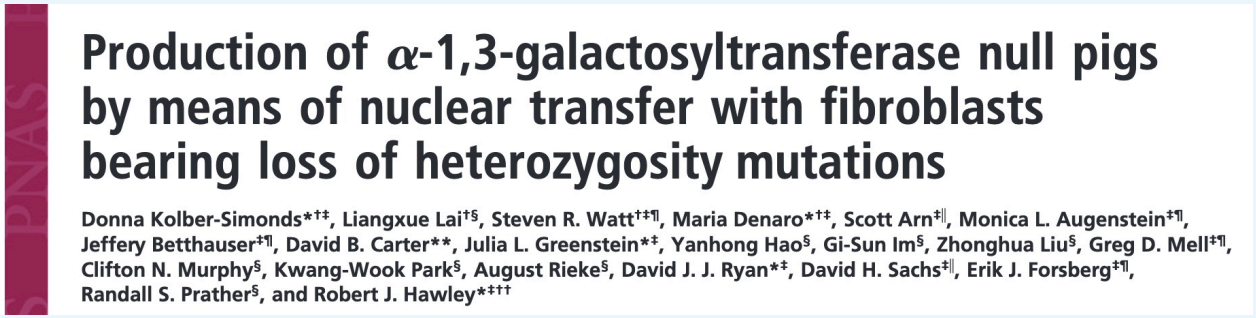
Homologous replacement of an endogenous GGTA1 allele by gene trap targeting vector



Enucleated pig oocyte

Galactose-α1,3-galactose [Gal]

- Heterozygous **GGTA1** knockout pigs
- Several congenital abnormalities detected….



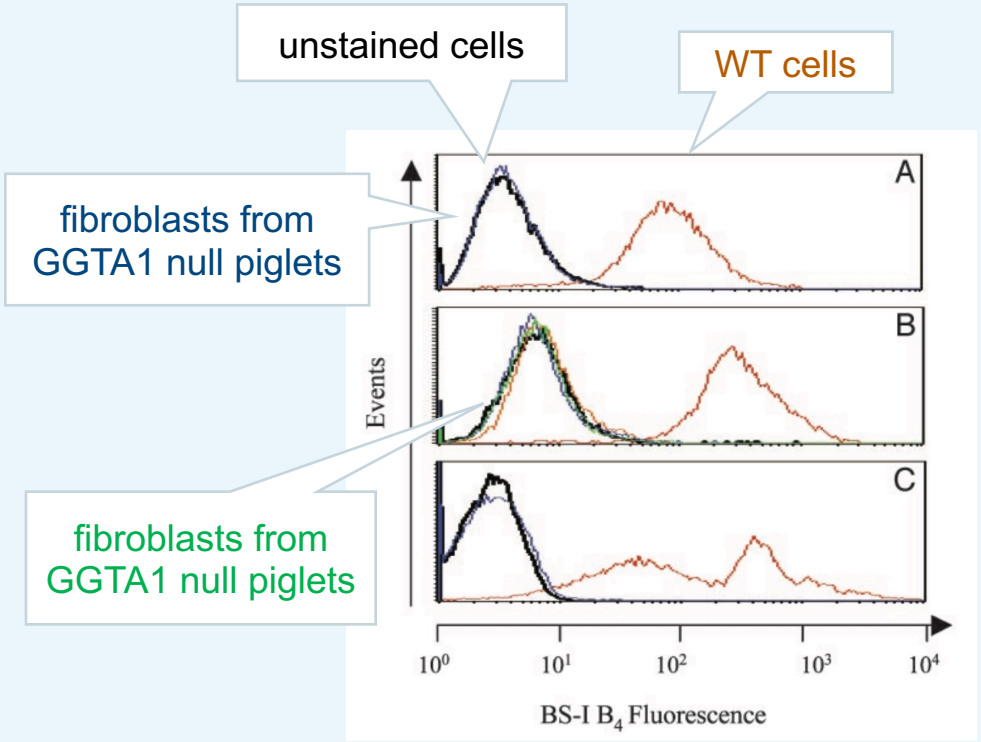
Production of α-1,3-galactosyltransferase null pigs by means of nuclear transfer with fibroblasts bearing loss of heterozygosity mutations

Donna Kolber-Simonds*†‡, Liangxue Lai†§, Steven R. Watt†‡¶, Maria Denaro*†‡, Scott Arn‡‖, Monica L. Augenstein‡¶, Jeffery Betthauser‡¶, David B. Carter**, Julia L. Greenstein*‡, Yanhong Hao§, Gi-Sun Im§, Zhonghua Liu§, Greg D. Mell‡¶, Clifton N. Murphy§, Kwang-Wook Park§, August Rieke§, David J. J. Ryan*‡, David H. Sachs‡‖, Erik J. Forsberg‡¶, Randall S. Prather§, and Robert J. Hawley*‡††

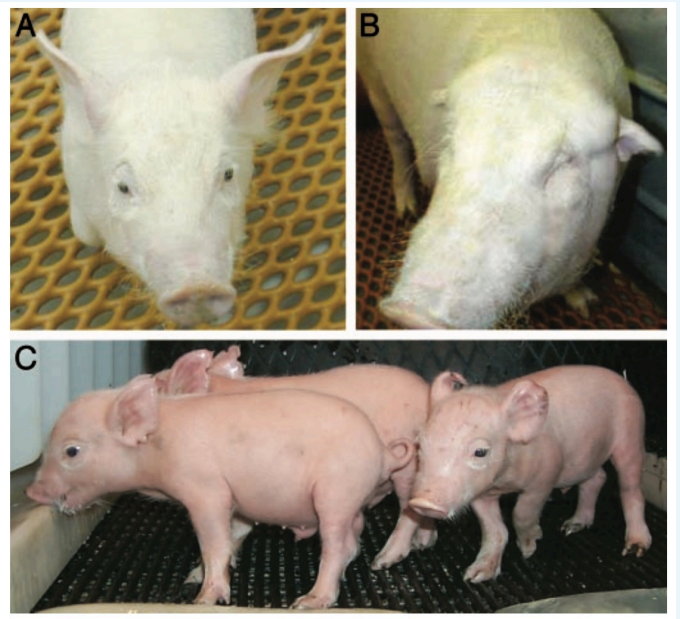
- Selection of spontaneous null mutant cells from fibroblast cultures from heterozygous animals → serial rounds of nuclear transfer
- Unexpectedly high rate of loss of the WT allele
- **No defects**, no phenotypic differences to WT piglets

→ Healthy homozygous **GGTA1** knockout pigs

Galactose-α1,3-galactose [Gal]



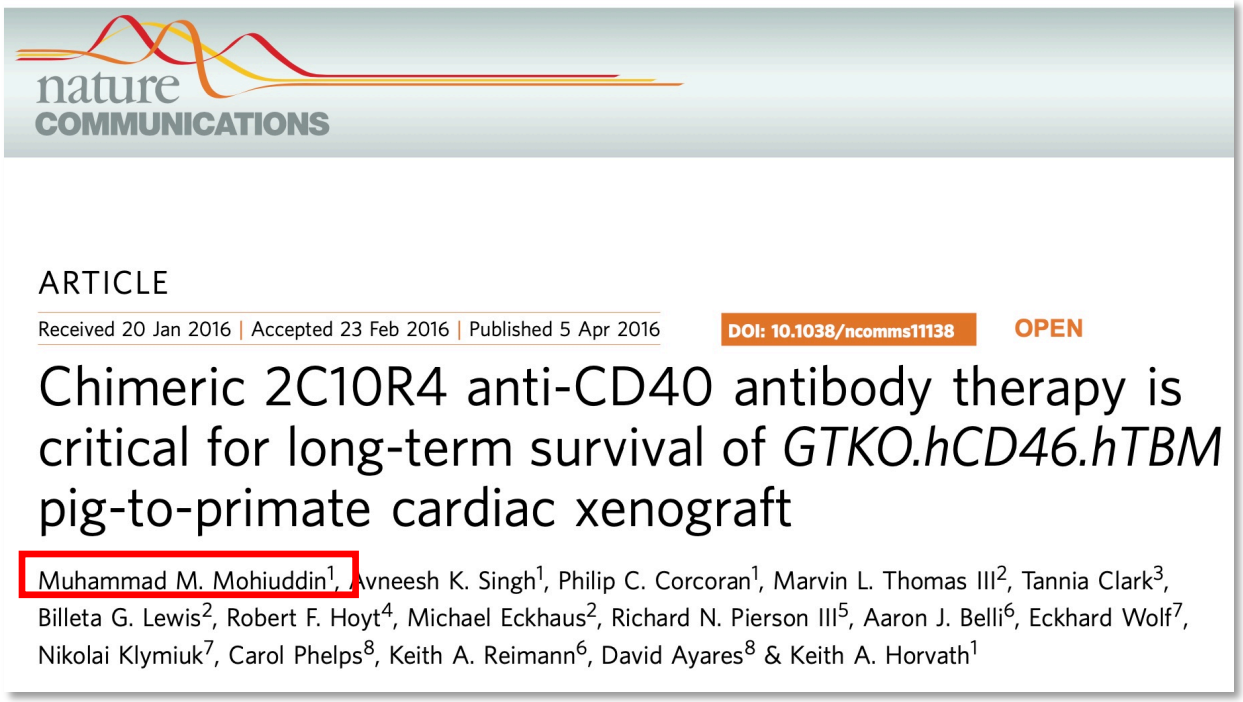
Flow cytometry analysis of Gal-α1,3-Gal epitopes on GGTA1 null piglets using BS-I-B4 lectin



