DEPARTMENT OF CARDIO-
THORACIC AND VASCULAR
SCIENCES AND PUBLIC HEALTH

UNIVERSITÀ
DEGLI STUDI
DI PADOVA

Xenotrapianto:
dalla fantascienza alla concreta possibilità

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Clinical Xenotransplantation: Major advantages

- Unlimited supply of organs, tissues, and cells
- Organs will be available electively
- Avoids the detrimental effects of brain death on donor organs
- "Infection-free" sources of organs, tissues, and cells
- Obviates the "cultural" barriers to deceased human organ donation present in some countries

[adapted from Wijkstrom M et al, Kidney Int 2017]

The great progress of the xenotransplantation science

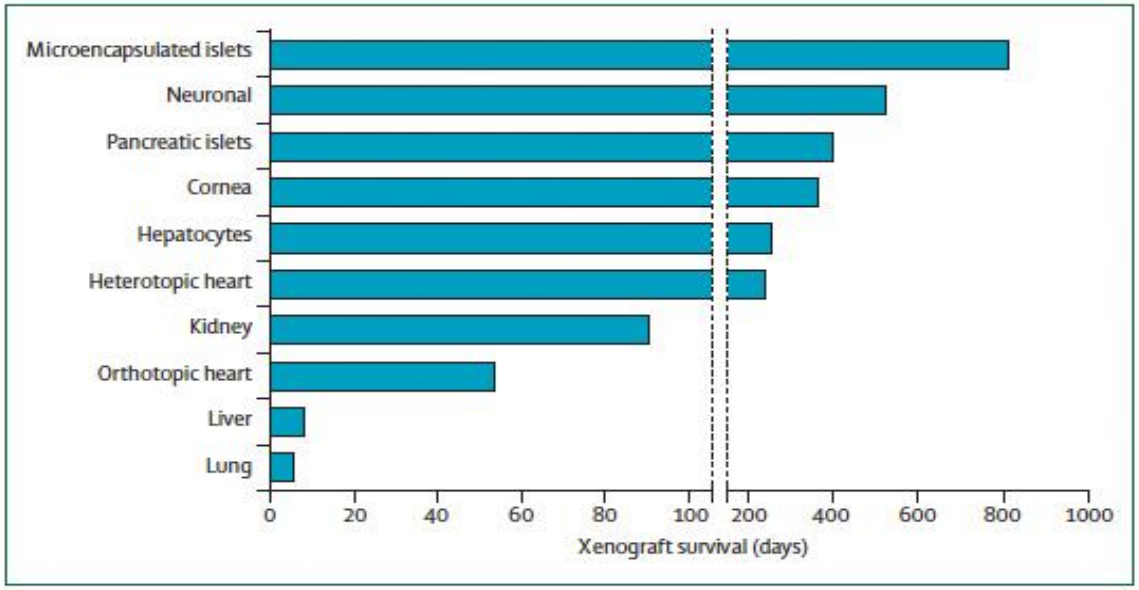
Xenotransplantation preclinical research as of **2012**

THE LANCET

www.thelancet.com

Clinical xenotransplantation: the next medical revolution?

Best preclinical results reported as of **2012**



[Ekser et al., Lancet 2012]

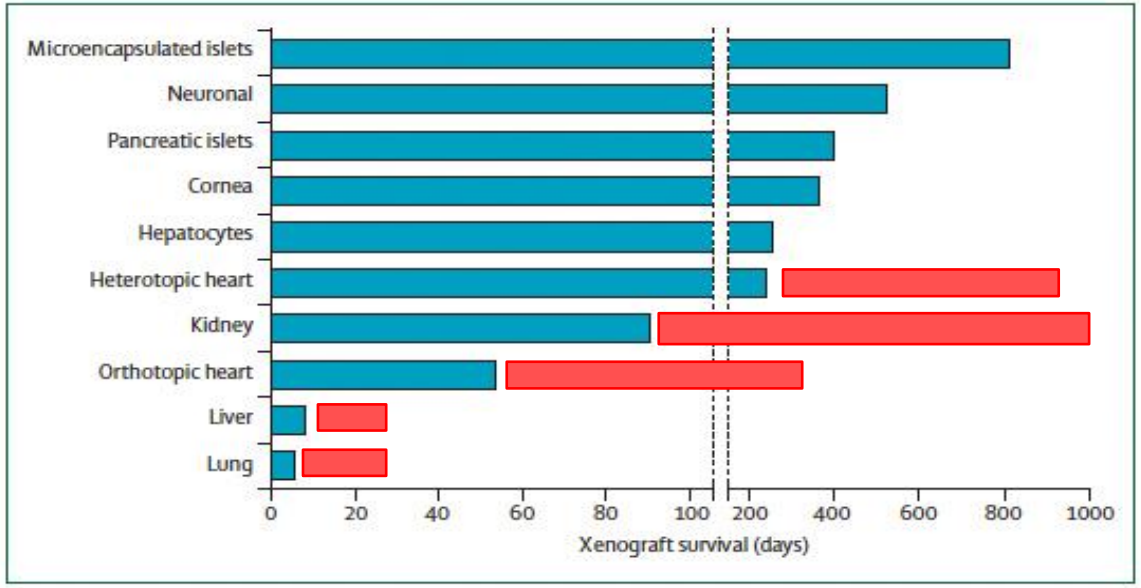
Xenotransplantation preclinical research as of **03/2023**

THE LANCET

www.thelancet.com

Clinical xenotransplantation: the next medical revolution?

Best preclinical results reported as of **2023**



[Adapted from Ekser et al., Lancet 2012]

The reasons underlying such a major progress in preclinical xenotransplantation

- A better understanding of the xenotransplantation science

- Genetic engineering of the pig

- Different immunosuppressive approach

Genetic engineering of the pig: Possible targets of intervention

- Complement regulation (CD55, CD46, CD59)
- Immunogenicity (αGALT-KO)
- Immunomodulation (CTLA4Ig, CIITA-DN, SIRPα…)
- Ischemia reperfusion injury (CD39, A20, HO1…)
- Coagulation (CD39, TM, TFPI, TF-KO…)
- Safety (no-PERV animals,….)

Rapid progress is now possible thanks to the advent of the CRISPR/Cas9 technology

Moving towards clinical xenotransplantation: Key points to address

- Immunology
- Physiology
- Biosafety
- Ethics and regulations

Clinical Xenotransplantation: Key points to address

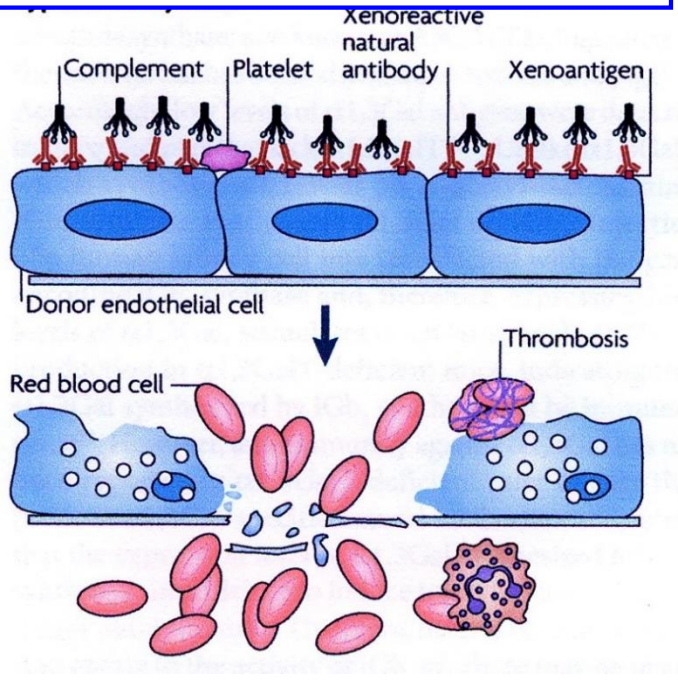
- Immunological barriers
- Physiology
- Biosafety
- Ethics and regulations

Xenotransplantation: the immunological barriers

- Antibody-mediated rejection
  - Hyperacute rejection (HAR)
  - Acute humoral xenograft rejection (AHXR)
- Cell-mediated rejection
- Chronic rejection
- Natural immunity

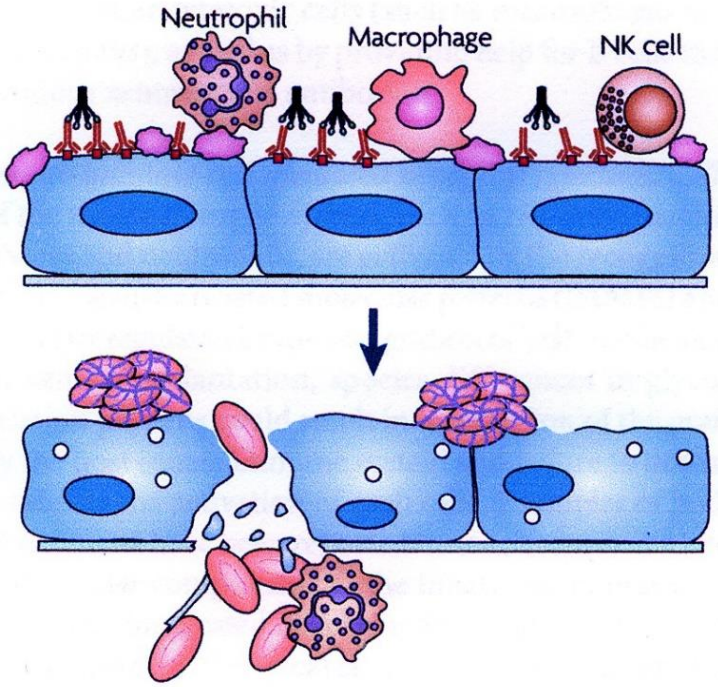
Humoral rejection of a xenograft

Hyperacute



- Occurs within minutes to hours
- Mediated by preexisting antibodies (e.g., anti-αGal)

