



DEPARTMENT OF CARDIO-
THORACIC AND VASCULAR
SCIENCES AND PUBLIC HEALTH



UNIVERSITÀ
DEGLI STUDI
DI PADOVA

Xenotransplantation: where do we stand?

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Definizione di xenotrapianto (Consiglio d'Europa 2003)

Per xenotrapianto si intende qualsiasi procedura di **trapianto**, impianto o infusione in un ricevente umano di:

- organi, tessuti o cellule vivi **di provenienza animale**

ma anche di

- fluidi corporei, cellule, tessuti o organi **umani entrati in contatto *ex vivo*** con cellule, tessuti o organi **animali vivi**

Questa definizione non include altri prodotti di derivazione animale regolati come device [**valvole cardiache**], farmaci [**insulina**] o biologics (**anticorpi monoclonali** nel tumore)

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

The great progress of the
xenotransplantation science

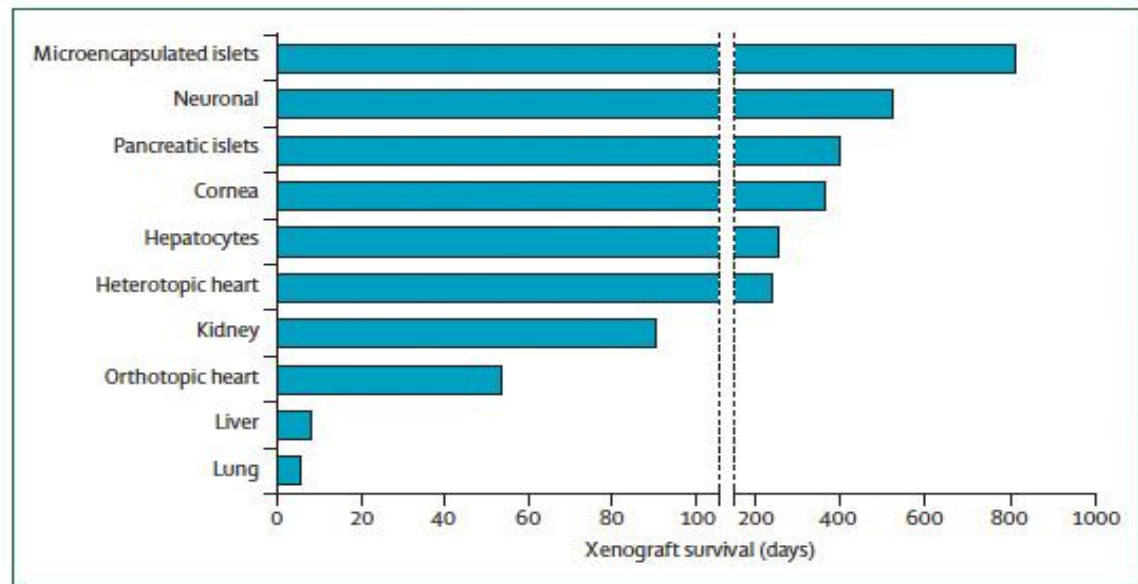
Xenotransplantation preclinical research as of **2012**

THE LANCET

www.thelancet.com

Clinical xenotransplantation: the next medical revolution?

Best preclinical
results reported
as of 2012



Xenotransplantation

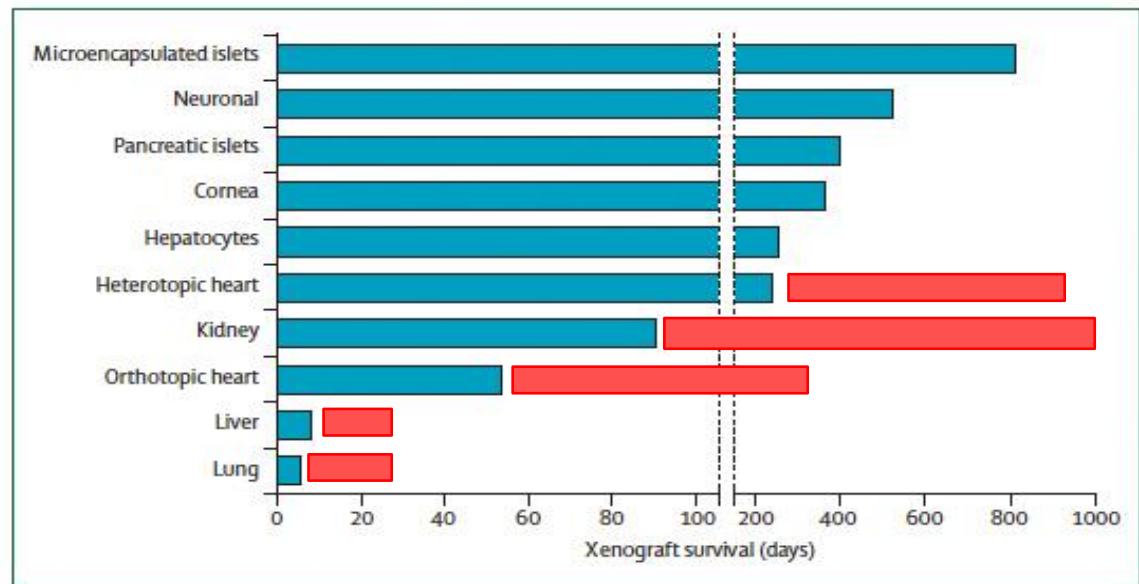
preclinical research as of 03/2023

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
- Different immunosuppressive approach
- Genetic engineering of the pig

Genetic engineering of the pig: Possible targets of intervention

- Complement regulation (CD55, CD46, CD59)
- Immunogenicity (α GALT-KO)
- Immunomodulation (CTLA4Ig, CIITA-DN, SIRP α)
- 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

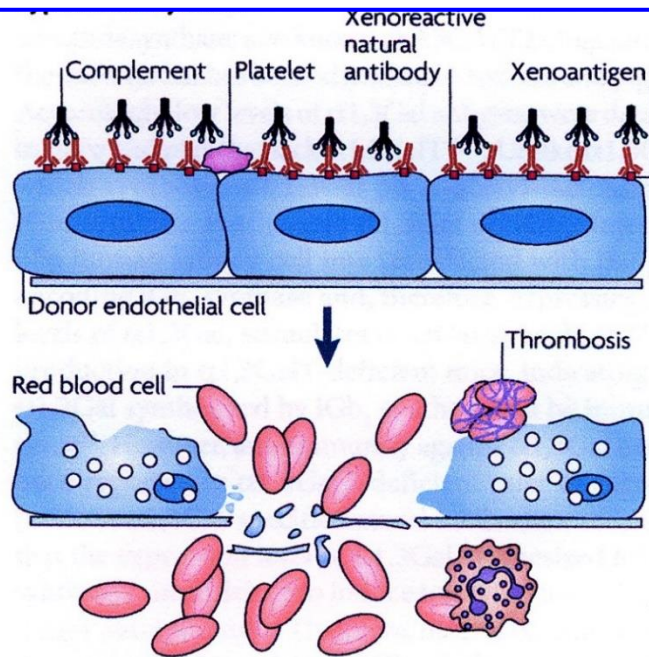
- Immunological barriers
- Physiology
- Biosafety
- Ethics and regulations

Xenotransplantation: the immunological barriers

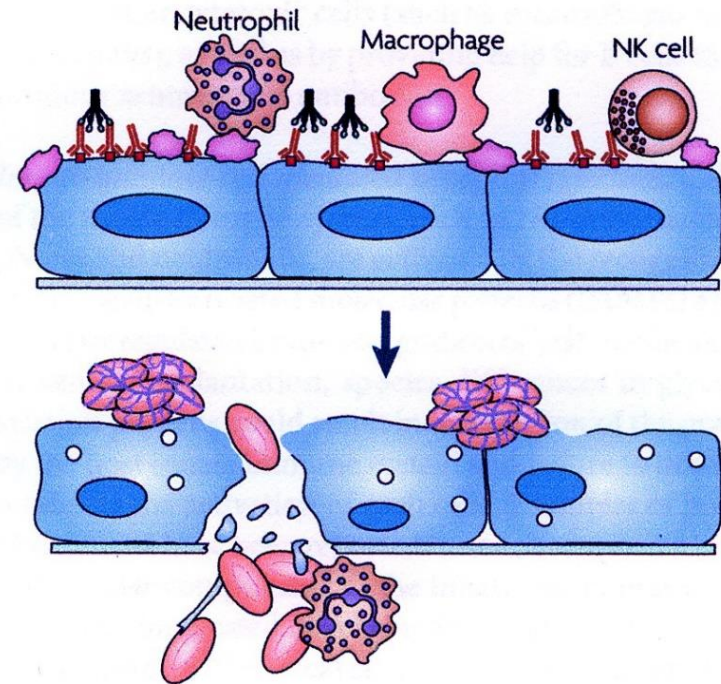
- Antibody-mediated rejection
 - Hyperacute rejection (HAR)
 - Acute humoral xenograft rejection (AHXR)
- Cell-mediated rejection
- Chronic rejection
- Natural immunity