B: first round NT pig with ear and eyes defects
C: offsprings of B

Pig-to-primate Xenografts

Pig-to-primate Xenografts



nature COMMUNICATIONS

ARTICLE

Received 20 Jan 2016 | Accepted 23 Feb 2016 | Published 5 Apr 2016

DOI: 10.1038/ncomms11138 OPEN

Chimeric 2C10R4 anti-CD40 antibody therapy is critical for long-term survival of *GTKO.hCD46.hTBM* pig-to-primate cardiac xenograft

Muhammad M. Mohiuddin[1], Avneesh K. Singh[1], Philip C. Corcoran[1], Marvin L. Thomas III[2], Tannia Clark[3], Billeta G. Lewis[2], Robert F. Hoyt[4], Michael Eckhaus[2], Richard N. Pierson III[5], Aaron J. Belli[6], Eckhard Wolf[7], Nikolai Klymiuk[7], Carol Phelps[8], Keith A. Reimann[6], David Ayares[8] & Keith A. Horvath[1]

Pig-to-primate Xenografts

- GTKO pigs (α-1,3-galactosyltransferase knockouts) have been tested
- Combination with other gene knockouts and modifications
- Combination with intensive immunotherapy

Delayed xenograft rejection

Thrombotic microangiopathy and consumptive coagulopathy

α-Gal knockout

GTKO.hCD46.hTBM

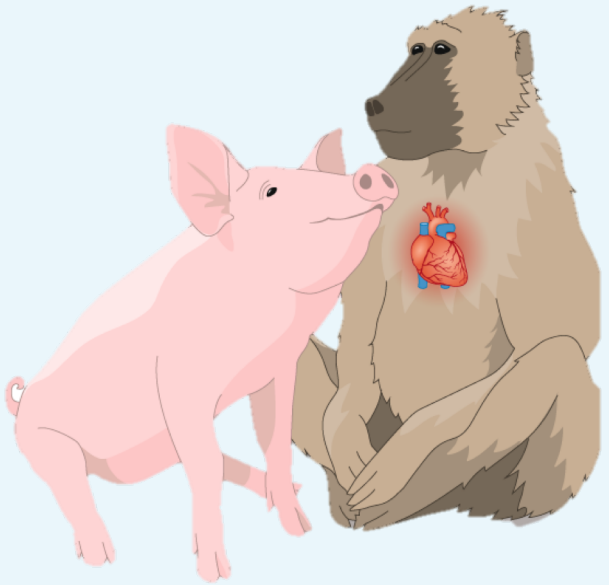
Additional human complement regulatory protein (hCD46) expression
→ suppression of complement activation

Additional expression of human thromboregulatory protein (hTBM)
→ reduction of thrombo-dysregulation

Mohiuddin et al.: Nat Comm, 2016.

Pig-to-primate Xenografts

Five baboons received GTKO.hCD46.hTBM pig hearts



Mohiuddin et al.: Nat Comm, 2016.

Immunosuppressive regimen

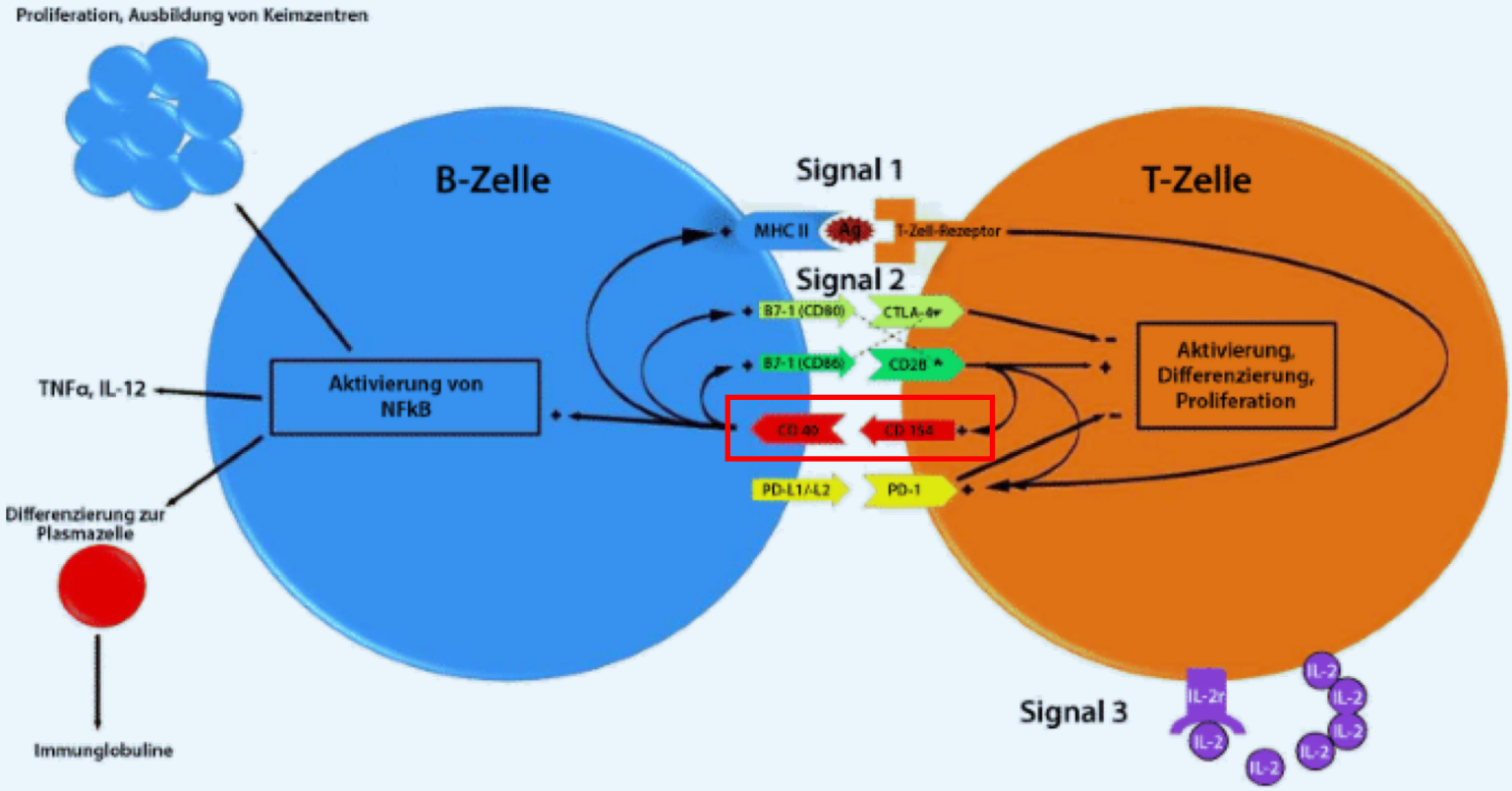
Table 1 | Immunosuppressive regimen.

Agent	Dose	Timing	Route	Pre-treatment	Purpose
Induction					
Anti-CD20	19 mg kg^{-1}	Days −7, 0, 7 and 14	i.v. infusion	Solu-Medrol, Benadryl, H2 blocker	To deplete B cells
ATG	5 mg kg^{-1}	Days −2, and −1	i.v. infusion	Solu-Medrol, Benadryl, H2 blocker	To reduce number of T cells
Anti-CD40 (clone 2C10R4)	50 mg kg^{-1} for 100 days–1 year, then slowly tapered off		Slow i.v. infusion	None	Co-stimulation blockade. Suppression of both B- and T-cell response
CVF	50–100 U kg^{-1}	Days −1, 0 and 1	i.v.	None	To inhibit complement activity
Maintenance					
Anti-CD40 (clone 2C10R4)	10–50 mg kg^{-1}*	Weekly	Slow i.v. infusion	None	Co-stimulation blockade. Suppression of both B- and T-cell response
MMF	20 mg kg per 2 h	BID, daily	i.v. infusion	None	BID daily
Solu-Medrol	2 mg kg^{-1}	BID tapered off in 7 weeks	i.v.	None	Suppress inflammation
Aspirin	81 mg		Oral	None	Prevent platelet aggregation
Heparin	50–400 U h^{-1}	Continuous	i.v. infusion	None	Maintain ACT 2 × normal and prevent inflammation
Supportive					
Ganciclovir	5 mg kg^{-1} per day	Daily	i.v. infusion		For CMV prophylaxis
Cefazolin	250 mg	Daily for 7 days and whenever needed	i.v.	None	Antibiotic cover
Epogen	200 U kg^{-1}	Day −7 to 7 then weekly	i.m. or i.v.	None	To increase haematocrit

BID, twice daily; CMV, Cytomegalovirus; CVF, cobra venom factor; i.m., intramuscular; i.v., intravenous.
*Anti-CD40 antibody dose was reduced either from 50 to 25 mg kg^{-1} on day 100 ($n=2$) or completely tapered off starting from day 365 ($n=2$).

Mohiuddin et al.: Nat Comm, 2016.