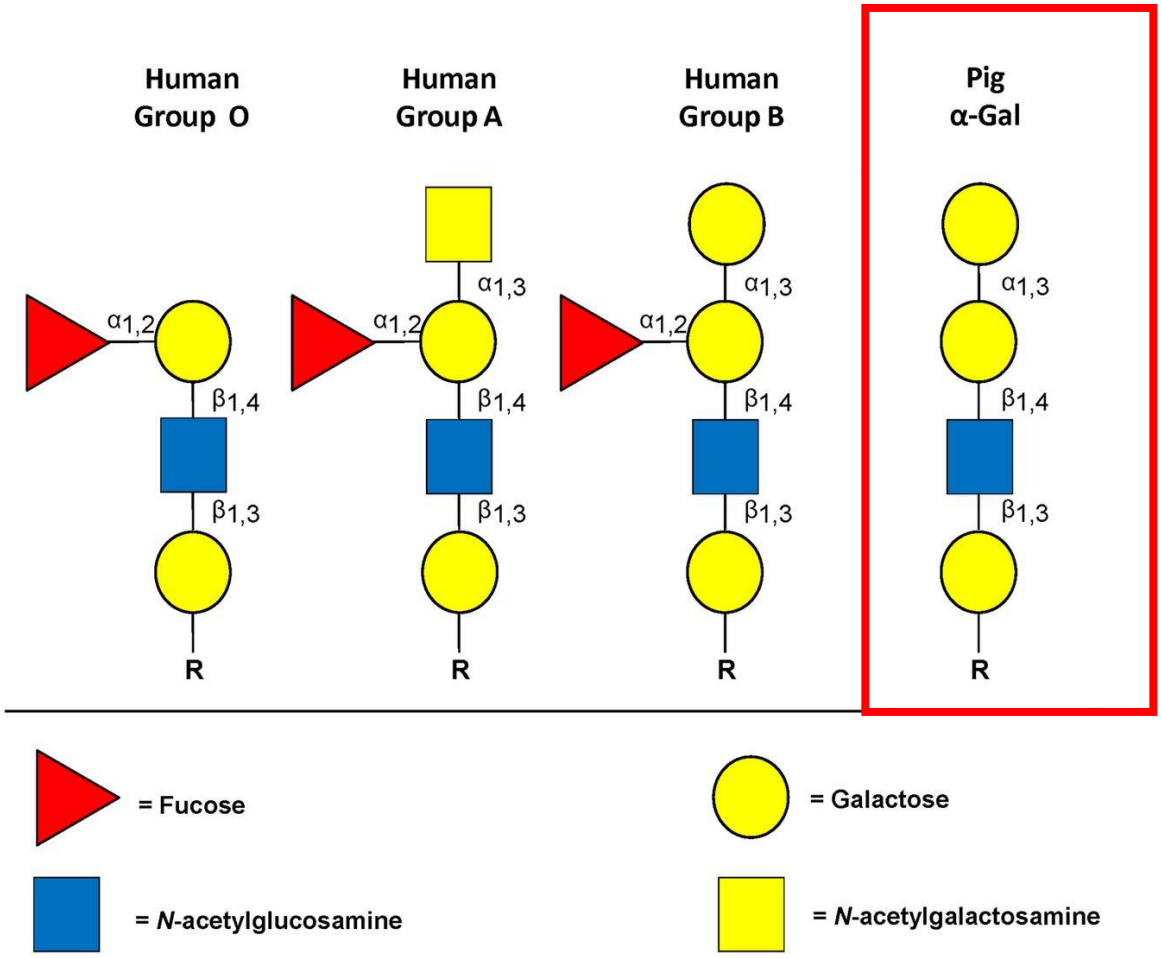
Acute



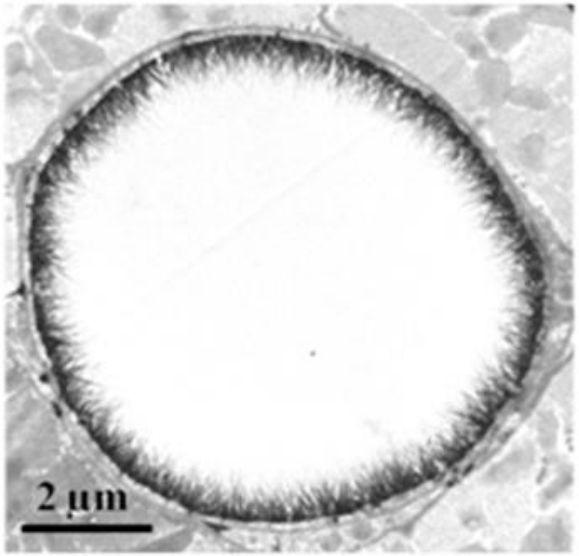
- Occurs within a few days or weeks
- Mediated by elicited antibodies (anti-Gal or non anti-Gal)

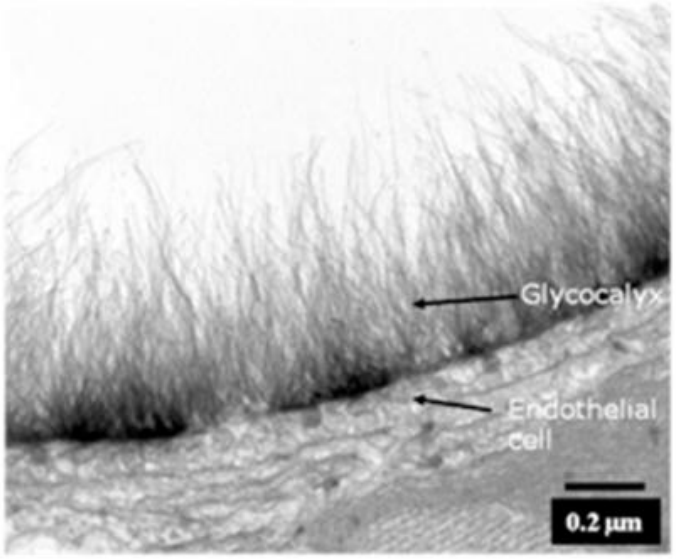
[Y Yang, Nature Med 2007]