Humoral rejection of a xenograft

Hyperacute



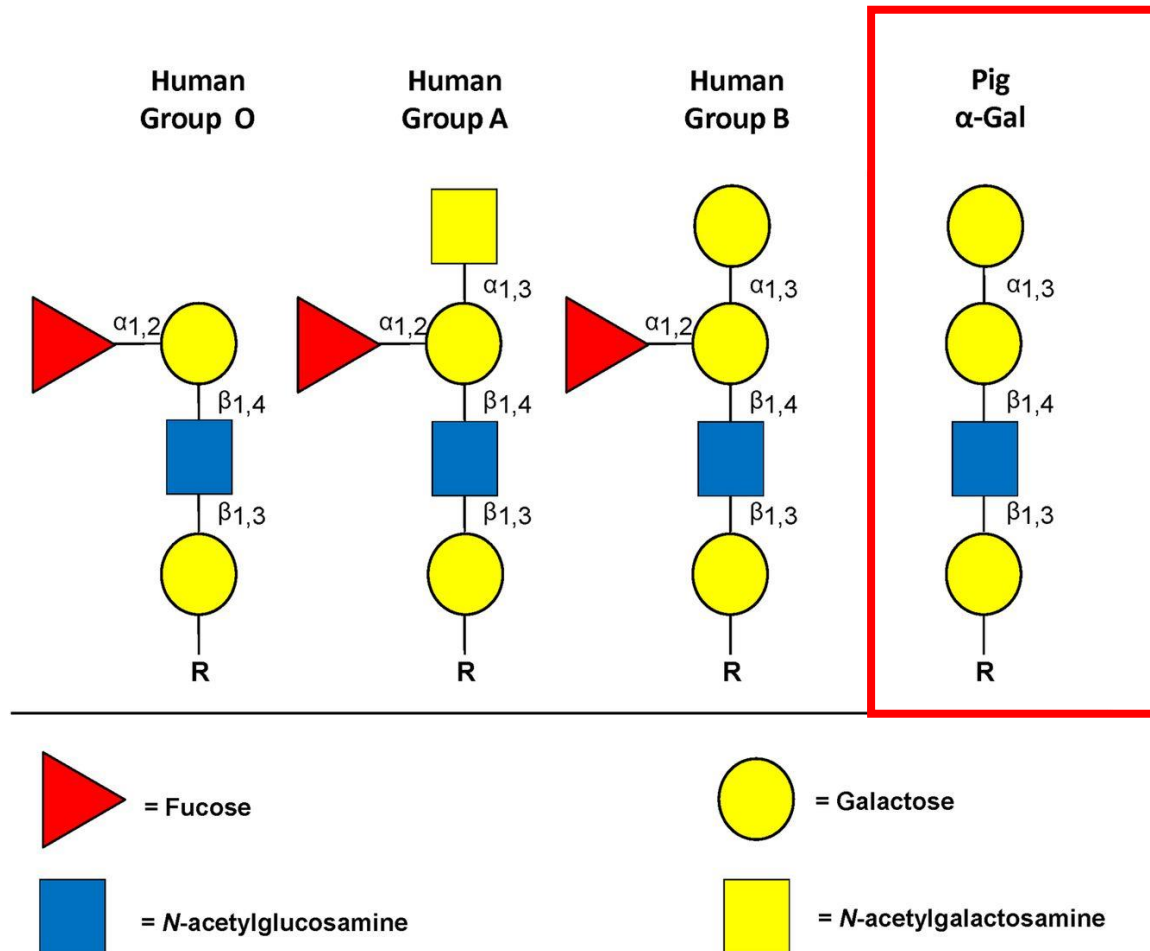
Acute



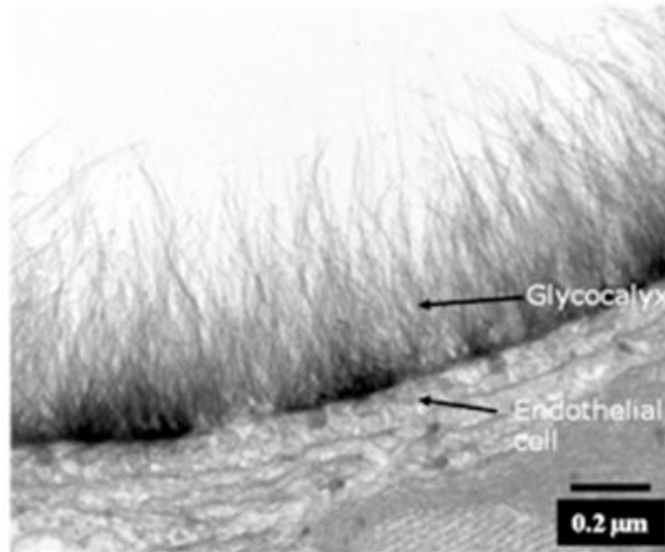
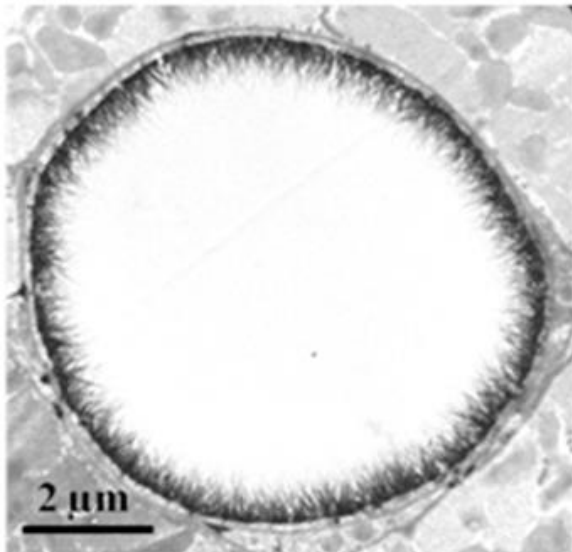
- Occurs within minutes to hours
- Mediated by preexisting antibodies (e.g., anti- α Gal)

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

Xenograft immunogenicity: the importance of the sugars



Xenograft immunogenicity: new aspects

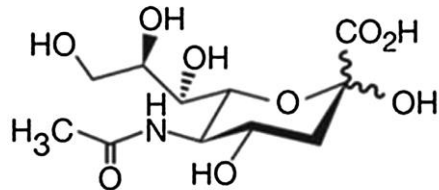


<http://www.hubrecht.eu/research/dekoning/research.html>

Xenograft immunogenicity: recent aspects

“Human”

N-Acetylneuraminic acid (Neu5Ac)

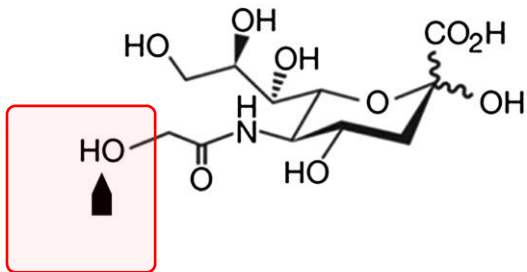


CMP-Neu5Ac
Hydroxylase



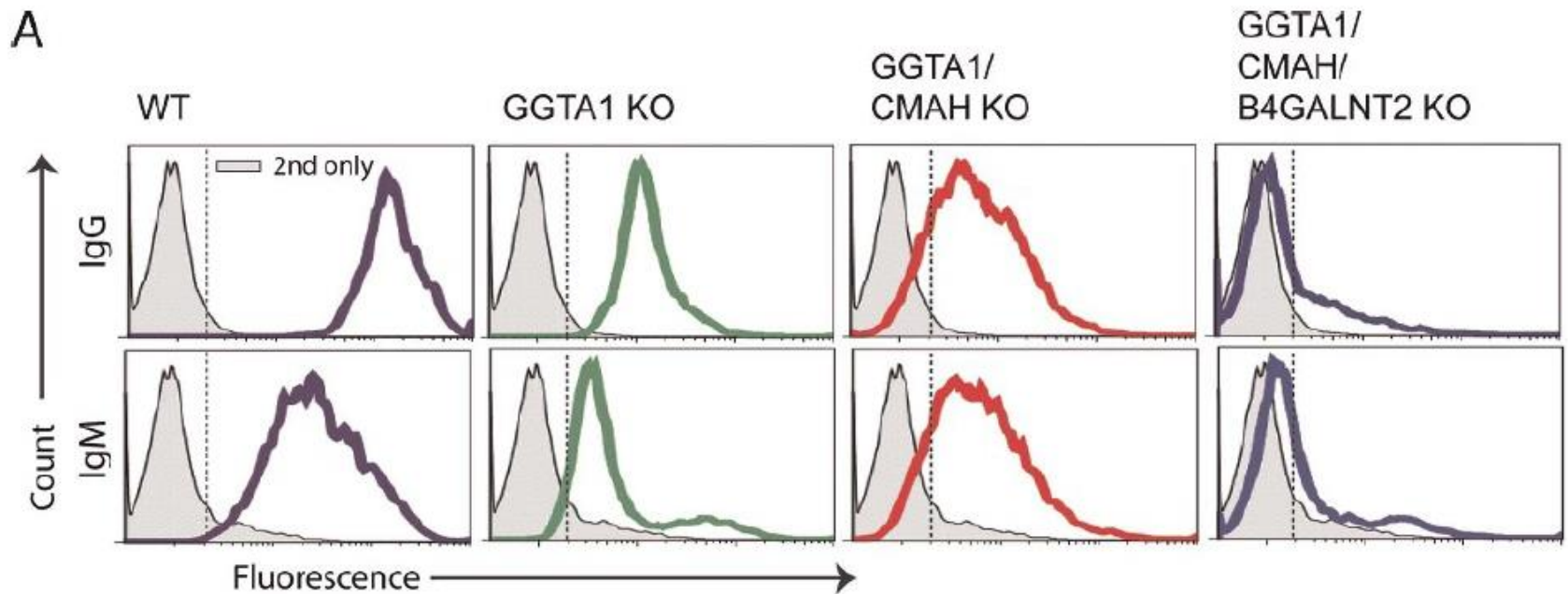
“Non-Human”