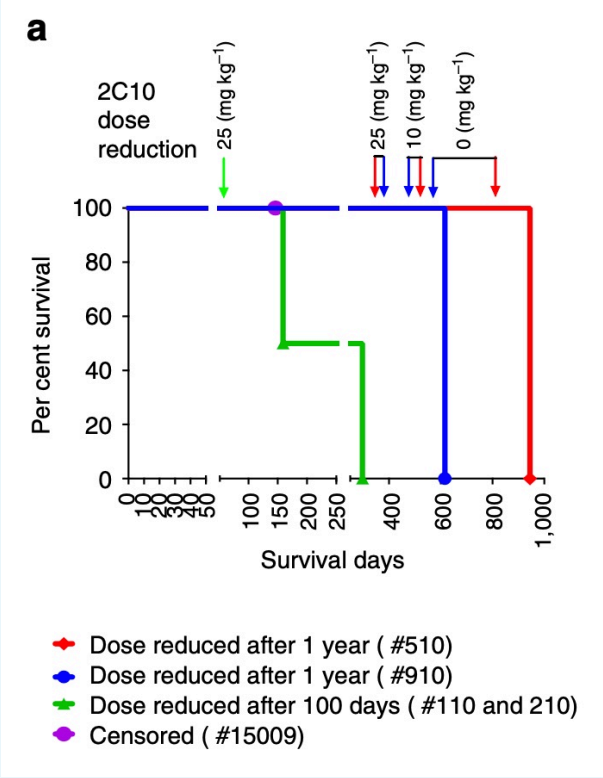
Immunosuppressive regimen

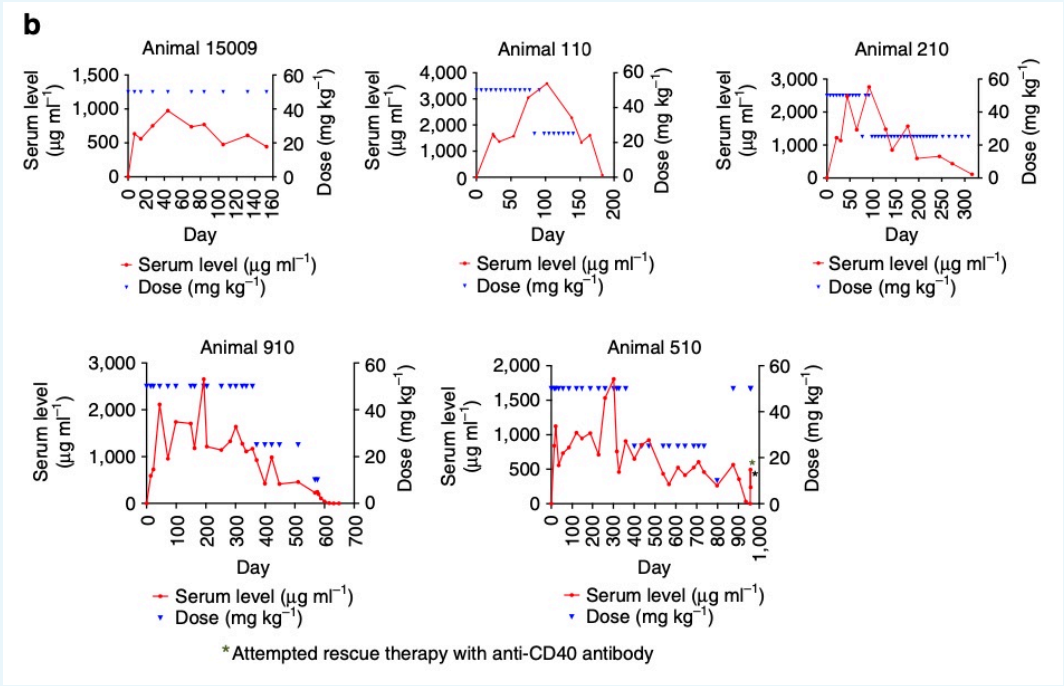


Induction and maintenance:
anti-CD154-CD40 co-stimulation blockade antibody

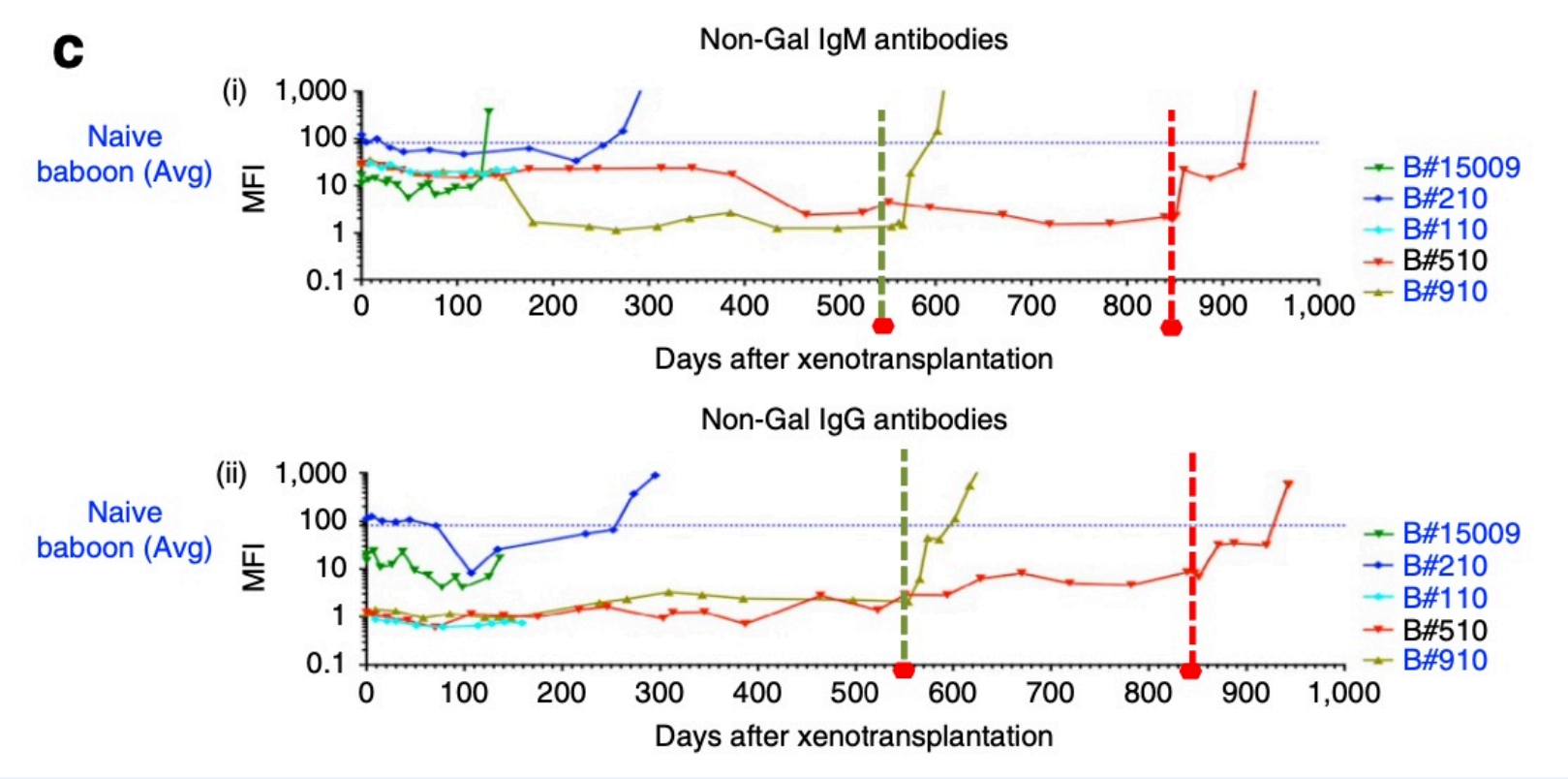
Mohiuddin et al.: Nat Comm, 2016.