Xenograft immunogenicity: the importance of the sugars



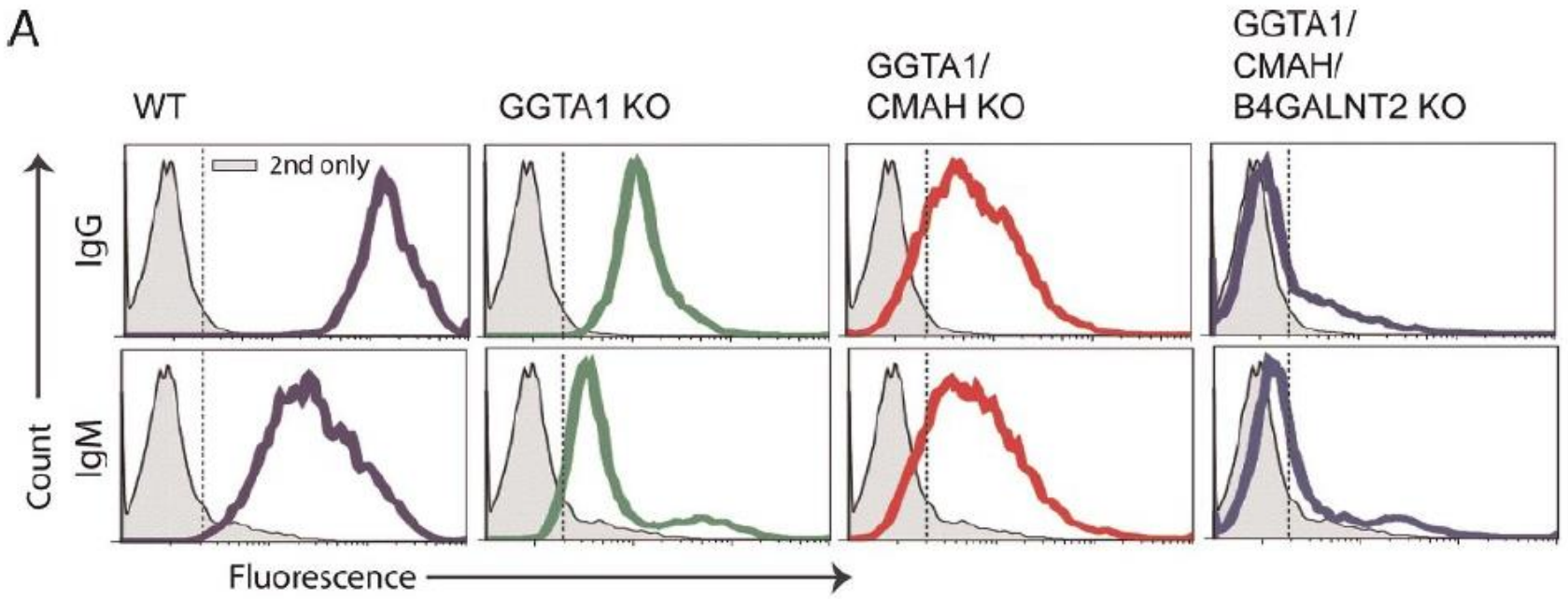
Xenograft immunogenicity: new aspects





http://www.hubrecht.eu/research/dekoning/research.html

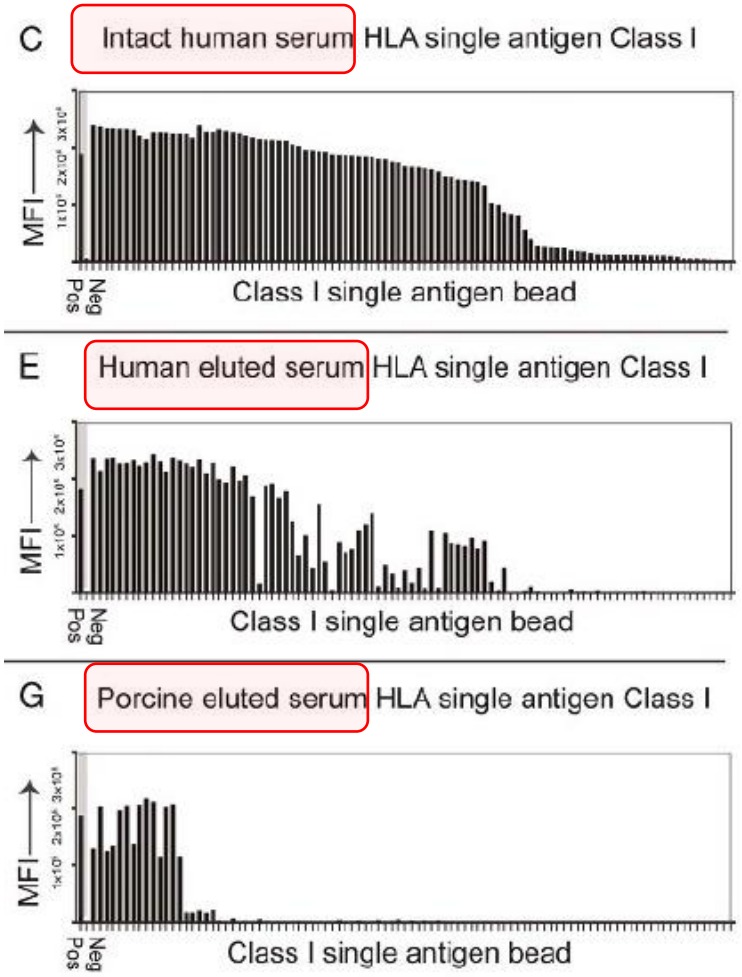
Binding of human sera to pig PBMC: The incredible progress of genetic engineering



[Martens et al, Transplantation 2017]

Human anti-HLA antibodies cross-react with swine leukocyte antigens (SLA)

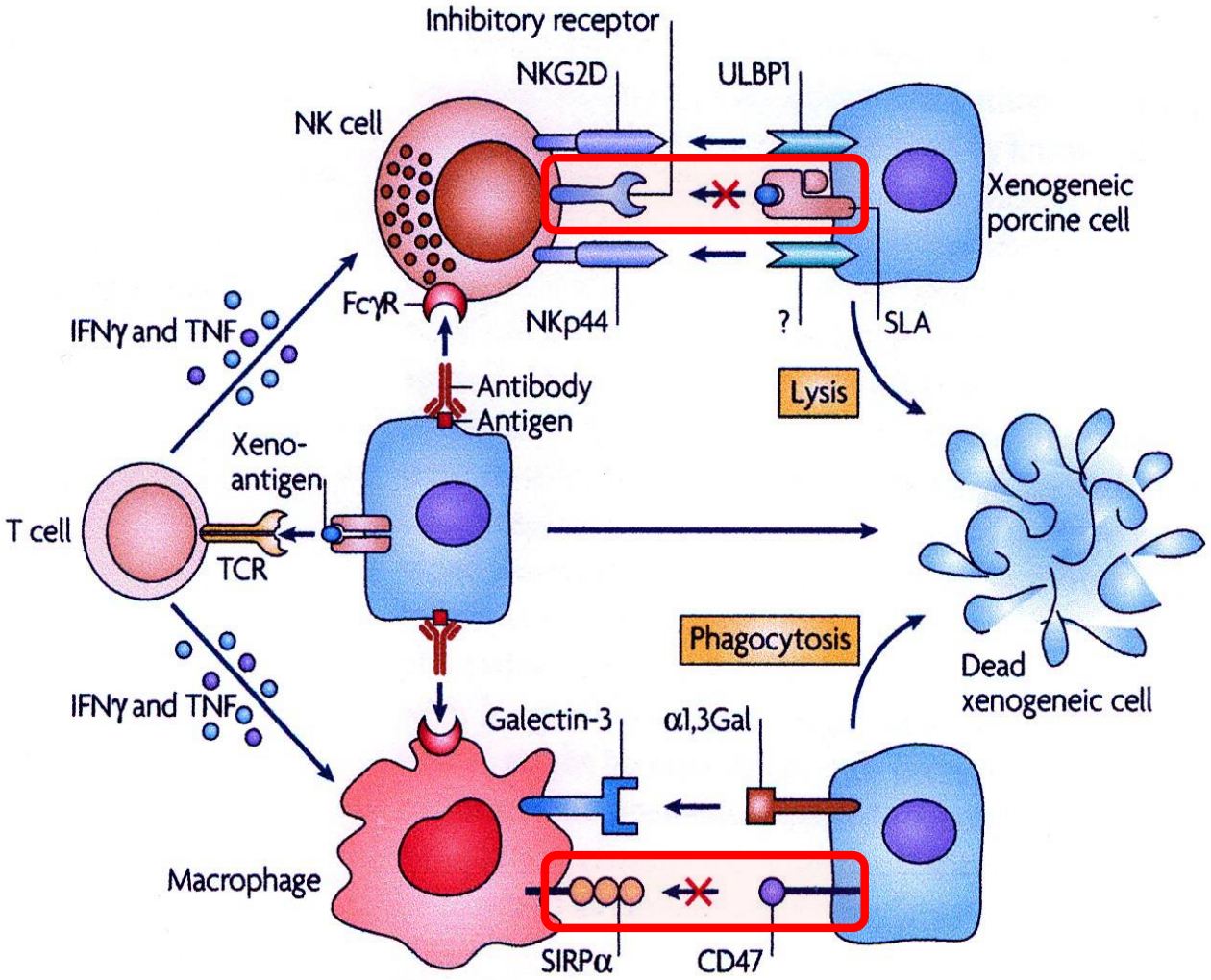
Human anti-HLA IgG cross-react with Swine class I SLA molecules



Very high MFI!

[Martens et al, Transplantation 2017]

Cell-mediated rejection



Involves:

1. T-Lymphocytes
2. NK
3. Macrophages
4. B-lymphocytes

[Y Yang, Nature 2005]

Clinical Xenotransplantation: Key points to address

- Immunology
- Physiology
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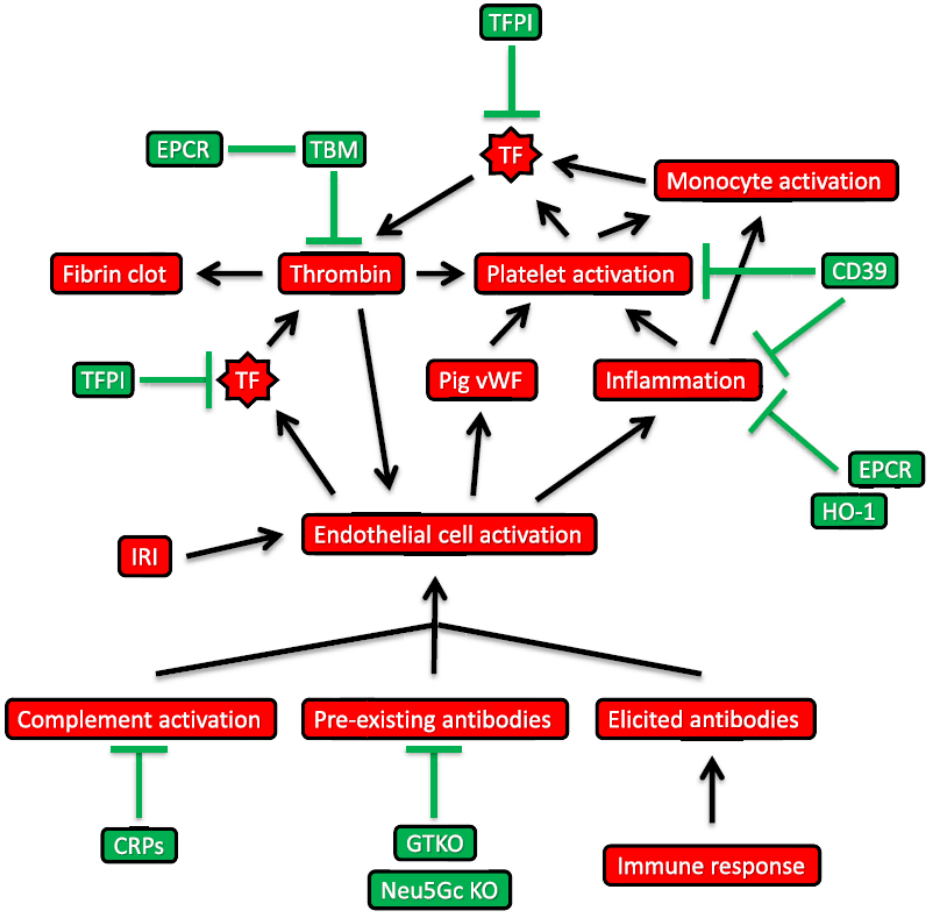
Coagulation dysfunction