N-Glycolylneuraminic acid (Neu5Gc)



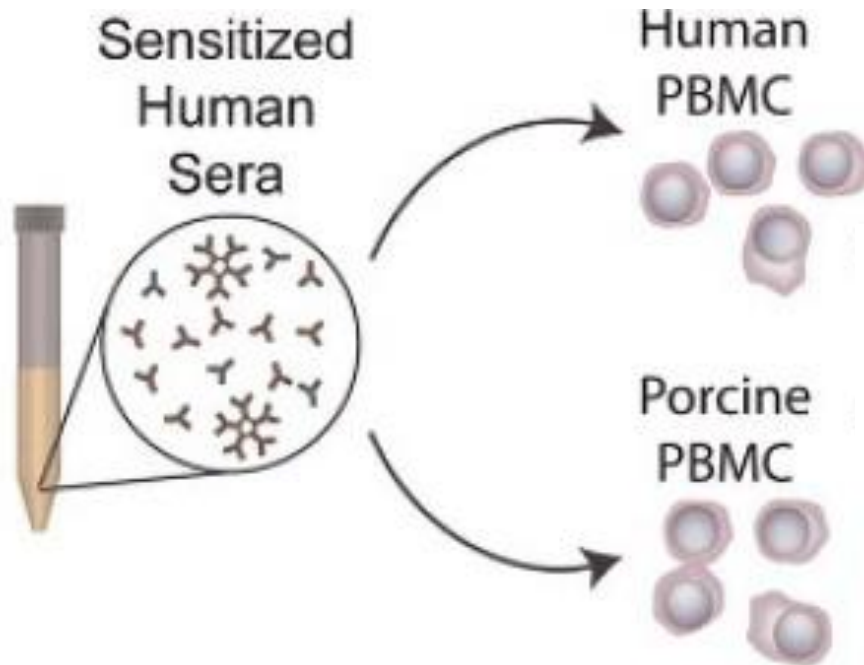
- Sialic acids (Sias) are sugars terminating the glycan chains of glycoproteins and glycolipids.
- N-acetylneuraminic acid (Neu5Ac) and its hydroxylated form Neu5Gc are the two major Sias in mammals
- However, the CMP-Neu5Ac hydroxylase (CMAH) is present in most mammals [including pig] but NOT IN HUMANS.
- As a consequence, Neu5Gc residues are immunogenic in man

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



[Martens et al, Transplantation 2017]

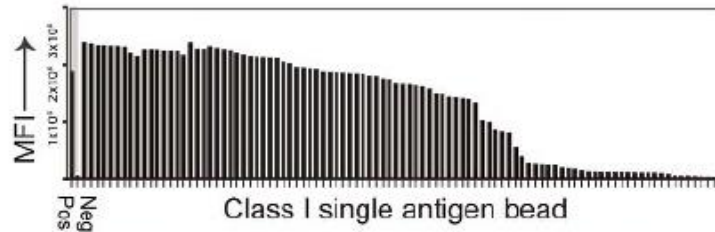
Human anti-pig IgG cross-react with class I human leukocyte antigens



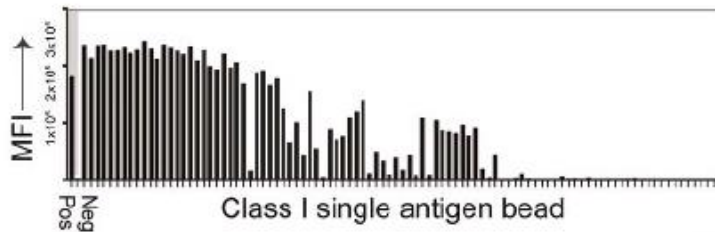
[Martens et al, Transplantation 2017]

Human anti-pig IgG cross-react with class I HLA molecules

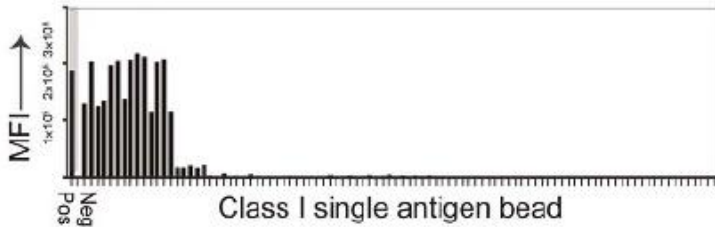
C Intact human serum HLA single antigen Class I



E Human eluted serum HLA single antigen Class I



G Porcine eluted serum HLA single antigen Class I



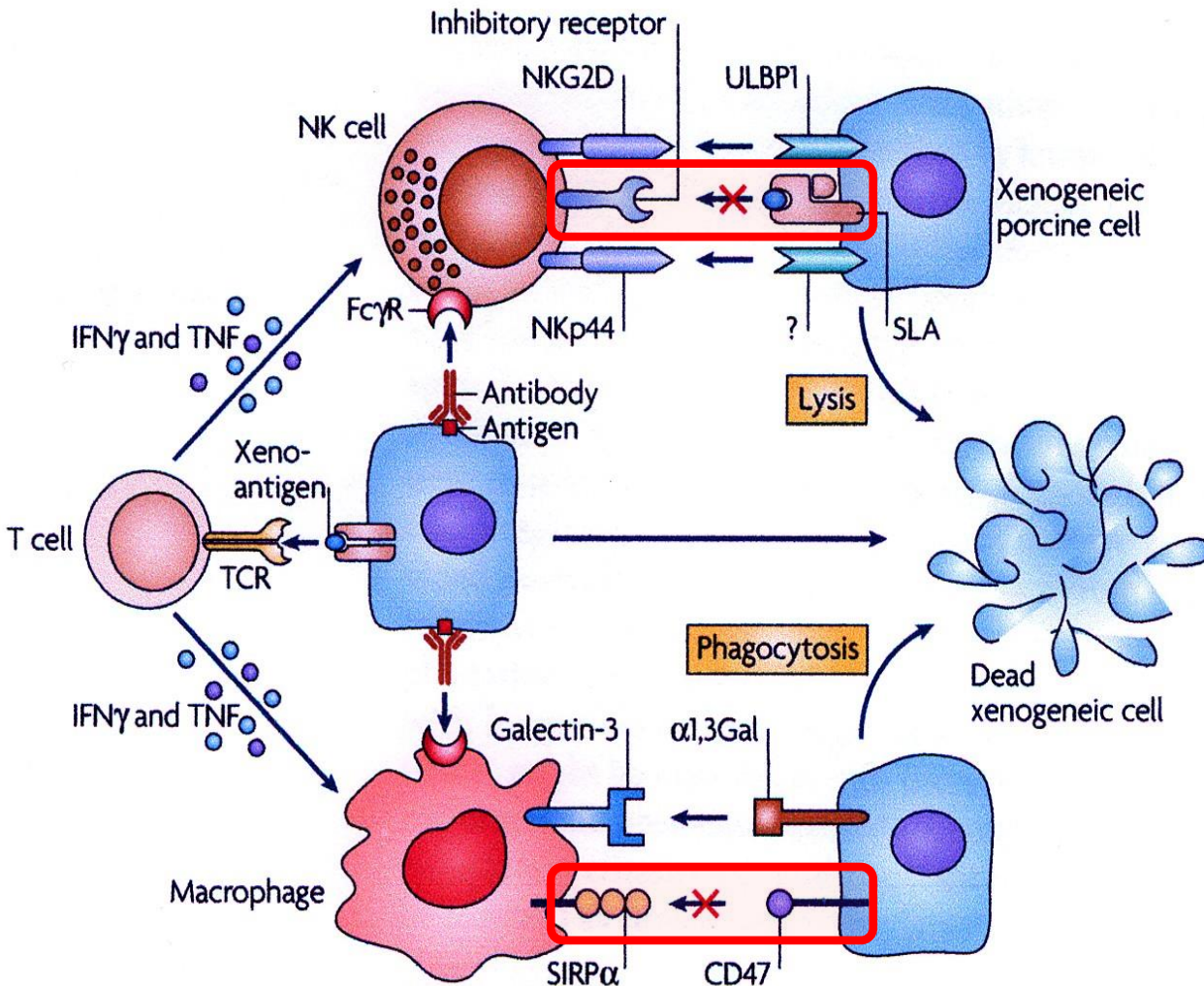
Very high MFI!