Pig-to-primate Xenografts: Results

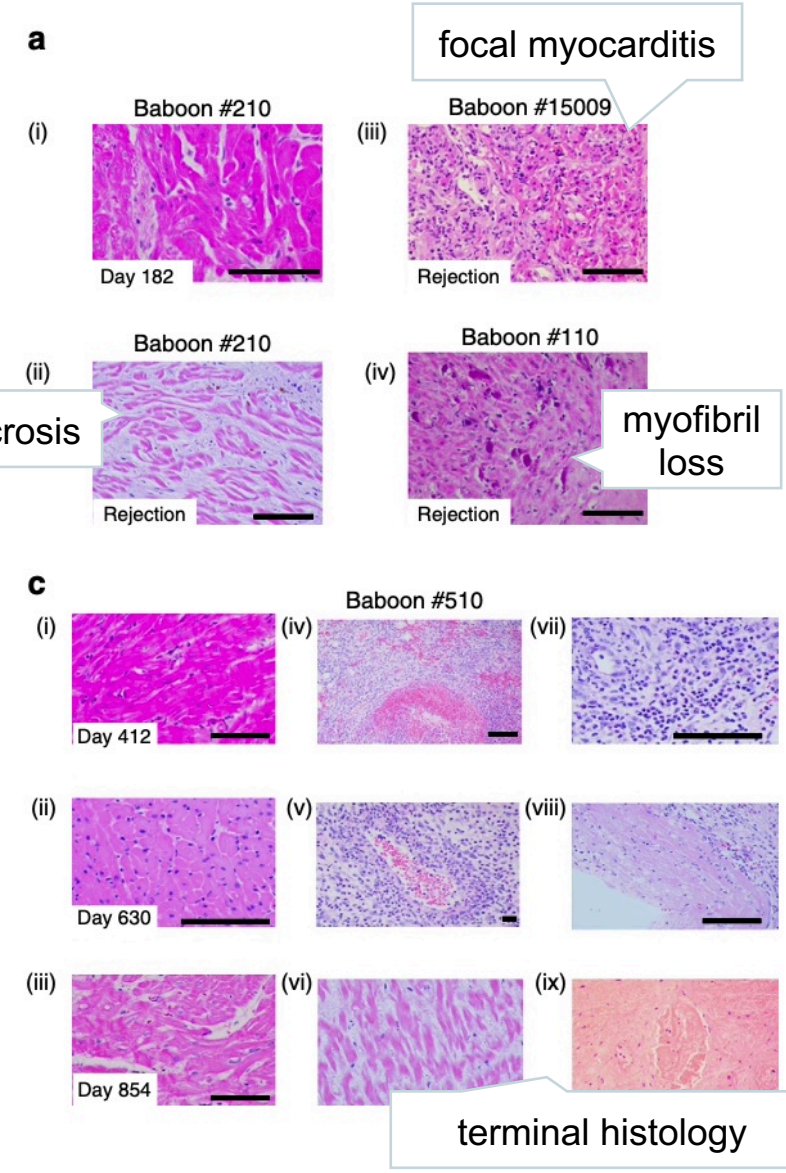




Pig-to-primate Xenografts: Results

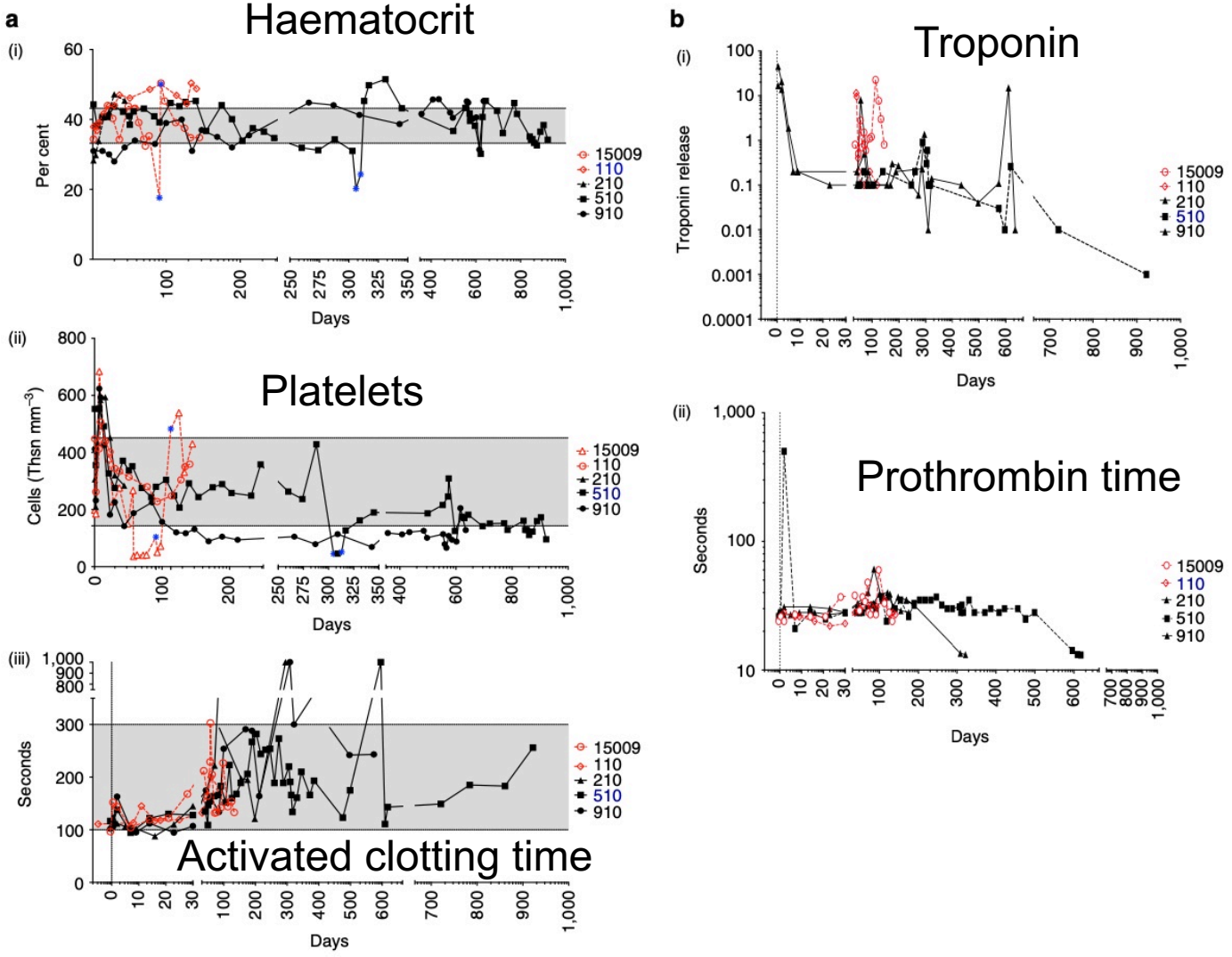


Pig-to-primate Xenografts: Results



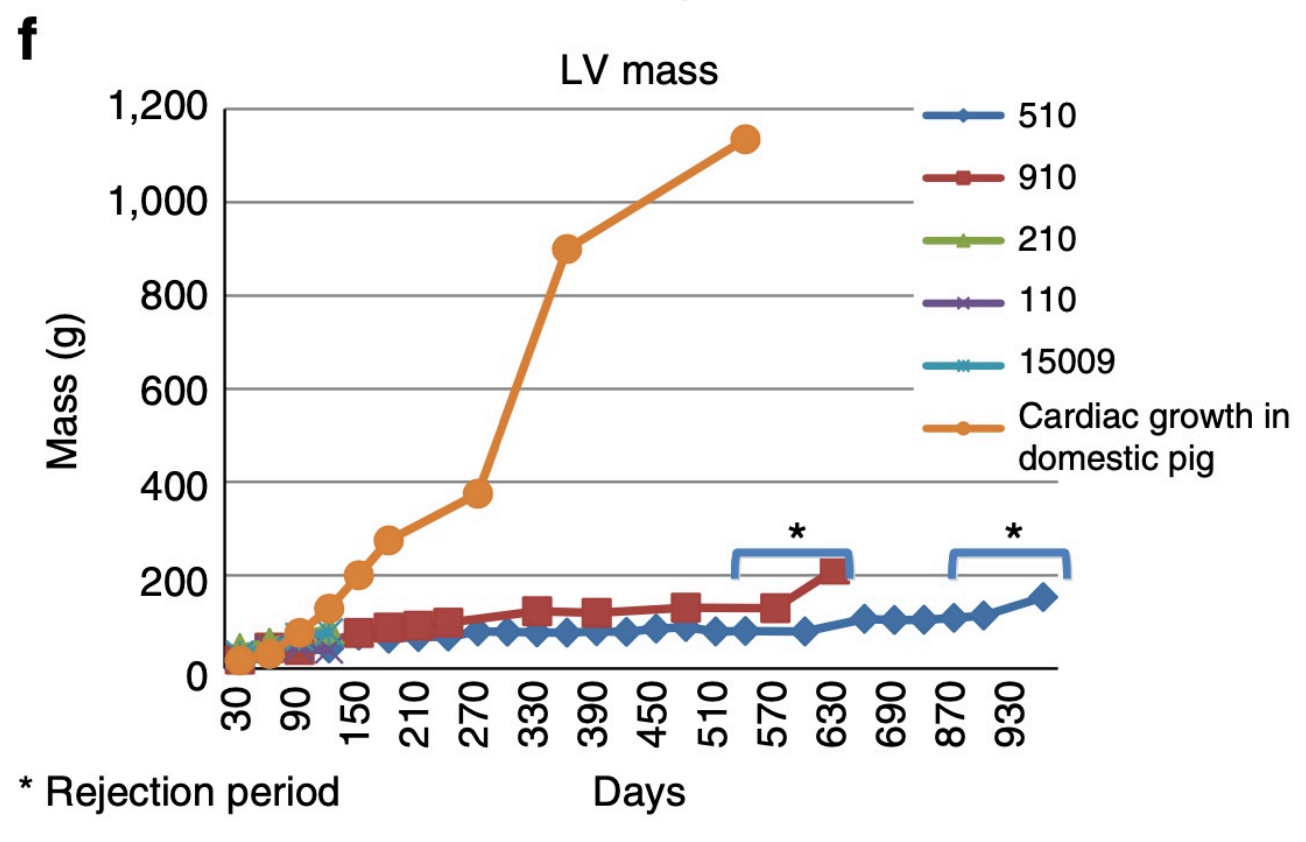
Mohiuddin et al.: Nat Comm, 2016.

Pig-to-primate Xenografts: Results



Mohiuddin et al.: Nat Comm, 2016.

Pig-to-primate Xenografts: Results



Mohiuddin et al.: Nat Comm, 2016.