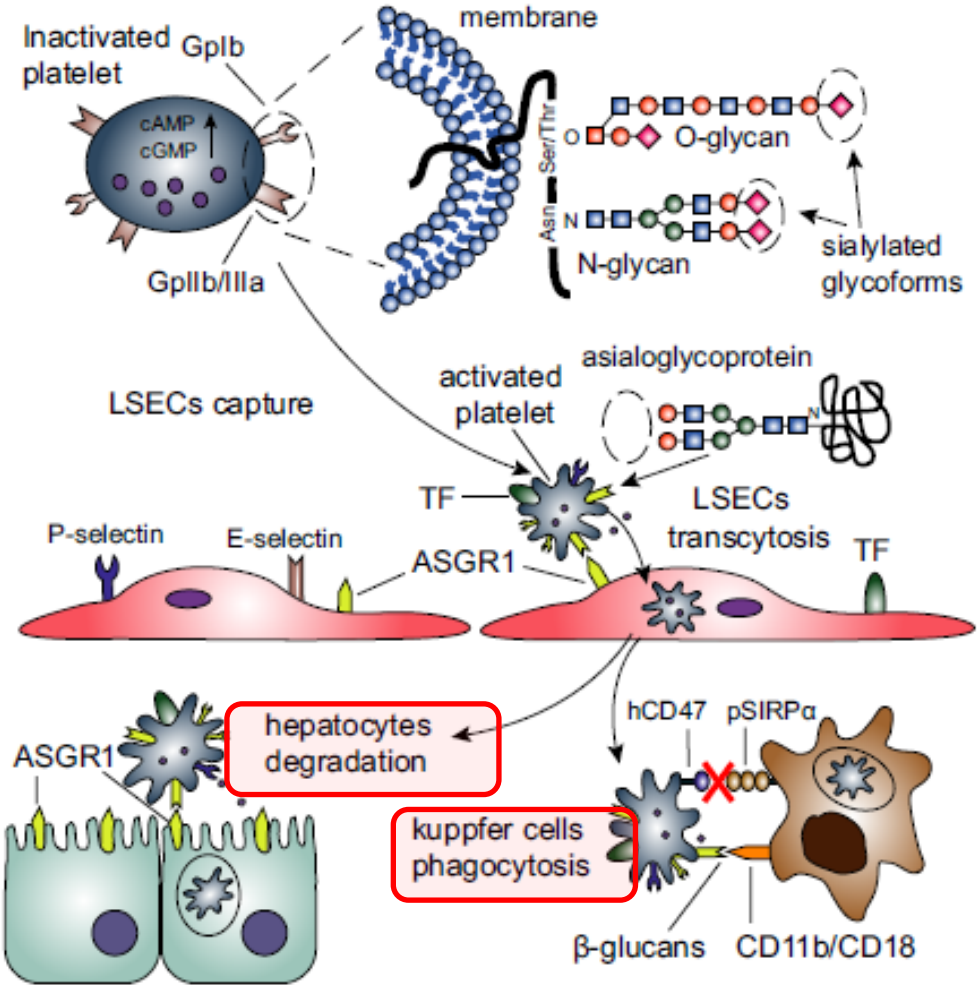


Leads to:

- thrombotic Microangiopathy
- DIC (Thrombocitopenia, consumption of fibrinogen, increase of PT)

[Cowan et al, Int. J Surgery 2015]

Thrombocytopenia



[Zhang et al, Xenotransplant 2019]

Moving towards clinical xenotransplantation: Key points to address

- Immunology
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Risk of infection Following transplantation (I)

The risk of infection after transplantation is the ultimate result of the interaction between:

- epidemiology of infection (the dose, intensity, and virulence of organisms in the **recipient** and also in the **graft**)
- net state of immunosuppression (nature, intensity, and duration of **immunosuppression**)

[Fishman, NEJM 2022]

Risk of infection following transplantation (II)

- Potential transmission of infections is the basis of:
  - antimicrobial **prophylaxis** (e.g., for Pneumocystis jirovecii, hepatitis viruses, CMV, HIV, or fungi)
  - Microbiologic screening before allotransplantation that is, however, generally **limited** to a panel of **serologic** and **molecular** tests *(microbiologic **cultures** available **only after** organ transplantation)*
  - post-transplantation **surveillance** protocols (e.g., nucleic acid testing) for CMV or EBV

- ***Donor-derived*** infections are **uncommon** in **allo**transplantation.

  Unexpected *donor-derived infections* occur in approximately **0.2%** of transplantations (and include infections due to organisms that are undetected in current donor screening)

- The use of organs from **donors with known, latent infections is routine.**

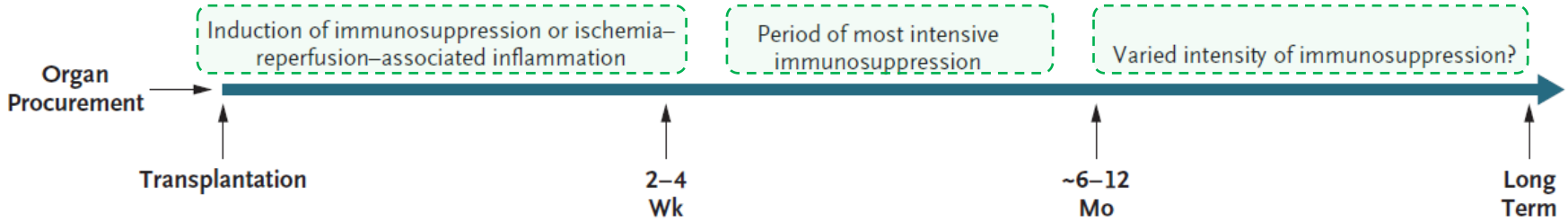
[Fishman, NEJM 2022]

Microbiological screening of swine donors for xenotransplantation

Swine Pathogen Type	Examples	Microbiologic Assays Available?	Exclude as Xenograft Donor?
Pathogens of immunologically normal humans	Influenza viruses, SARS-CoV-2, mycobacteria, rabies virus, mycoplasma species	Yes	Yes; some infections may clear with therapy
Pathogens in immunosuppressed human transplant recipients	*Toxoplasma gondii*, strongyloides species, aspergillus species	Yes	Decision is based on risk of infection with a specific organism
Porcine organisms similar to common pathogens in immunosuppressed human transplant recipients	PCMV, PLHV, porcine adenovirus	Some	Decision requires validation of assays in human blood or tissues; herpesviruses are generally species-specific
Unique swine pathogens	PERV	Some	Perhaps no; probably low risk; requires study in xenograft recipients

[Fishman, NEJM 2022]

Timeline of infections after xenotransplantation



	Nosocomial and Technical Infections	Opportunistic, Relapsed, or Residual Infections	Opportunistic and Community Infections
Common Human Pathogens	Anastomotic leaks or stenosis, *C. difficile* colitis, antimicrobial resistant bacteria, candida species (catheter-associated, aspiration, wound infection)	HSV, CMV, HBV, HCV, listeria, PCP, *Toxoplasma gondii*, VZV, BKPyV, nocardia, mycobacteria species, cryptococcus, RVI	CAP, RVI, UTI, aspergillus and other molds, endemic fungi, JC virus (PML), EBV (PTLD), skin cancers
Possible Pig-Derived Pathogens	Porcine bacteria, fungi (graft contamination)	PCMV, PLHV, PCV, PERV, others?	Unknown

[Fishman, NEJM 2022]

Porcine endogenous retroviruses (PERV)

- PERV is a ubiquitous genomic **provirus** of porcine cells.
- Three homologous PERVs (PERV-**A**, PERV-**B**, and PERV-**C**)
- in vitro, PERV-A and PERV-B can infect porcine cells and adenovirus-5–transformed, permissive human target cells.
- PERV-C infects **only porcine cells**.
- Increased efficiency of viral replication observed in the case of viral recombination (PERV-AC).

[Fishman, NEJM 2022]

Xenotransplantation and PERV zoonoses (I)

SCIENCE VOL 285 20 AUGUST 1999

Search for Cross-Species Transmission of Porcine Endogenous Retrovirus in Patients Treated with Living Pig Tissue

Khazal Paradis,[1*] Gillian Langford,[1] Zhifeng Long,[2]
Walid Heneine,[3] Paul Sandstrom,[3] William M. Switzer,[3]
Louisa E. Chapman,[3] Chris Lockey,[2] David Onions,[4]
The XEN 111 Study Group,[5] Edward Otto[2]

Xenotransplantation and PERV zoonoses (II)

Procedure	*N*	Age (years)	Duration of exposure	Months since treatment ± SD (range)
Extracorporeal liver perfusion	1	59	4.25 hours	40.5
Extracorporeal kidney perfusion	2	40 to 50	15 and 65 min	33.9 ± 2.8 (31.9 to 35.8)
Bioartificial liver perfusion	28	11 to 65	11.75 hours (2 to 30 hours)	25.8 ± 18.7 (2.4 to 60.8)
Pancreatic islet cells	14	19 to 59	1 to 460 days	59.7 ± 24.2 (18.8 to 92.9)
Skin	15	8 to 67	10 days (estimated average)	101.9 ± 34.4 (35.7 to 149.5)
Extracorporeal splenic perfusion	100	2 to 77	50 to 60 min	29.7 ± 28.5 (0 to 102)
Total	160	2 to 77		38.5 ± 34.8 (0 to 149.5)

- No PERV infection was detected in any of the patients
- Persistent microchimerism observed in 23 patients for up to 8.5 years.