[Martens et al, Transplantation 2017]

Cell-mediated rejection

Involves:

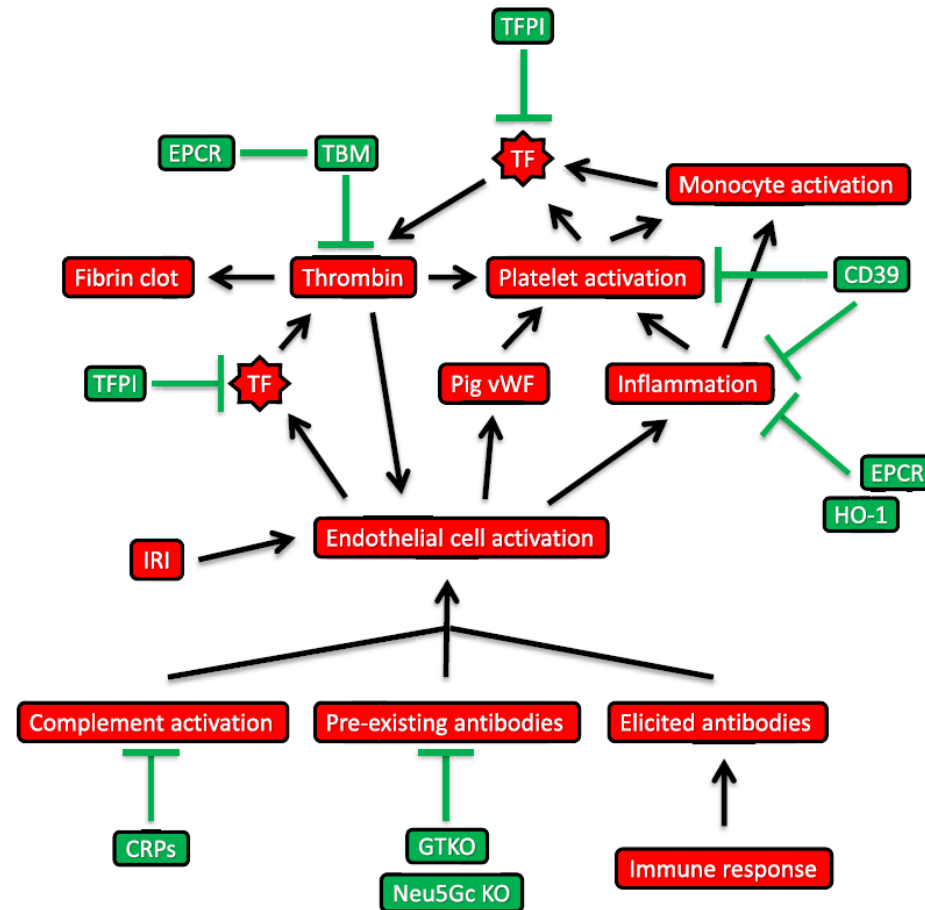
1. T-Lymphocytes
2. NK
3. Macrophages
4. B-lymphocytes



Clinical Xenotransplantation: Key points to address

- Immunology
- **Physiology**
- Biosafety
- Ethics and regulations

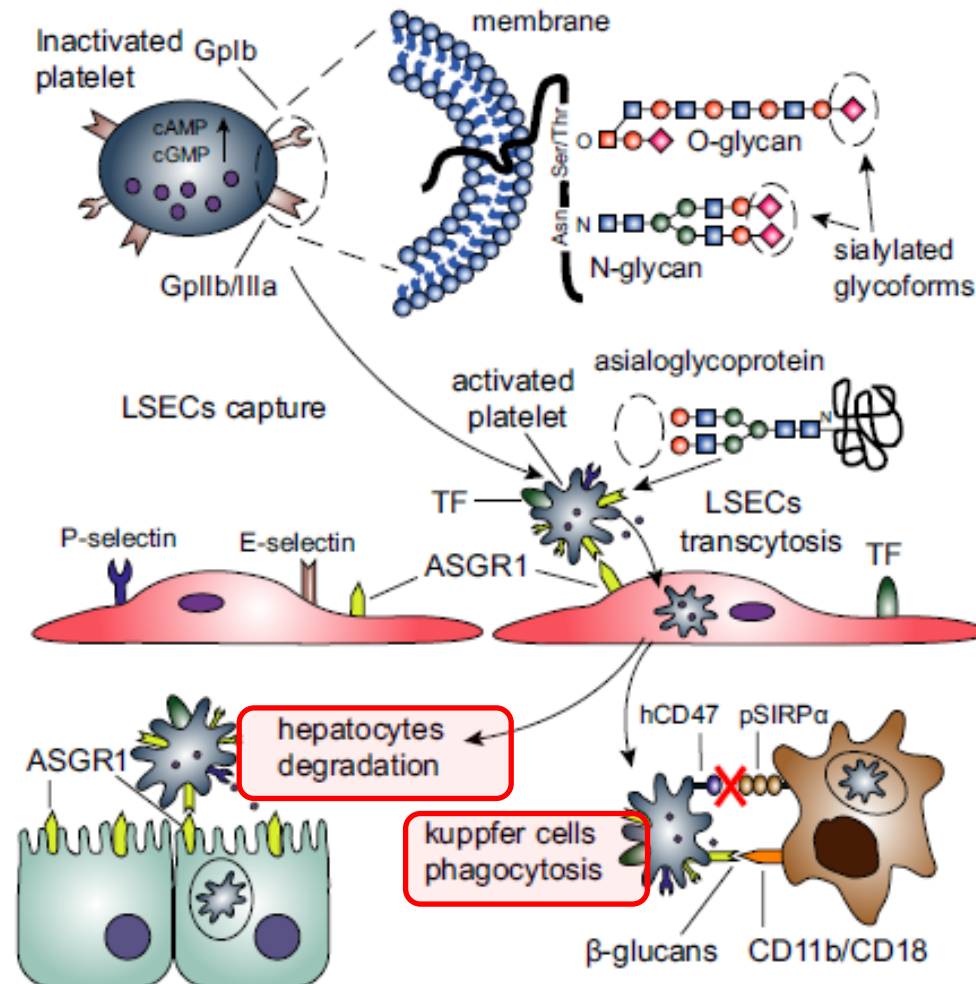
Coagulation dysfunction



Leads to:

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

Thrombocytopenia



Moving towards clinical xenotransplantation: Key points to address

- Immunology
- Physiology
- **Biosafety**
- Ethics and regulations

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**)

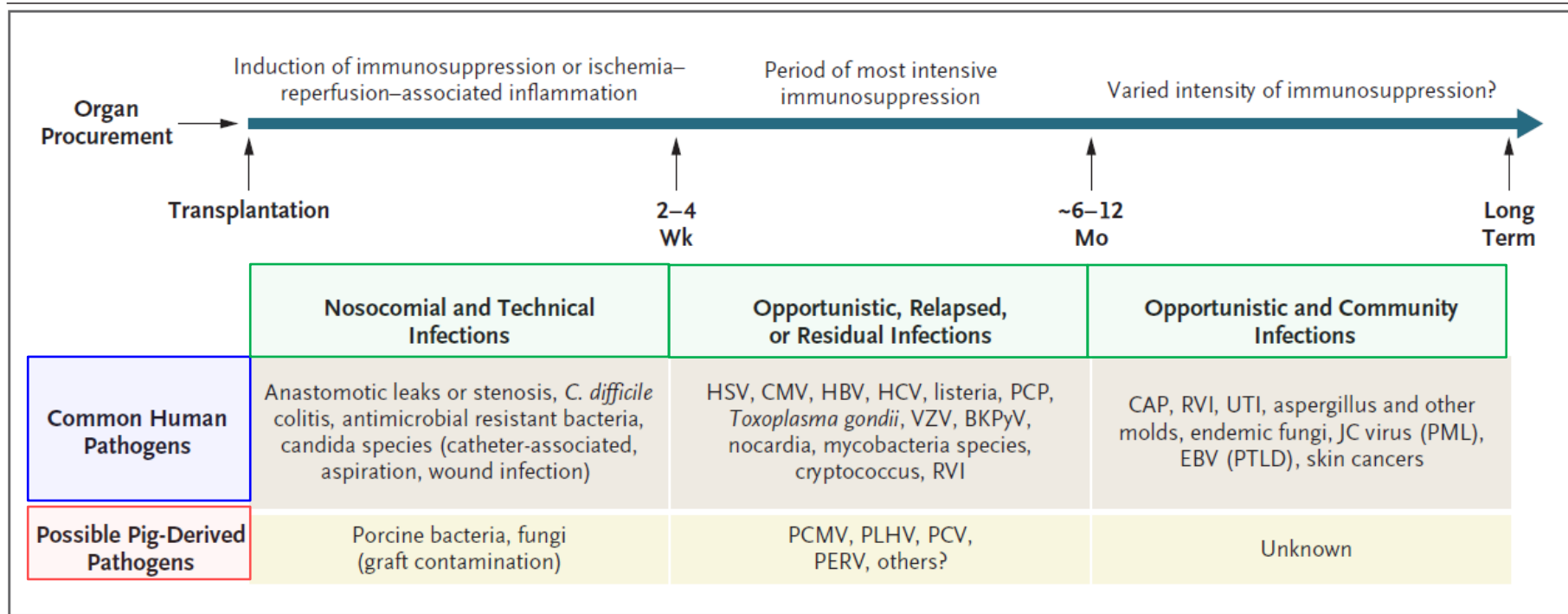
Risk of infection following transplantation (II)

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

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	<i>Toxoplasma gondii</i> , 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

Timeline of infections after xenotransplantation



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).