Pig-to-primate xenografts: Conclusion

- Genetic modifications (GTKO.hCD46.hTBM)
- Combination with an immunmodulatory drug regimen including **anti-thymocyte globulin** and **anti-CD20 antibody**, followed by maintenance with **mycophenolate mofetil** and intensively dosed **anti-CD40 antibody**
- consistent prevention of humoral rejection and systemic coagulation pathway dysregulation

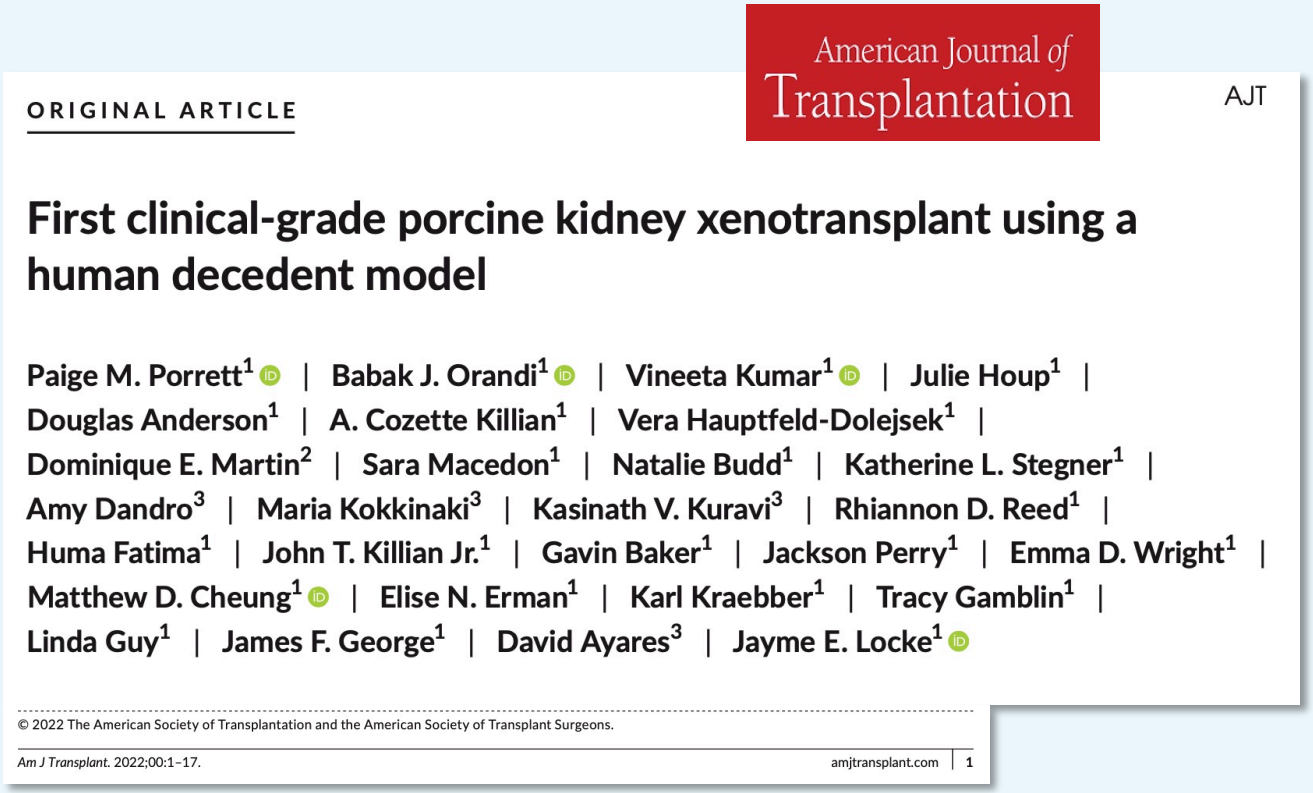
→ long-term cardiac xenograft survival beyond 900 days

- The reduction of CD40 antibody dose resulted in recrudescence of anti-pig antibody and graft-failure

Mohiuddin et al.: Nat Comm, 2016.

The first genetically modified pig kidney transplanted

The first genetically modified pig kidney transplanted



American Journal of Transplantation

AJT

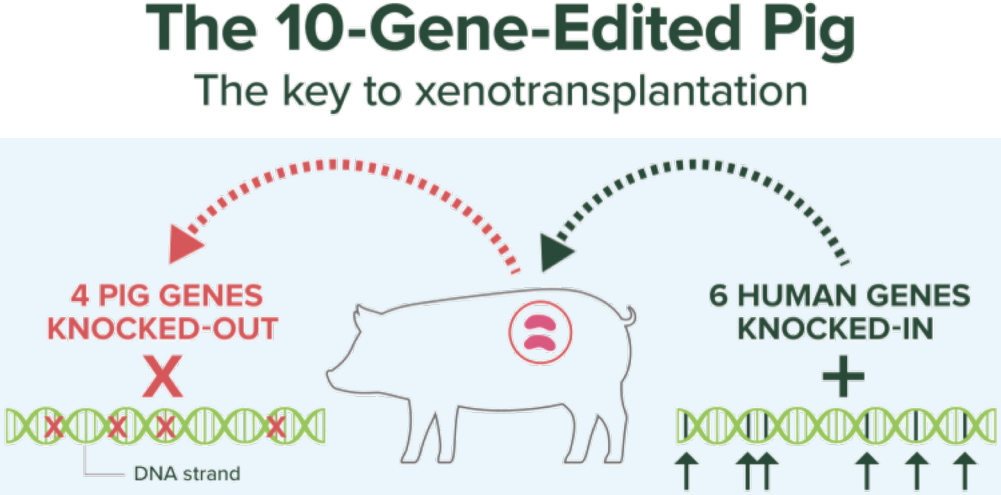
ORIGINAL ARTICLE

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

Paige M. Porrett[1] | Babak J. Orandi[1] | Vineeta Kumar[1] | Julie Houp[1] | Douglas Anderson[1] | A. Cozette Killian[1] | Vera Hauptfeld-Dolejsek[1] | Dominique E. Martin[2] | Sara Macedon[1] | Natalie Budd[1] | Katherine L. Stegner[1] | Amy Dandro[3] | Maria Kokkinaki[3] | Kasinath V. Kuravi[3] | Rhiannon D. Reed[1] | Huma Fatima[1] | John T. Killian Jr.[1] | Gavin Baker[1] | Jackson Perry[1] | Emma D. Wright[1] | Matthew D. Cheung[1] | Elise N. Erman[1] | Karl Kraebber[1] | Tracy Gamblin[1] | Linda Guy[1] | James F. George[1] | David Ayares[3] | Jayme E. Locke[1]

- Preparation for a clinical trial of xenotransplantation
- *In vivo* pre-clinical human model to **test safety and feasibility**

The 10GE pig



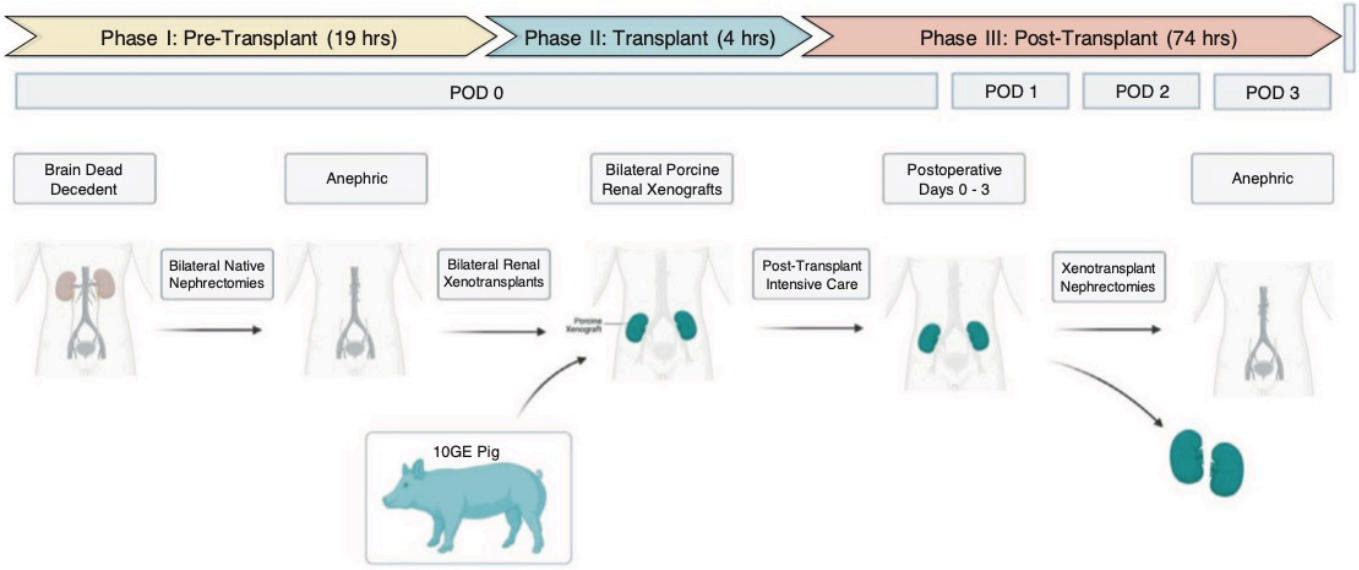
Deletion of

- 3 branched carbohydrates
  (including **α-Gal**)
- A **growth hormone receptor** gene

Knoched-in from human

- 2 human **complement inhibitor** genes (hDAF, hCD46)
- 2 human **anticoagulant** genes (hTBM, hEPCR)
- 2 human **immunomodulatory** genes (hCD47, hHO1)

Study timeline and event summary



- Family authorization
- Prospective crossmatch
- Xenograft procurement
- Pre-transplant xenograft histology
- Removal of bilateral decedent native kidneys