[Paradis et al, Science 1999]

Xenotransplantation and PERV zoonoses (III)

Virus Research 227 (2017) 34–40

Research Article

No PERV transmission during a clinical trial of pig islet cell transplantation

Vladimir A. Morozov[a], Shaun Wynyard[b], Shinichi Matsumoto[c], Adrian Abalovich[d], Joachim Denner[a,*], Robert Elliott[e]

[a] Robert Koch Institute, Berlin, Germany
[b] Diatranz Otsuka Limited, Auckland, New Zealand
[c] Otsuka Pharmaceutical Factory Inc., Naruto, Japan
[d] Hospital Eva Peron de San Martin, Buenos Aires, Argentina
[e] Elliott Enterprises, Auckland, New Zealand

No PERV transmission in islet xenografts recipients followed for up to 113 weeks.

PERV and antiretroviral agents

Antimicrobial Agents and Chemotherapy
Volume 44, Issue 12, 1 December 2000, Pages 3432-3433
https://doi.org/10.1128/AAC.44.12.3432-3433.2000

Antiviral Agents

Antiretroviral Agents Inhibit Infection of Human Cells by Porcine Endogenous Retroviruses

S. K. Powell[1], M. E. Gates[1], G. Langford[2], M.-L. Gu[1], C. Lockey[1], Z. Long[1], and E. Otto[1,*]

[Powell et al, Antimicrobial Agent and Chemotherapy 2000]

The CRISPR/Cas9 technology

Inactivation of porcine endogenous retrovirus in pigs using CRISPR-Cas9

[Niu et al, Science 2017]

Animal husbandry for xenotransplantation: The "designated pathogen-free" (DPF) status

Bacteria

Brucella suis

Leptospira species

Listeria monocytogenes

Nontuberculous mycobacteria (including *Mycobacterium bovis*)

M. tuberculosis

Mycoplasma hyopneumoniae (lung transplant)

Salmonella species (*S. enterica* serotype Typhi, serotype Typhimurium, serotype Choleraesuis)

Shigella

Stool enteric pathogens (yersinia, campylobacter)

Fungi

Aspergillus species (colonized or lesions)

Candida species (lesions)

Cryptococcus neoformans

Histoplasma capsulatum

Parasites (detected in stool or on serologic testing)

Ascaris suum

Cryptosporidium or microsporidium species

Echinococcus species

Other parasites

Giardia species

Isospora species

Strongyloides species

T. gondii

Trichinella spiralis

Trypanosoma species

Viruses

Adenovirus (swine)

Encephalomyocarditis virus (vaccine)

Hepatitis E virus

Influenza virus (swine and human)

PCMV

Porcine circovirus (types 1, 2, and 3)

PLHV

Porcine reproductive and respiratory syndrome virus

Porcine parvovirus

Pseudorabies virus

Rabies virus

[Fishman, NEJM 2022]

Microbial surveillance: The importance of baseline clinical samples

- Recipients
  - RNA
  - DNA
  - Cells
  - Antibodies
- Close contacts

- stored for 20 years or for the recipient's lifetime
- accessible by clinical teams and public health authorities

Microbial surveillance: Routine testing of xenograft recipients

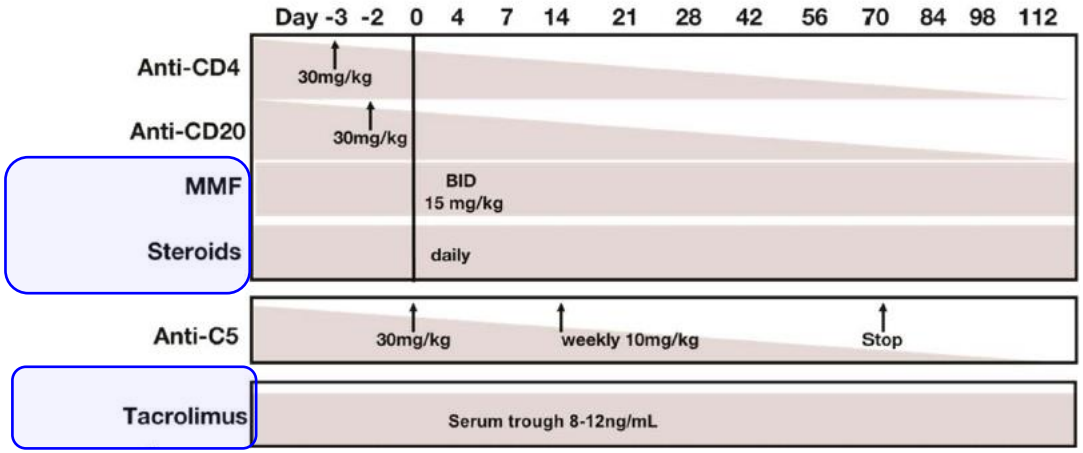
Virus	Testing Method†
PERV-A, -B, -C, -AC	QNAT, antibody-based tests (serologic testing, ELISA, Western blot testing)
PLHV-2	QNAT
PCMV	QNAT, serologic testing
HCMV, according to risk status	QNAT
Human EBV, according to risk status	QNAT
BK polyomavirus (in kidney recipients), per protocol	QNAT
Pig-cell chimerism in circulation (PBMCs)	QNAT (e.g., P-MHC class I gene; p-mtCOII gene) in recipient PBMC DNA[23,35]

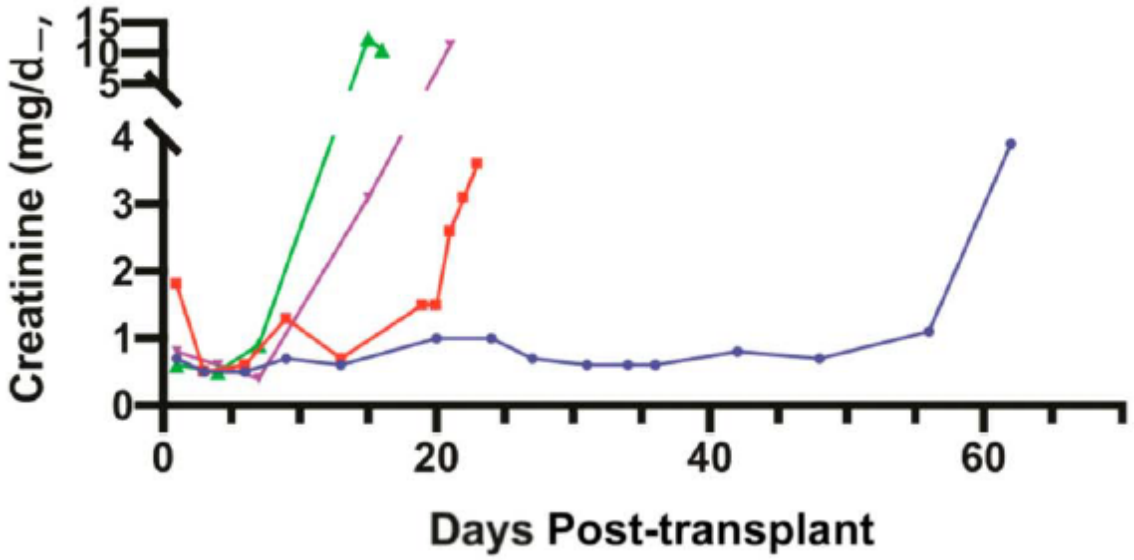
Yet unidentified pathogens (?)

[Fishman, NEJM 2022]

Best results in pig-to primate xenotransplantation

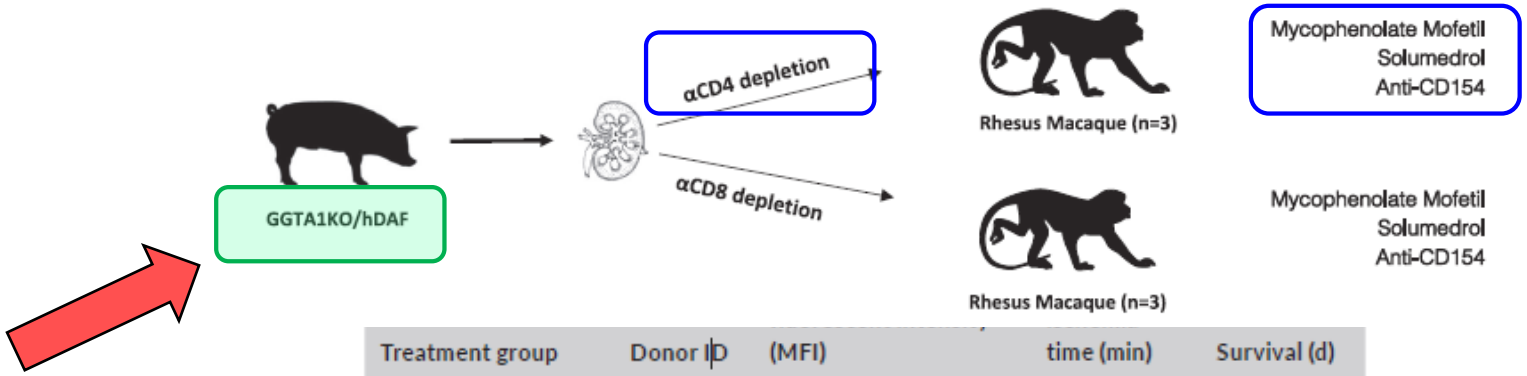
Tacrolimus-based immunosuppression **fails** to enable longterm renal xenograft survival





[Tector et al, Kidney 360 2022]

Longterm xenograft survival reproducibly achievable with low anti-pig antibody, anti-CD4 and anti-CD154



Treatment group	Donor ID	(MFI)	time (min)	Survival (d)
High titer	D1	8983	195	6
$\alpha CD4^+\alpha CD8^+$ anti-CD154	D1	2960	90	310
	D2	1041	93	160
	D3	1699	45	406
		1049	147	18
	D4	2273	51	115
		966	180	>400
$\alpha CD4^+$ anti-CD154	D5	1195	200	499
	D6	1340	235	414
	D7	1446	62	>70
$\alpha CD8^+$ anti-CD154	D5	1969	40	15
	D6	2159	150	6
	D7	979	132	6