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 Otto²

Xenotransplantation and PERV zoonoses (II)

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

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 Diatrax Otsuka Limited, Auckland, New Zealand

^c Otsuka Pharmaceutical Factory Inc., Naruto, Japan

^d Hospital Eva Peron de San Martín, 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

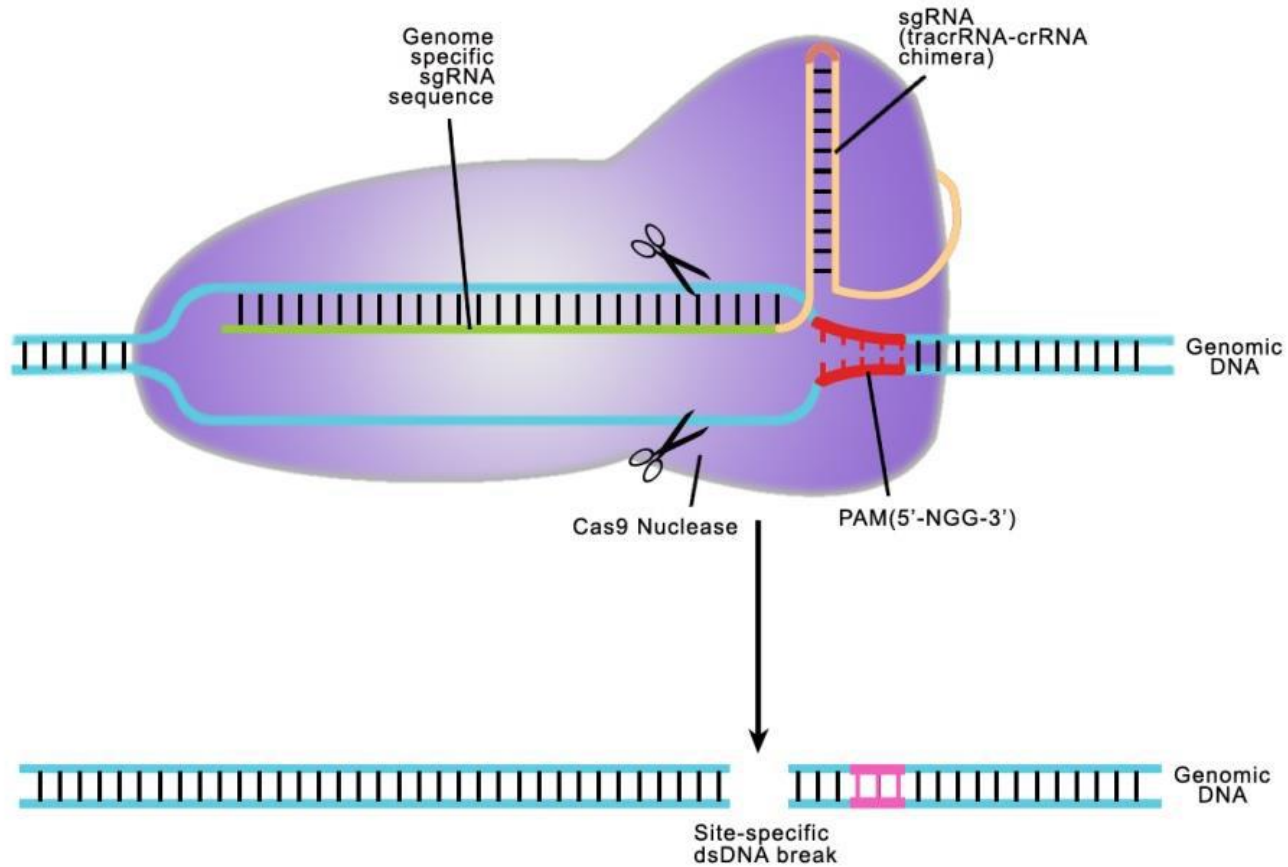
Antimicrobial Agents and Chemotherapy
Volume 44, Issue 12, 1 December 2000, Pages 3432-3433
<https://doi.org/10.1128/AAC.44.12.3432-3433.2000>

Antiviral Agents

Antiretroviral Agents Inhibit Infection of Human Cells by Porcine Endogenous Retroviruses

S. K. Powell¹, M. E. Gates¹, G. Langford², M.-L. Gu¹, C. Lockey¹, Z. Long¹, and E. Otto^{1,*}

The CRISPR/Cas9 technology



[Fu et al, Nat. Biotechnology 2014]

The CRISPR/Cas9 technology

Inactivation of porcine endogenous retrovirus in pigs using CRISPR-Cas9



[Niu et al, Science 2017]

Coronaviruses

- There is no evidence that **swine carry coronaviruses** that are pathogenic for humans.
 - Coronaviruses in swine are **largely enteric pathogens**.
 - Six coronaviruses cause clinically significant infection in pigs.
 - **Porcine respiratory** coronavirus causes **mild** respiratory infections in swine.
- pigs could not be infected intranasally with **Covid-19**; more than 3 weeks after infection, all animals were negative for viral RNA, and no pigs had seroconversion

Animal husbandry for xenotransplantation: The “designated pathogen-free” 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

Animal husbandry for xenotransplantation: The “designated pathogen-free” status

- **microbiologic cultures**
- **serologic assays**
(past infections, often latent in normal animals
[e.g., PCMV infection])
- **quantitative nucleic acid assays**
(actively replicating pathogens which may
emerge with immunosuppression).



**Sentinel
animals**

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

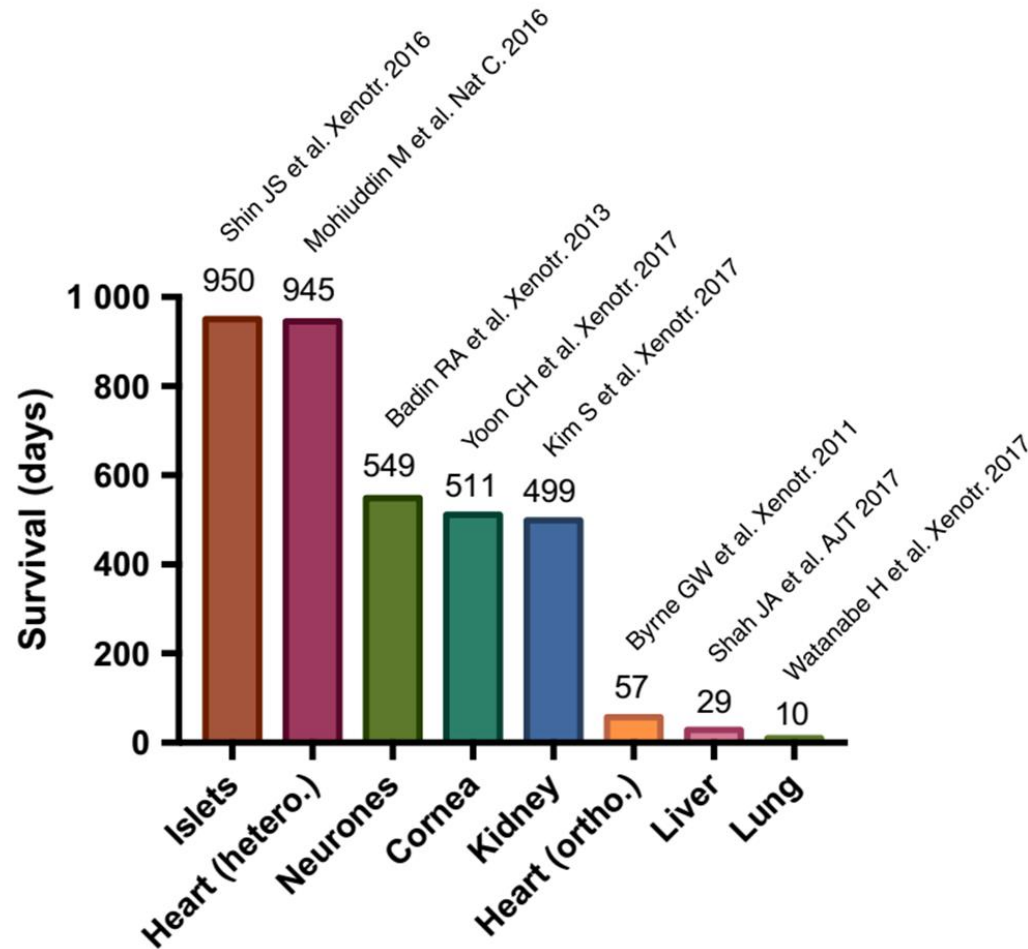
Virus	Testing Method†
PERV-A, -B, -C, -AC	QNAT, antibody-based tests (serologic testing, ELISA, Western blot testing)
PLHV-2	QNAT
PCMV	QNAT, serologic testing
HCMV, according to risk status	QNAT
Human EBV, according to risk status	QNAT
BK polyomavirus (in kidney recipients), per protocol	QNAT
Pig-cell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

Yet unidentified pathogens (?)