- Induction immunosuppression
- Transplantation of right and left kidney xenografts
- Visual assessment for hyperacute rejection
- Visual assessment of xenograft perfusion
- Post-implantation xenograft biopsi

- Induction and maintenance immunosuppression
- Assessment of xenograft fu
- Visual inspec
- HLA
- ssessment for transmission of porcine endogenous retroviruses
- Serial xenograft biopsies
- Bilateral xenograft nephrectomies

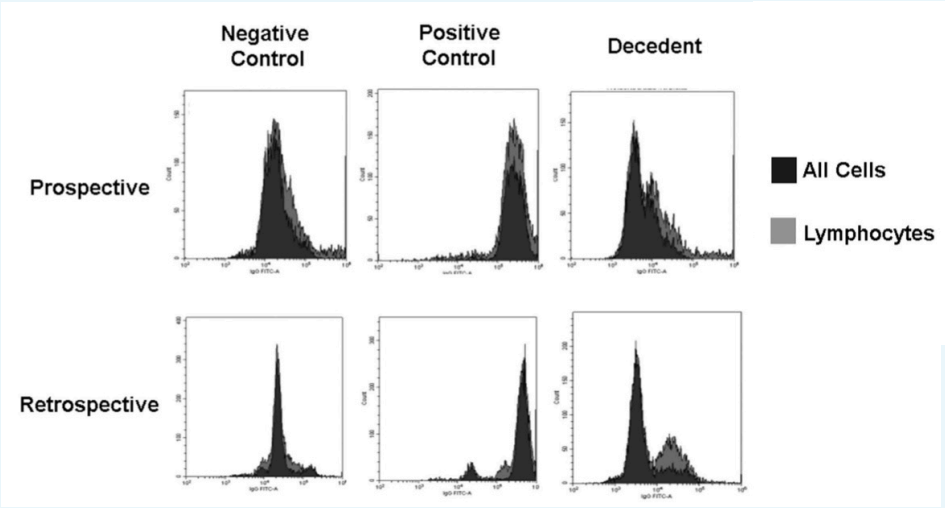
Primary endpoint: kidney function for 3 days

Pig-to-human specific flow crossmatch

- Determine tissue compatibility prior transplantation
- Pre-transplant crossmatch requiremed for human transplantation
- Flow cytometry: **anti-FITC secondary antibody (goat) → to detect antibodies in the serum that were bound to porcine lymphocytes**

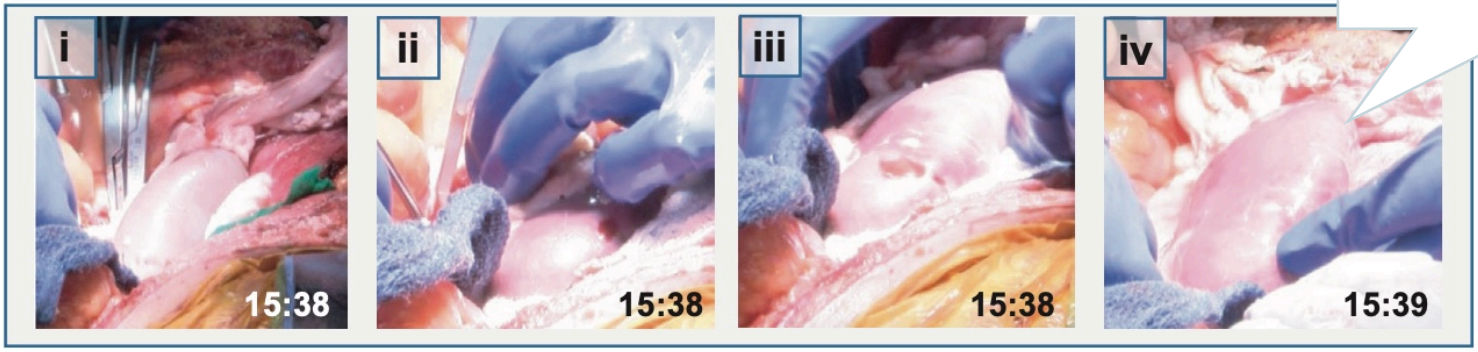
Frozen PBMCs:

Freshly isolated PBMCs:

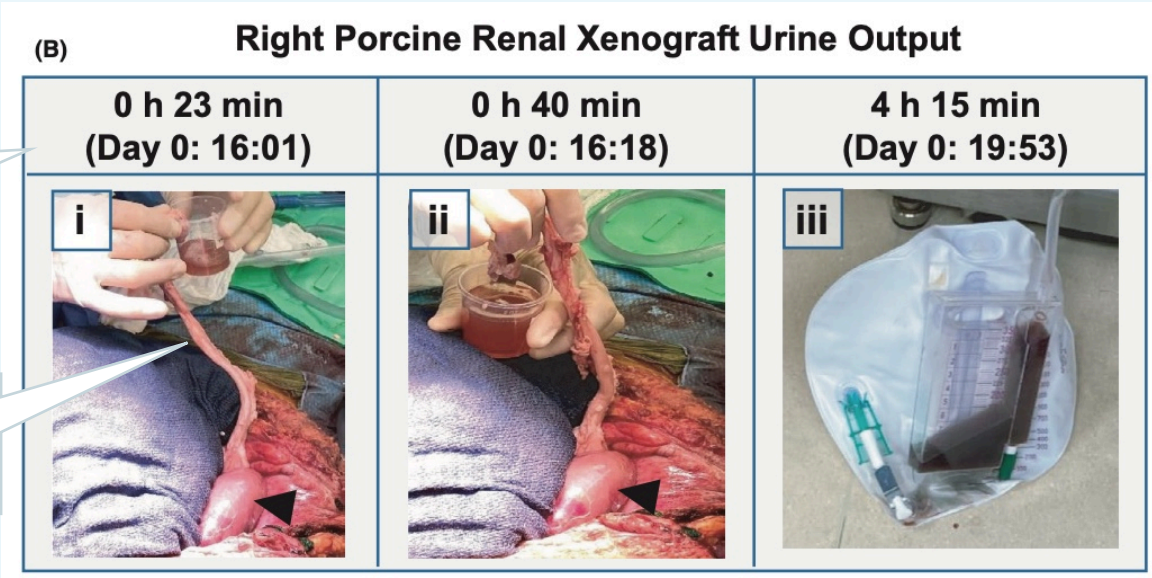


Donor pig and recipient human characterics

intraoperatively viable kidneys bilateral



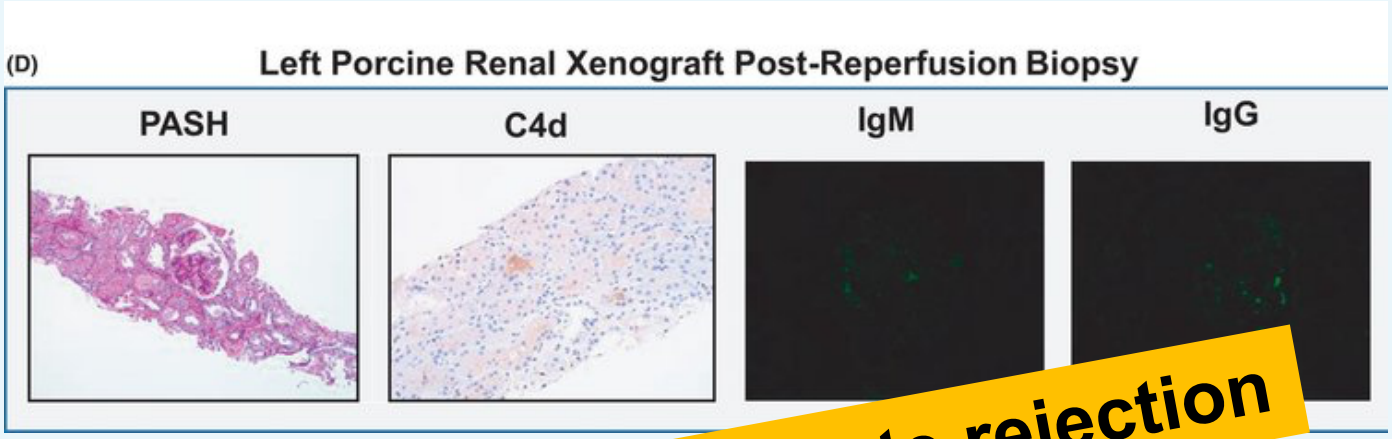
Sequential urine output after reperfusion