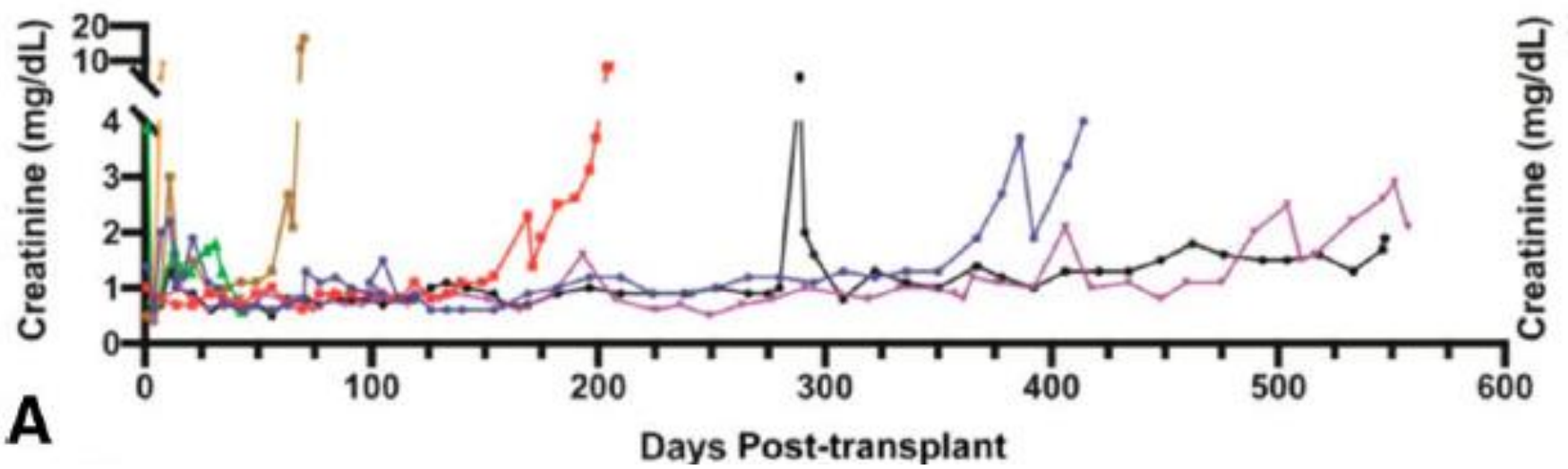
[Kim et al, AJT 2019]

Anatomic and physiologic comparisons and potential physiologic barriers

Renal Physiology Component	Comparison and Potential Barrier
Anatomy	Pigs have fewer nephrons and a lower percentage of long-looped nephrons, and thus have a reduced ability to concentrate urine.
Global function	Markers of renal function, including **glomerular filtration rate and renal plasma flow**, **are comparable between humans and pigs, and remain adequate for at least several months.** It remains unknown, however, how long these parameters would be stable following kidney xenotransplantation.
Sodium handling	The renin-angiotensin-aldosterone system (RAAS) **remains functional**. Major electrolyte levels, including sodium, potassium, and chloride, are maintained in NHPs with pig kidneys NHPs experience episodes of hypovolemia post-xenotransplantation which may be associated with physiologic differences in renin across species.
Water handling	**Human antidiuretic hormone** (ADH) has a different structure compared to pig ADH and is **less potent in pigs.** This may lead to decreased water reabsorption and a reduced ability to concentrate urine after xenotransplantation.
Erythropoietin (EPO) production	Pig EPO has a high degree of homology to human EPO and appears to be functional in recent pig-to-NHP studies.
Renal response to hormones	Pig kidney grafts have been shown to grow rapidly after xenotransplantation independent of rejection in some studies. Pig kidneys are able to process human growth hormones, catecholamines, and prostaglandins.
Acid-base balance	Humans and pigs have **comparable blood pH levels, but the composition of metabolites differs** as pigs have higher bicarbonate and phosphate levels. While a pig kidney can excrete acid and reabsorb bicarbonate at acceptable rates, it may not excrete as much phosphate, which could lead to an anion-gap acidosis.
Calcium/phosphorus handling	Following renal xenotransplantation in NHPs, **serum calcium levels rise to high normal values** while **phosphate levels fall**. It remains to be seen how the pig graft will respond to human FGF-23, parathyroid hormone, or vitamin D.

[Hansen-Estruch et al, AJT 2022]

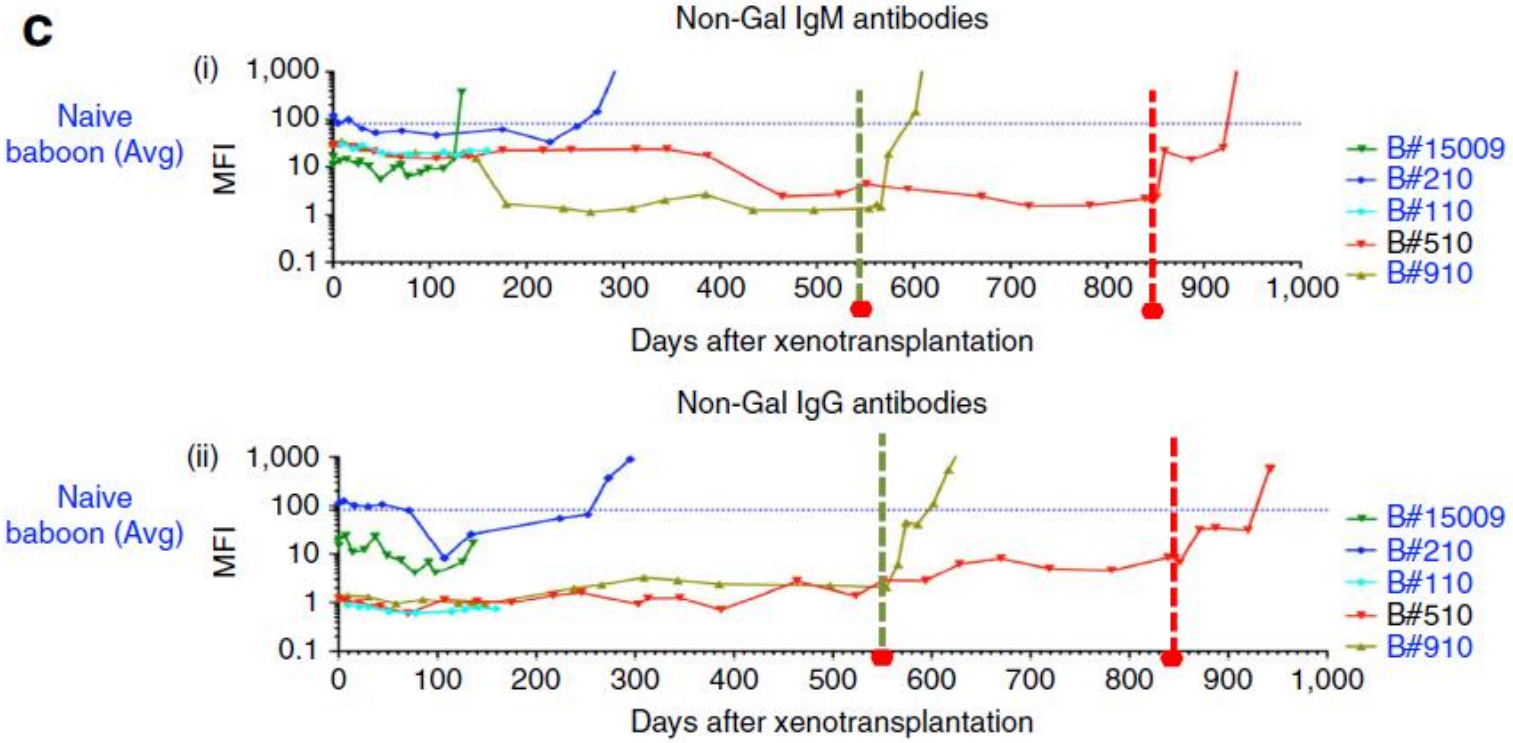
Anti-C5 plus CD40/CD40L pathway inhibitors considerably extend renal xenograft survival



Longest survival reported: > 4 years (Adams 2023)

[Tector et al, Kidney 360 2022]

Longterm Cardiac xenograft survival: antibody titres and outcomes

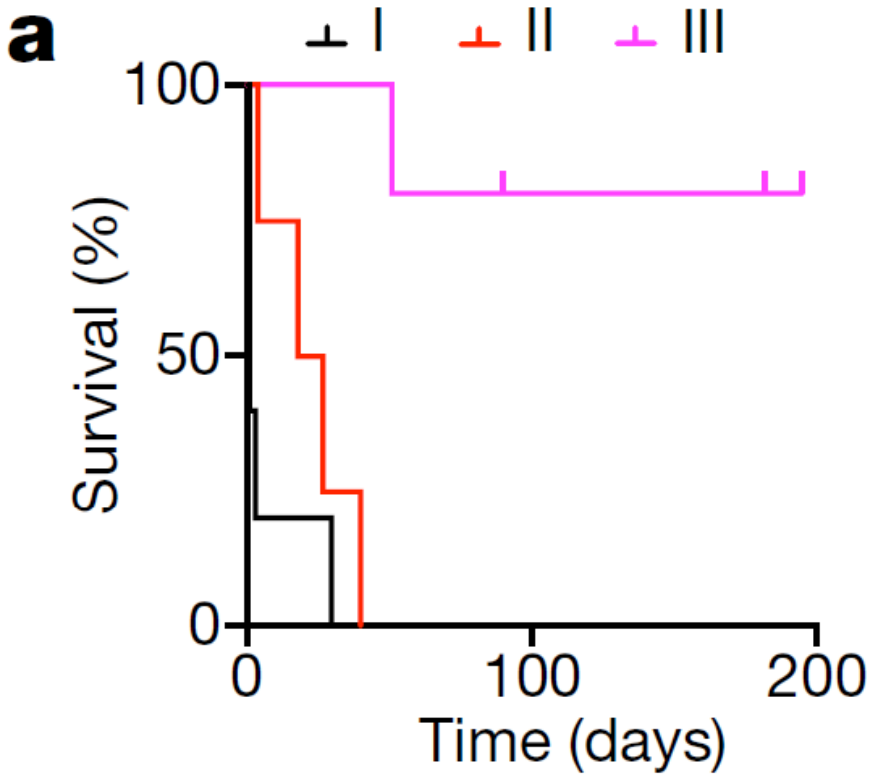


…reduction of the αCD40 Mab dose resulted in recrudescence of anti-pig antibodies and graft failure.