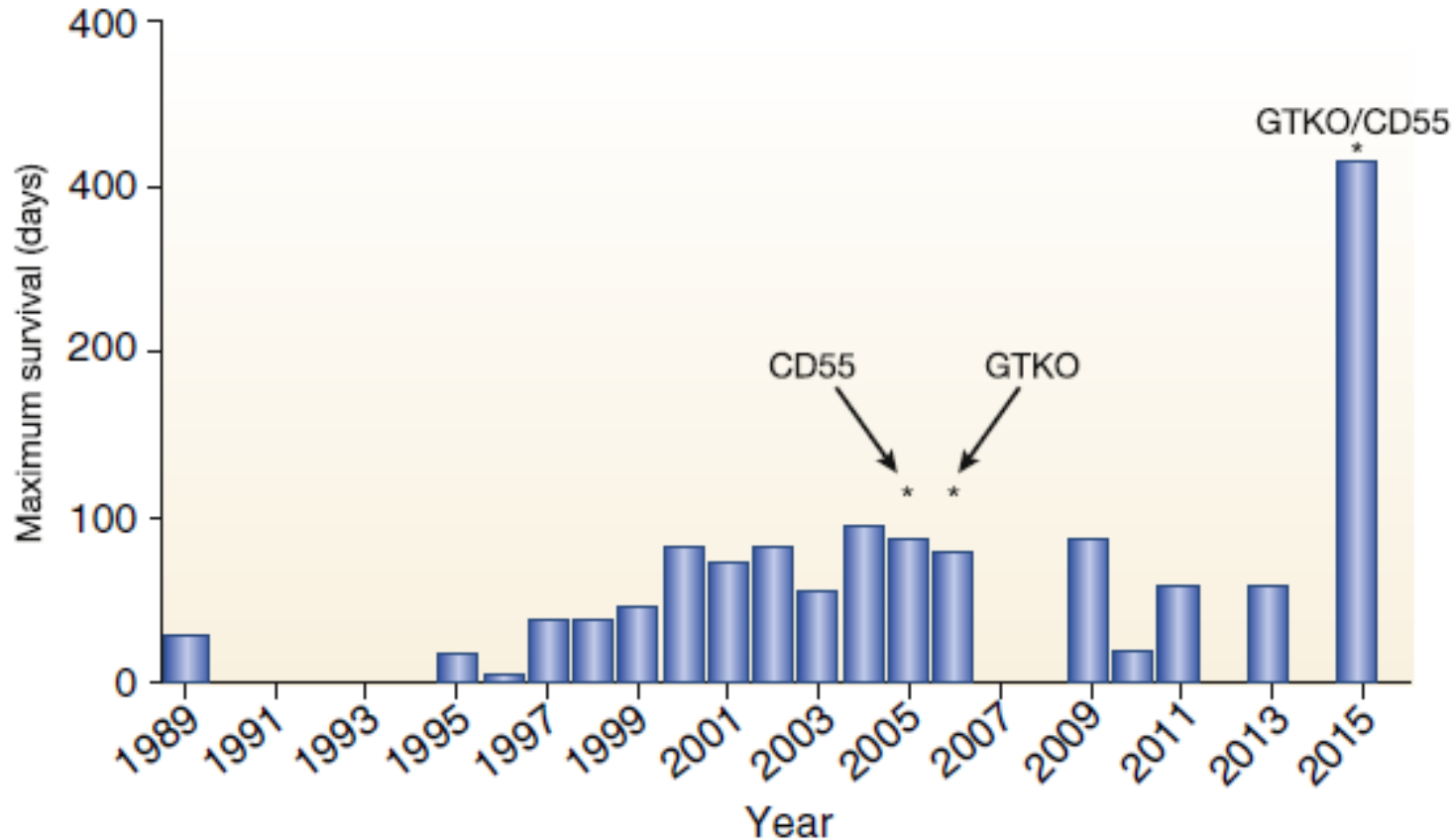
[Fishman, NEJM 2022]

Best results in pig-to primate
xenotransplantation

Best results in pig-to primate xenotransplantation

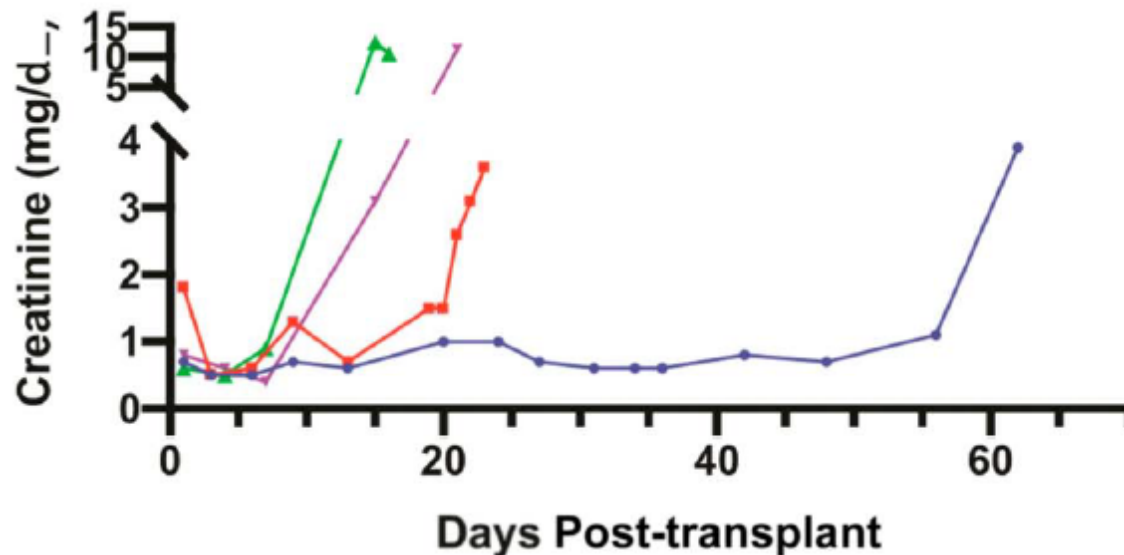
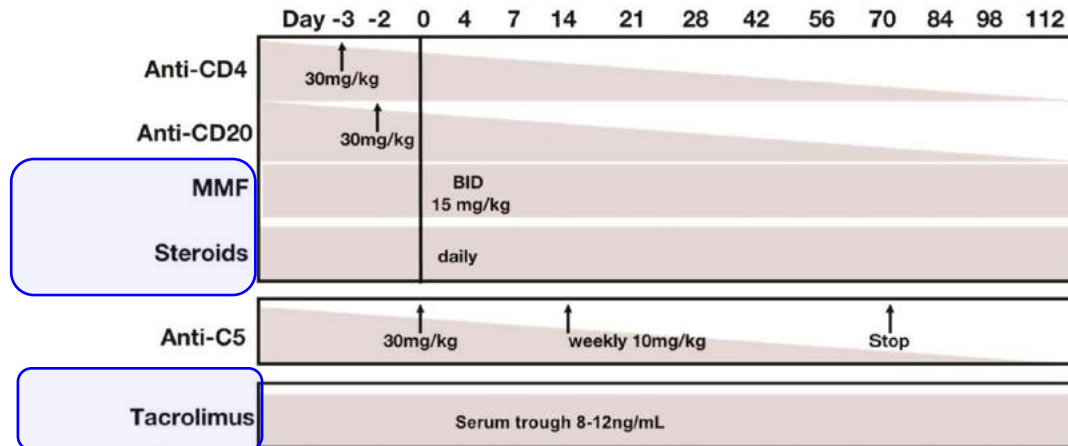


Progress in pig-to-primate Kidney Xenotransplantation

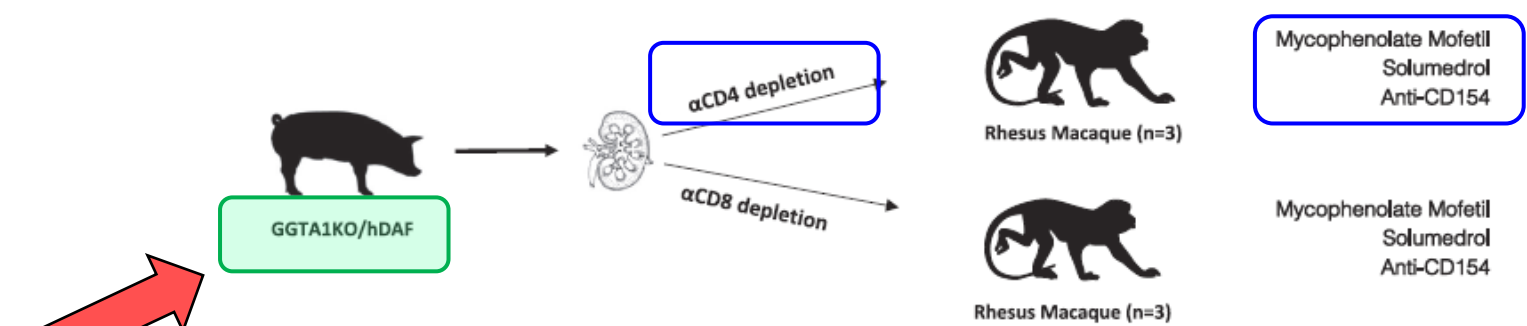


[Adapted from Wijkstrom et al, *Kidney International* 2017]