right ureter before anastomosis to the decedent bladder

No hyperacute rejection in both kidneys during 60min observation time

Post-implantation xenograft biopsies

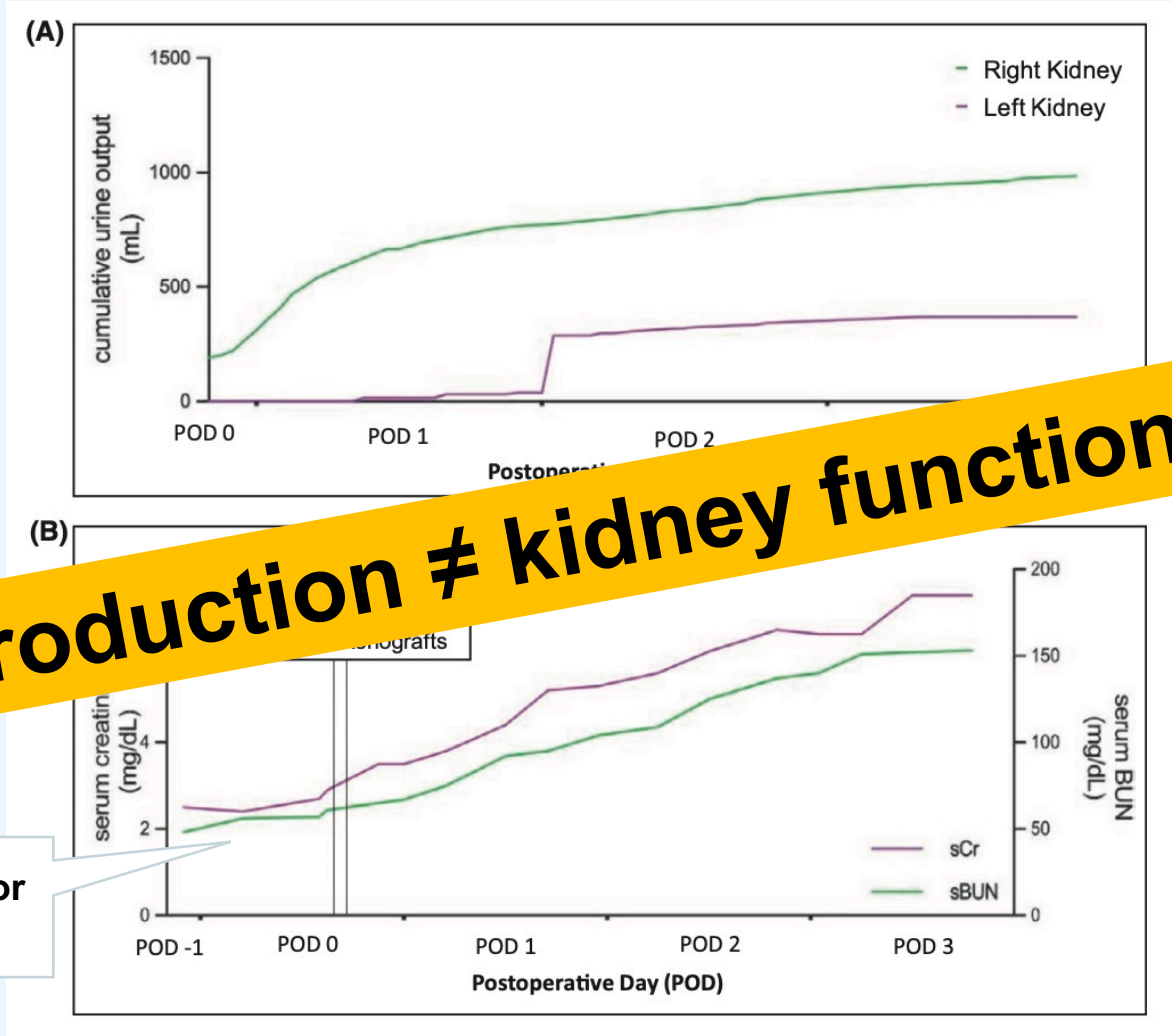


No evidence for acute rejection

Immunosuppression

• **Anti-Thymocyte Globulin**	IgG against human T-lymphocytes, prevent rejection by removing/modulating human T-cells
• **Rituximab**	anti-CD20 antibody, B-cell depletion
• **Tacrolimus** • **Mycophenolat mofetil** • **Methylprednisolone**	Immunosuppressan

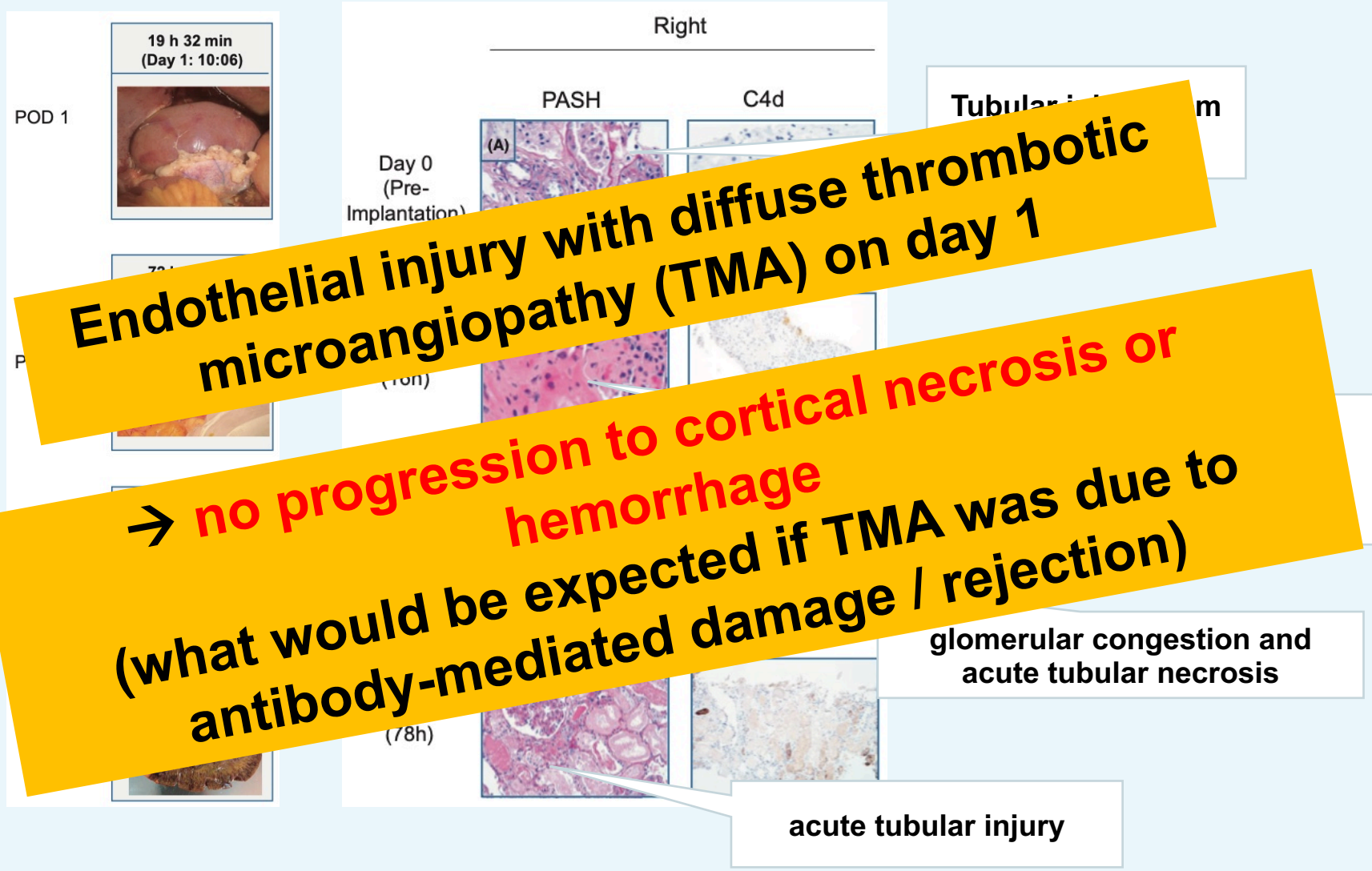
Renal xenotransplant function in the human



urine production ≠ kidney function

Functional kidney prior transplantation

Serial histologic examination of the kidneys



Endothelial injury with diffuse thrombotic microangiopathy (TMA) on day 1

→ no progression to cortical necrosis or hemorrhage

(what would be expected if TMA was due to antibody-mediated damage / rejection)

Serial histologic examination of the kidneys

Right xenograft

Left xenograft

negative for IgM, IgG, C4d, C1q and C3

TMA not mediated by complement or antibody

unknown mechanism…?

Donor pig and recipient human characterics

TABLE 2 Decedent demographics and pertinent history

Characteristic	Decedent
Sex	Male
Race	White
Age	57 years
BMI	35.2 kg/m^2
Cause of death	Head trauma
Mechanism of injury	Blunt trauma
Past medical history	Hypertension, Hyperlipidemia
Past surgical history	Trauma exploratory laparotomy
Blood type	AB+
Calculated panel reactive antibody (cPRA)	0%

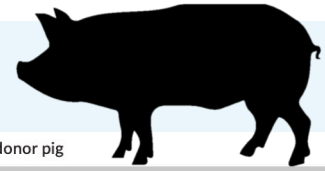
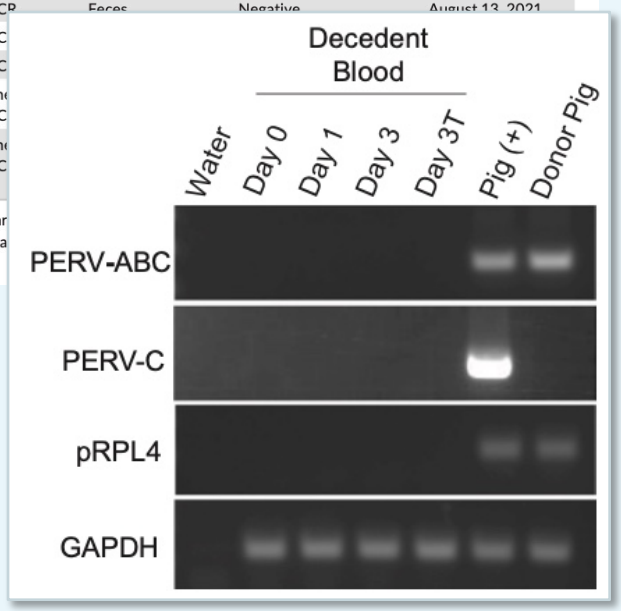