[Mohiuddin et al, Nature Com 2016]

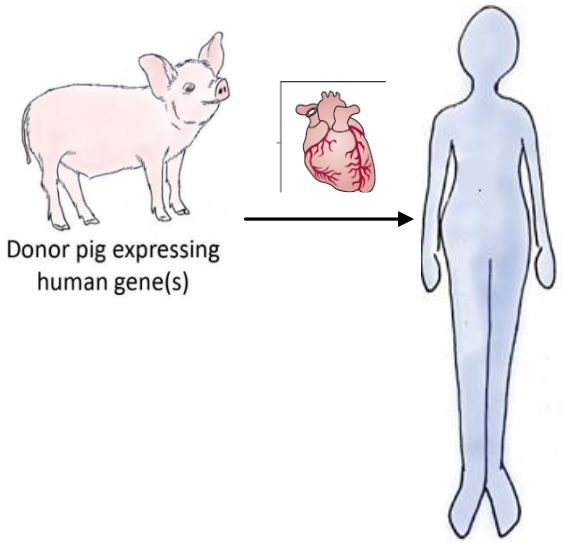
LETTER NATURE | www.nature.com/nature

Consistent success in life-supporting porcine cardiac xenotransplantation



[Langin et al, Nature 2018]

Heart Xenotransplantation: Physiological compatibility



1. No significant differences in the anatomy
2. Orthotopic cardiac xenografts tend to grow to a size exceeding the primate's organs (GH-R KO)
3. Cardiac output and stroke volume comparable in pig and human
4. Mean arterial pressure, heart rate, and myocardial blood flow are also nearly identical in the two species
5. The different action potential of cardiomyocytes related to morphological differences in the atrioventricuar node between species might potentially result in increased arrhythmogenicity of a pig heart
    - Not evidenced in pre-clinical studies

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- Immunology
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First genetically engineered pig-to-human heart xenotransplant

Genetically Modified Porcine-to-Human Cardiac Xenotransplantation

[Griffith et al, NEJM 2022]

First genetically engineered pig-to-human heart xenotransplant

Table 1 | Gene editing of pigs for xenotransplantation

Gene name	Edit	Purpose
Aalpha-1,3-galactosyltransferase	Knockout	Protect the pig organ from antibody-mediated damage and hyperacute rejection
Monophospho-N-glycolylneuraminic acid hydroxylase	Knockout	Protect the pig organ from antibody-mediated damage and delayed humoral xenograft rejection
β-1,4 N-acetylgalactosaminyltransferase 2	Knockout	Protect the pig organ from antibody-mediated damage and delayed humoral xenograft rejection
CD55	Transgene	Protect the pig organ from complement-mediated damage
CD46	Transgene	Protect the pig organ from complement-mediated damage
CD59	Transgene	Protect the pig organ from complement-mediated damage
Thrombomodulin	Transgene	Protect the pig organ from coagulation dysregulation
Endothelial protein C receptor	Transgene	Protect the pig organ from coagulation dysregulation and inflammation
CD47	Transgene	Protect the pig organ from macrophage-mediated damage
Hemeoxygenase-1	Transgene	Protect the pig organ from inflammation and apoptosis
Growth hormone	Knockout	Prevent organ growth after xenotransplantation
HLA-E	Transgene	Protect the pig organ from natural killer cell–mediated damage
PERV genes	Knockout	Reduce risk of transmission of pig viruses to humans

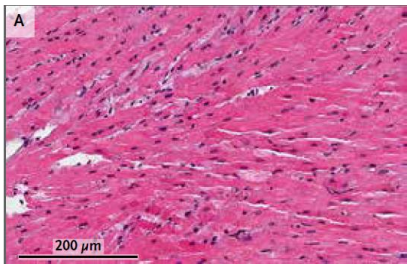
[Montgomery et al, Nature Med 2022]

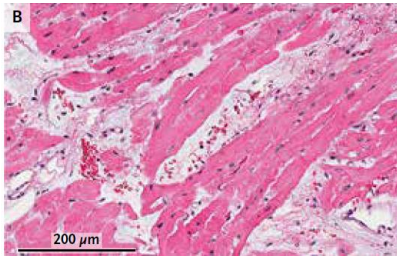
First pig-to-human heart xenotransplant: immunosuppression

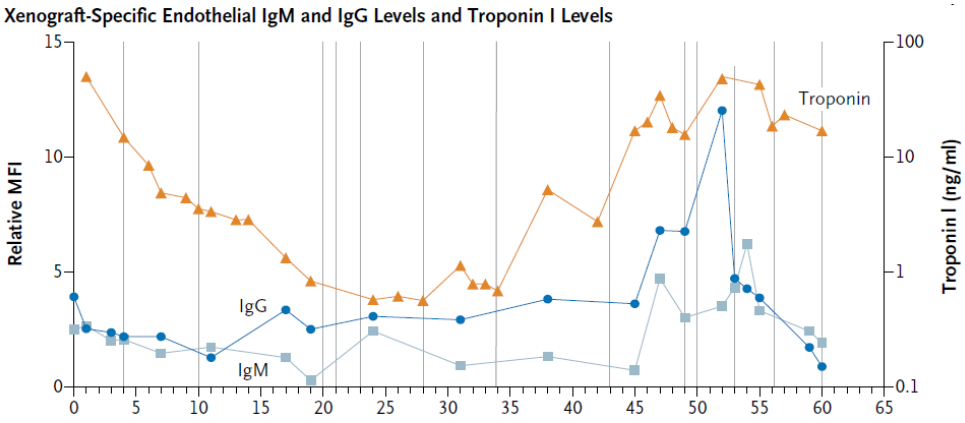
Induction:	Anti-CD20
	ATG
	Berinert (C1-inhib)
	MPS
	Anti-CD40
Maintenance:	Anti-CD40
	MMF
	Steroids

[Griffith et al, n, Nature Med 2022]

Clinical heart xenotransplantation







- Pig heart with **10 gene edits** into a 57yrs old man
- I.S. based on CD40 blockade (**anti-CD40 mAb**)
- The patient was weaned from ECMO, and the xenograft functioned normally
- **Sudden diastolic thickening** and failure of the xenograft occurred **on day 49**
  - Edematous xenograft (nearly 2x weight)
  - scattered myocyte necrosis, interstitial edema, and red-cell extravasation, without evidence of microvascular thrombosis
- **findings not consistent with typical rejection**; **weak IgG, IgM and C4d deposition on d56**
- Studies are under way to identify the mechanisms responsible for these changes **(+ role of pCMV?)**

For the first time, survival of a human being on a porcine xenograft for 60 days

[Griffith et al, NEJM 2022]

First genetically engineered pig-to-human kidney xenotransplants

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

[Montgomery et al, NEJM 2022]

First genetically engineered pig-to-human kidney xenotransplants

Table 1 | Gene editing of pigs for xenotransplantation

Gene name	Edit	Purpose
Aalpha-1,3-galactosyltransferase	Knockout	Protect the pig organ from antibody-mediated damage and hyperacute rejection
Monophospho-N-glycolylneuraminic acid hydroxylase	Knockout	Protect the pig organ from antibody-mediated damage and delayed humoral xenograft rejection
β-1,4 N-acetylgalactosaminyltransferase 2	Knockout	Protect the pig organ from antibody-mediated damage and delayed humoral xenograft rejection
CD55	Transgene	Protect the pig organ from complement-mediated damage
CD46	Transgene	Protect the pig organ from complement-mediated damage
CD59	Transgene	Protect the pig organ from complement-mediated damage
Thrombomodulin	Transgene	Protect the pig organ from coagulation dysregulation
Endothelial protein C receptor	Transgene	Protect the pig organ from coagulation dysregulation and inflammation
CD47	Transgene	Protect the pig organ from macrophage-mediated damage
Hemeoxygenase-1	Transgene	Protect the pig organ from inflammation and apoptosis
Growth hormone	Knockout	Prevent organ growth after xenotransplantation
HLA-E	Transgene	Protect the pig organ from natural killer cell–mediated damage
PERV genes	Knockout	Reduce risk of transmission of pig viruses to humans