TABLE 4 Results of pathogen screening of the donor pig

Test	Methodology	Sample	Result	Date
Hepatitis E	Real-time PCR	Feces	Negative	September 7, 2021
Herpes virus gamma	PCR	Buffy coat	Negative	September 7, 2021
Influenza A	Real-time PCR	Nasal Swab	Negative	August 13, 2021
Mycoplasm hyopneumoniae	Real-time PCR	Nasal Swab	Negative	August 16, 2021
Porcine circovirus 2,3 (duplex)	Real-time PCR	Serum	Negative	August 18, 2021
Porcine cytomegalovirus	Real-time PCR	Buffy coat	Negative	August 17, 2021
Porcine endogenous retrovirus A	PCR	Buffy coat	Positive 20.23 Ct40	August 19, 2021
Porcine endogenous retrovirus B	PCR	Buffy coat	Positive 21.65 Ct40	August 19, 2021
Porcine endogenous retrovirus C	PCR	Buffy coat	Negative	August 19, 2021
Porcine Epidemic Diarrhea Virus (S gene)	Real-time PCR	Feces	Negative	August 13, 2021
Porcine deltacoronavirus	Real-time PC			
Transmissible Gastroenteritis virus	Real-time PC			
Porcine reproductive and respiratory syndrome virus (PRRSV) European	Thermo Fish Real-time PC			
Porcine reproductive and respiratory syndrome virus (PRRSV) North American	Thermo Fish Real-time PC			

Note: Most recent testing results in advance of the procurement ar
porcine and/or human health and do not all have zoonotic potentia
Laboratory. Test dates reflect completion of the assay.



No transmission of porcine retroviruses was detected

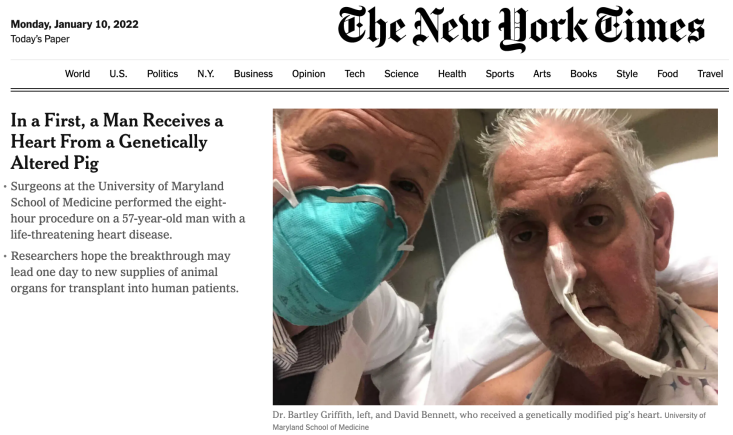
Pig to human kidney transplantation: Conclusion

- Genetic engineering sufficient to prevent hyperacute rejection
- Determine tissue compatibility prior pig-to-human transplantation
- The decedent model identified numerous limitations
  - A brain-dead recipient is a hostile environment for a transplant
  - Trying to get function in the face of brain-death is challenging
  - Short timeframe: no hyperacute rejection, other/slower types of rejection still possible

Mohiuddin et al.: Nat Comm, 2016.

The «first» heart xenotransplantation

The „first“ heart xenotransplantation



Monday, January 10, 2022
Today's Paper

The New York Times

World U.S. Politics N.Y. Business Opinion Tech Science Health Sports Arts Books Style Food Travel

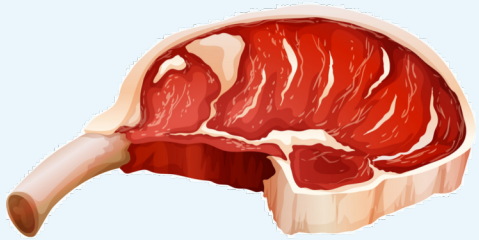
In a First, a Man Receives a Heart From a Genetically Altered Pig

- Surgeons at the University of Maryland School of Medicine performed the eight-hour procedure on a 57-year-old man with a life-threatening heart disease.
- Researchers hope the breakthrough may lead one day to new supplies of animal organs for transplant into human patients.

Dr. Bartley Griffith, left, and David Bennett, who received a genetically modified pig's heart. University of Maryland School of Medicine

- The recipient Mr. Bennett was deemed to not be a suitable candidate for heart allotransplantation or ventricular assist device → risk of death 100%
- Ethics approval
- The FDA authorized the surgery on Dec 31 2021 for “compassionate use“

FDA approval for GalSafe pigs in December 2020



α-Gal syndrome
„meat allergy“

FDA U.S. FOOD & DRUG ADMINISTRATION

Search Menu

Home / News & Events / FDA Newsroom / Press Announcements / FDA Approves First-of-its-Kind Intentional Genomic Alteration in Line of Domestic Pigs for Both Human Food, Potential Therapeutic Uses

FDA NEWS RELEASE

FDA Approves First-of-its-Kind Intentional Genomic Alteration in Line of Domestic Pigs for Both Human Food, Potential Therapeutic Uses

Alteration intended to eliminate alpha-gal sugar on surface of pigs' cells

Share Tweet Linkedin Email Print

More Press Announcements

For Immediate Release: December 14, 2020

Today, the U.S. Food and Drug Administration approved a first-of-its-kind intentional genomic alteration (IGA) in a line of domestic pigs, referred to as GalSafe pigs, which may

Content current as of: 12/14/2020

- Porcine heart transplants are NOT approved by the FDA
- The FDA authorized the surgery on Dec 31 2021 for “compassionate use“

The „first“ heart xenotransplantation

DOI: 10.1111/xen.12733

COMMENTARY

Xenotransplantation WILEY

World first pig-to-human cardiac xenotransplantation

Wayne J. Hawthorne[1,2]

[1] The Centre for Transplant and Renal Research, Westmead Institute for Medical Research, Westmead, New South Wales, Australia

[2] Department of Surgery, School of Medical Sciences, University of Sydney, Westmead Hospital, Westmead, New South Wales, Australia

DOI: 10.1111/xen.12734

COMMENTARY

Xenotransplantation WILEY

Clinical cardiac xenotransplantation first in the clinical arena

Martin Bender[1] | Matthias Längin[1] | Bruno Reichart[2] | Maren Mokelke[3] | Julia Radan[3] | Elisabeth Neumann[3] | Sebastian Michel[3] | Reinhard Ellgass[3] | Peter J. Cowan[4,5] | Eckhard Wolf[6] | Jan-Michael Abicht[1] | Paolo Brenner[3]

[1] Department of Anaesthesiology, University Hospital, LMU Munich, Munich, Germany

[2] Transregional Collaborative Research Center 127, Walter Brendel Centre of Experimental Medicine, LMU Munich, Munich, Germany

[3] Department of Cardiac Surgery, University Hospital, LMU Munich, Munich, Germany

[4] Immunology Research Centre, St. Vincent's Hospital Melbourne, Melbourne, Victoria, Australia

[5] Department of Medicine, University of Melbourne, Melbourne, Victoria, Australia

[6] Institute of Molecular Animal Breeding and Biotechnology, Gene Center, LMU Munich, Munich, Germany

The New York Times

March 9th 2022

Patient in Groundbreaking Heart Transplant Dies

David Bennett Sr. had received a heart from a genetically modified pig, a procedure that may yet offer hope to millions of Americans needing transplants.

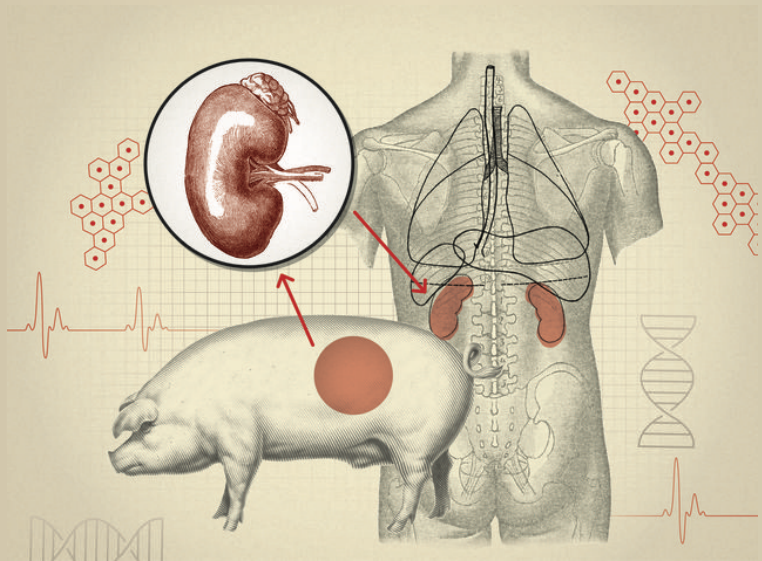
Future applications?

Future applications

- Xenotransplantation is the most pragmatic solution to end the organ shortage crisis, but safety and efficacy concerns have limited advancement in humans

- Hyperacute rejection was not observed in the genetically modified pigs

- **A long way to go**




JA ZUM
TRANSPLANTATIONSGESETZ
AM 15. MAI 2022