Thymo-kidney

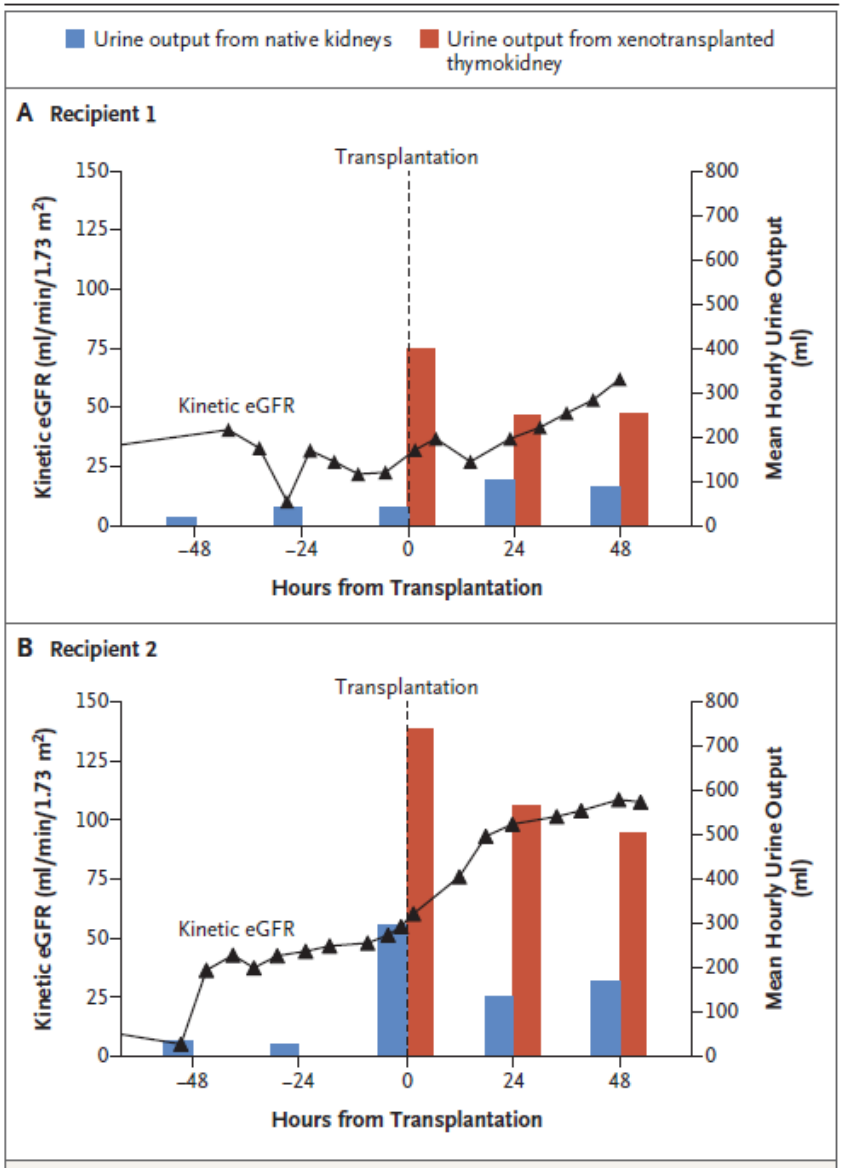
[Montgomery et al, n, Nature Med 2022]

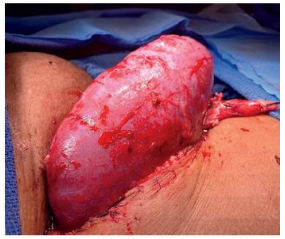
First pig-to-human kidney xenotransplants: immunosuppression

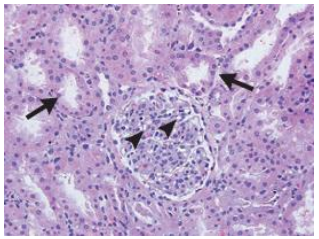
Induction:	no induction
Maintenance:	MMF Steroids

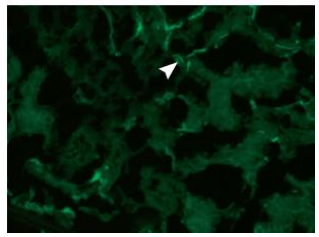
[Montgomery et al, n, Nature Med 2022]

Clinical renal xenotransplantation







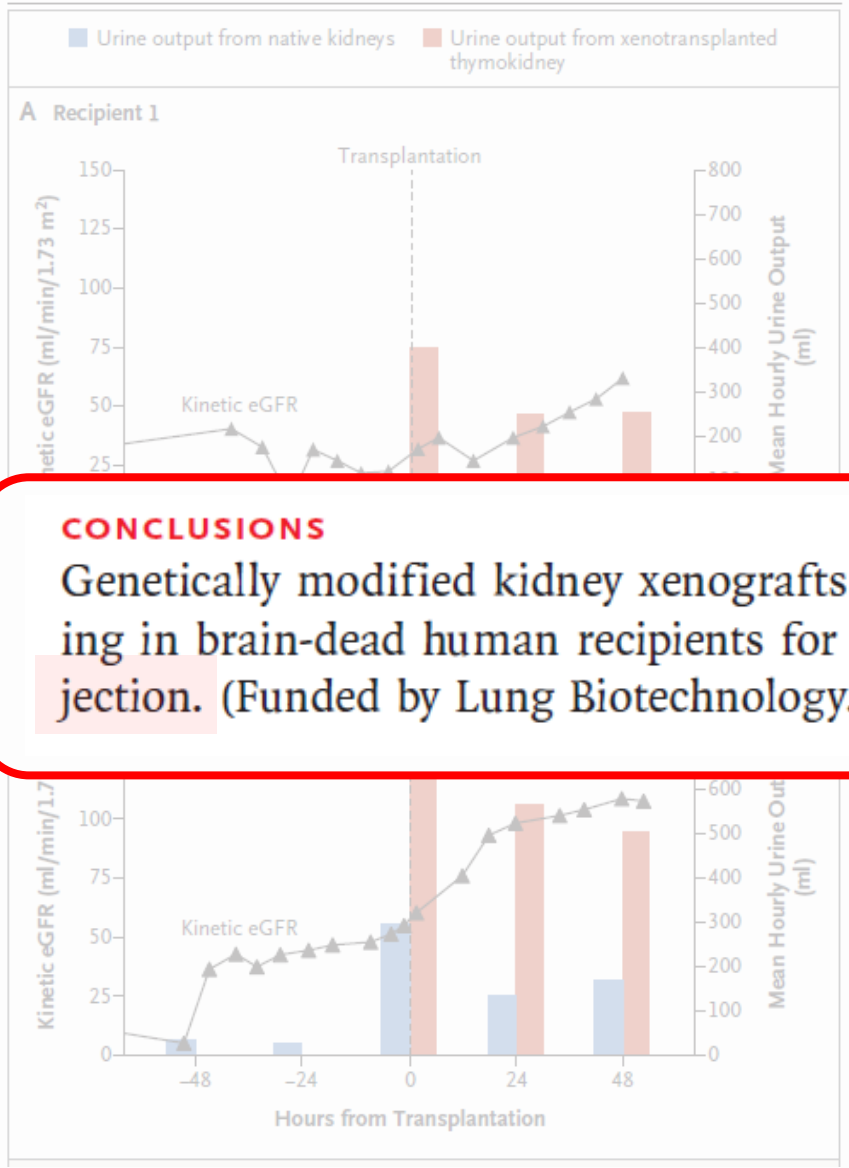


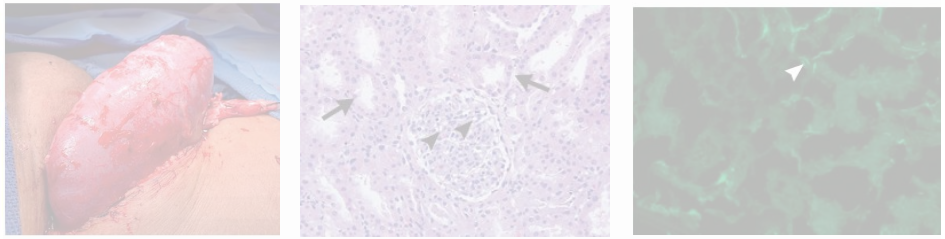
2 brain-dead (**decedent**) xenograft recipients:

- Thymokidneys from GTKO pigs
- I.S: MPS and MMF
- the xenografts remained **pink and well-perfused**, continuing to make urine throughout the 54-hour study.
- **creatinine** levels decreased
- the **eGFR** increased from 23 ml up to 109/min/1.73 m2
- **Biopsies** at 6, 24, 48, and 54 hours revealed **no signs of HAR or AMR**
- **Focal C4 deposition** in pt. 2 (at 54hr)

[Montgomery et al, NEJM 2022]

Clinical renal xenotransplantation





2 brain-dead (decedent) xenograft recipients:

- Thymokidneys from GTKO pigs
- I.S: MPS and MMF

CONCLUSIONS

Genetically modified kidney xenografts from pigs remained viable and functioning in brain-dead human recipients for 54 hours, without signs of hyperacute rejection. (Funded by Lung Biotechnology.)

- the **eGFR** increased from 23 ml up to 109/min/1.73 m2
- **Biopsies** at 6, 24, 48, and 54 hours revealed **no signs of HAR or AMR**
- **Focal C4 deposition** in pt. 2 (at 54hr)

[Montgomery et al, NEJM 2022]

Conclusions

- Recent progress has increased our understanding of the fundamental mechanisms underlying xenograft failure.
- Physiological incompatibilities between pigs and primates have been reported. However, none of these is perceived as unsurmountable (life-supporting pig-to-primate studies).
- At this stage no transmission of zoonoses to man has been reported in human recipients of porcine xenografts.
- Genetic engineering of the donor pig will enable the production of immunologically and physiologically-compatible organs with a better safety profile.
- The first genetically engineered pig organs have been transplanted into humans; more data is badly needed to understand whether xenotransplantation using pig donors is safe and may represent a solution to the shortage of human organs, tissues and cells