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



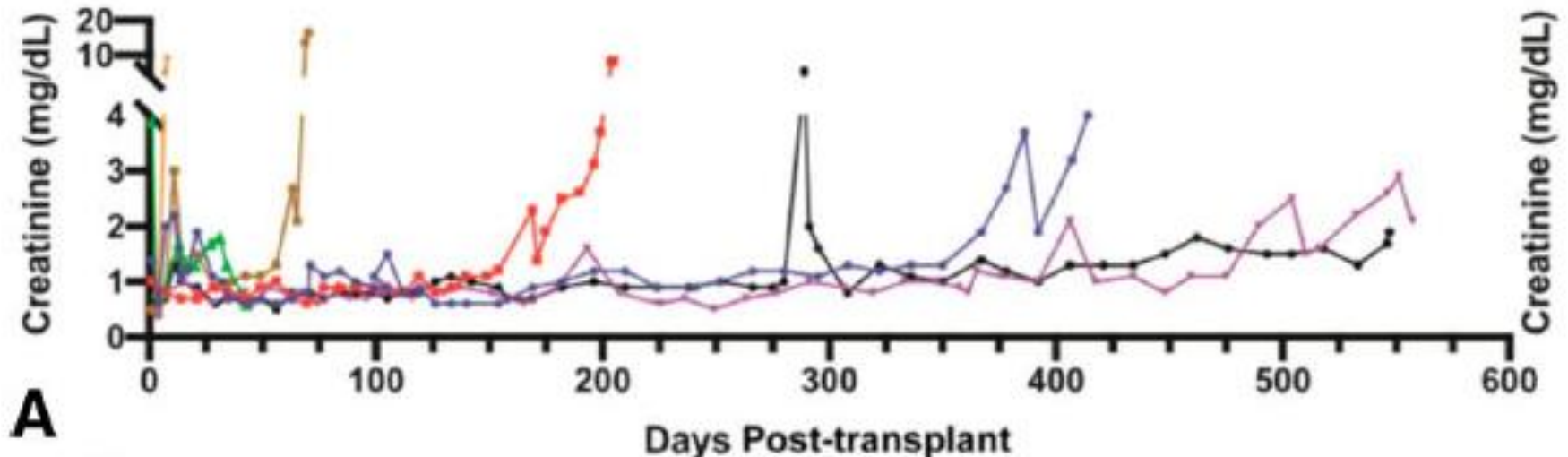
Longterm xenograft survival reproducibly achievable with low anti-pig antibody, anti-CD4 and antiCD154



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

[Kim et al, AJT 2019]

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

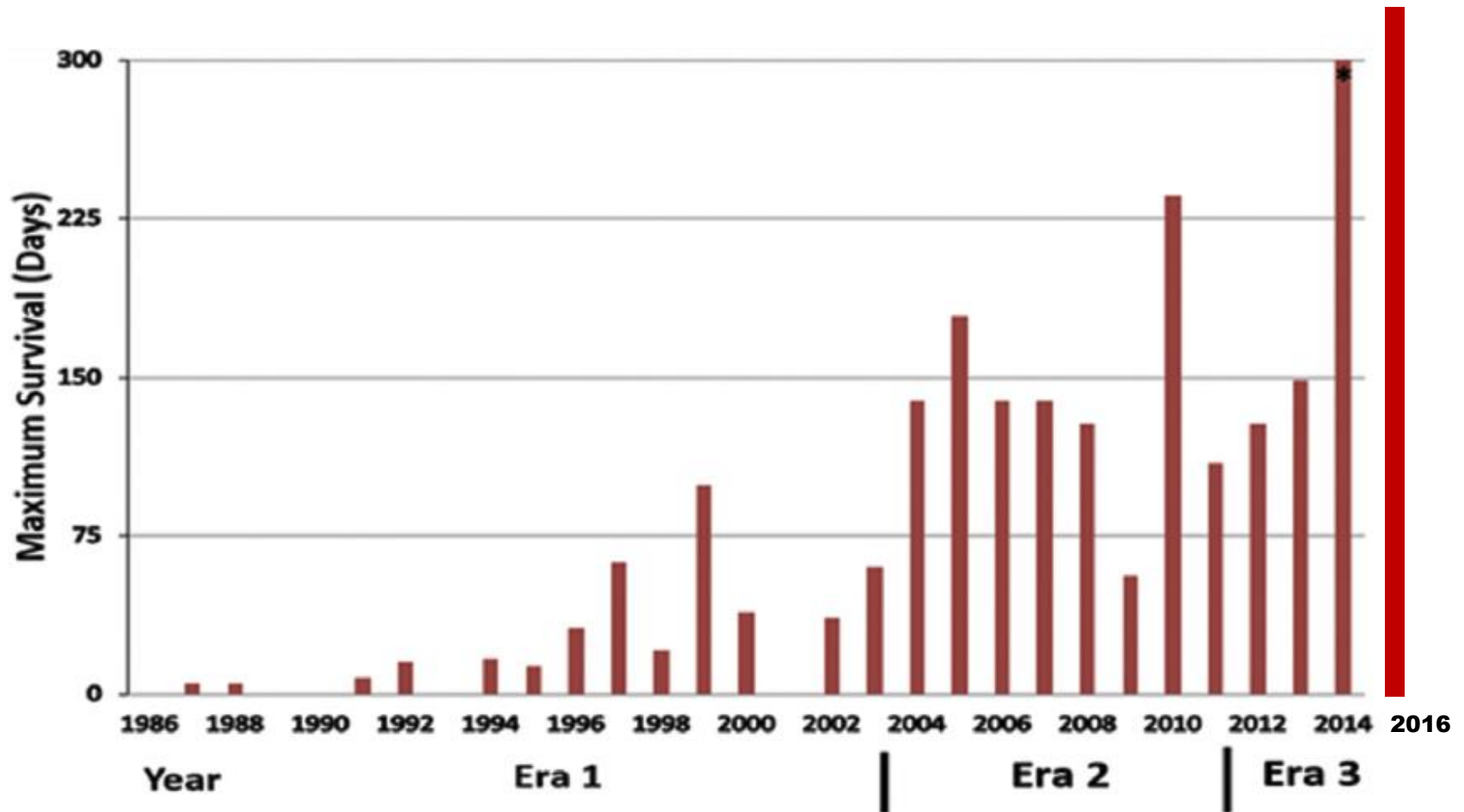


Longest survival reported: > 4 years (Adams at 2023)

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.

Progress in Heterotopic pig-to-primate cardiac Xenotransplantation



Longest survival reported: 945 days (2016)

Primate studies with longterm Cardiac xenograft survival

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

Primate studies with longterm Cardiac xenograft survival

Donors: GTKO/CD46/TBM

Recipients: Baboons

Immunosuppression:

Induction: Anti-CD20

ATG

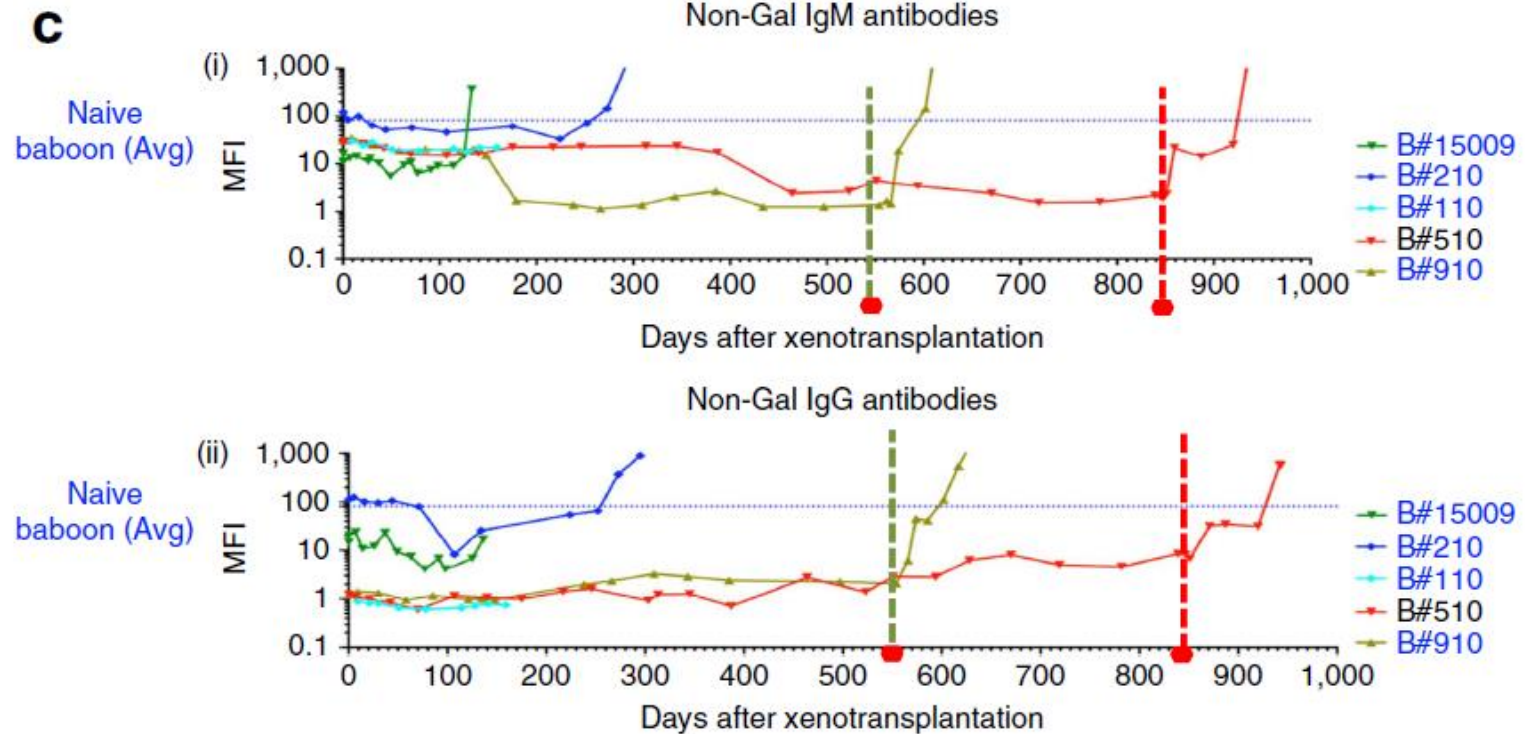
CVF

Maintenance: Anti-CD40

MMF/Steroids

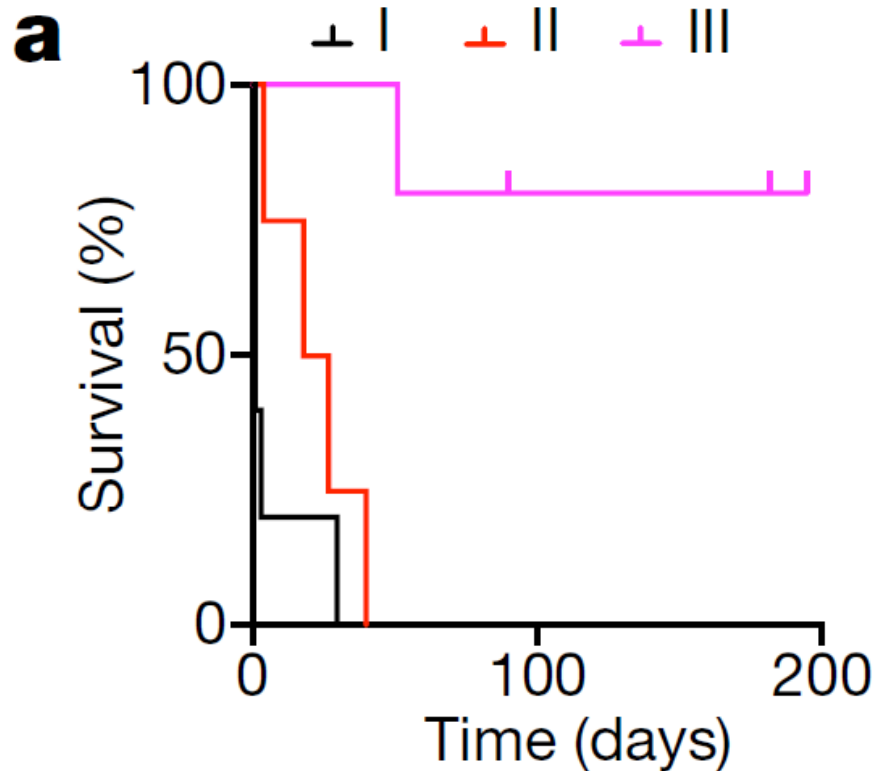
[Mohiuddin et al, Nature Com 2016]

Longterm Cardiac xenograft survival: antibody titres and outcomes

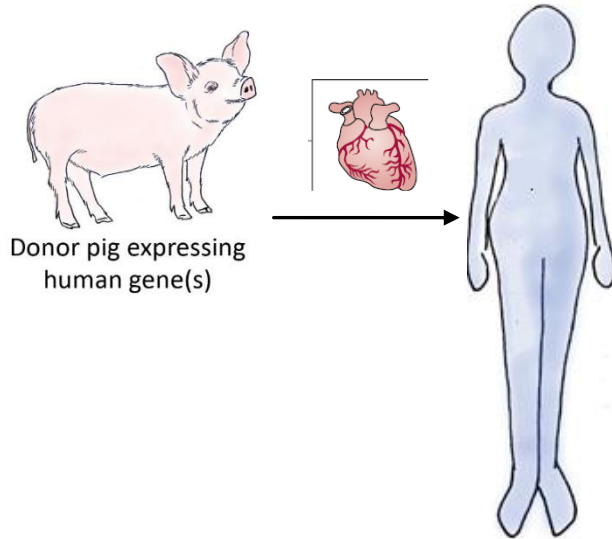


...reduction of the α CD40 Mab dose resulted in recrudescence of anti-pig antibodies and graft failure.

Consistent success in life-supporting porcine cardiac xenotransplantation



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 atrioventricular node between species might potentially result in increased arrhythmogenicity of a pig heart
 - Not evidenced in pre-clinical studies

Clinical Xenotransplantation: Key points to address

- Immunology
- Physiology
- Biosafety
- Ethics and regulations

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

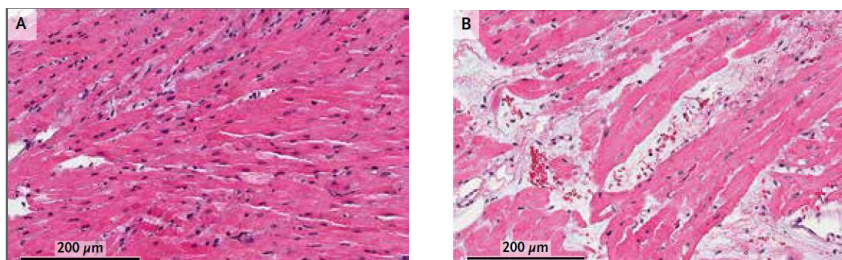
Table 1 | Gene editing of pigs for xenotransplantation

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

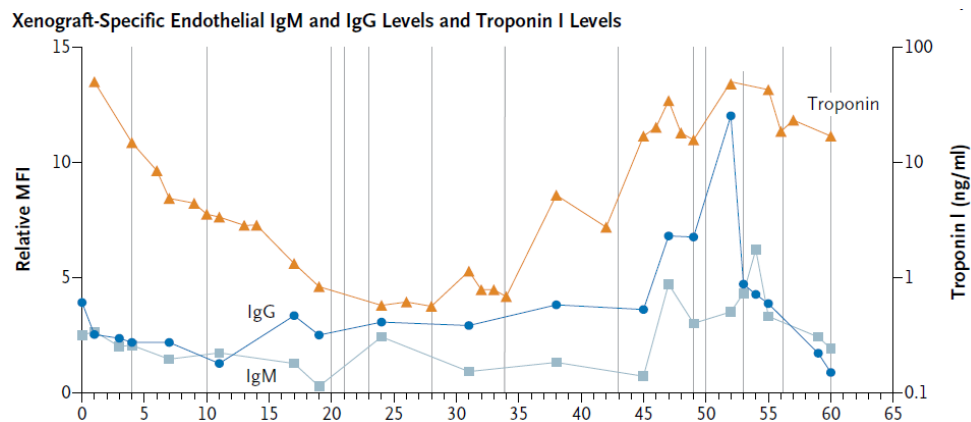
First pig-to-human heart xenotransplant: immunosuppression

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

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

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

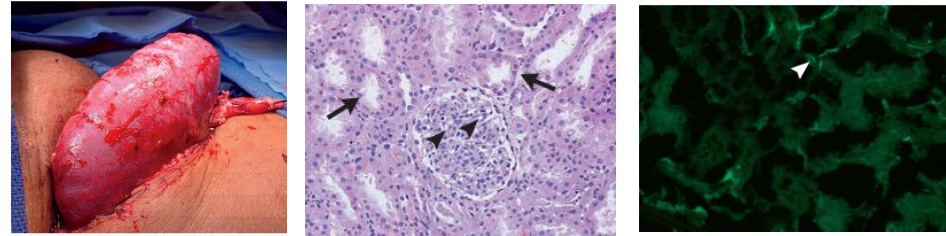
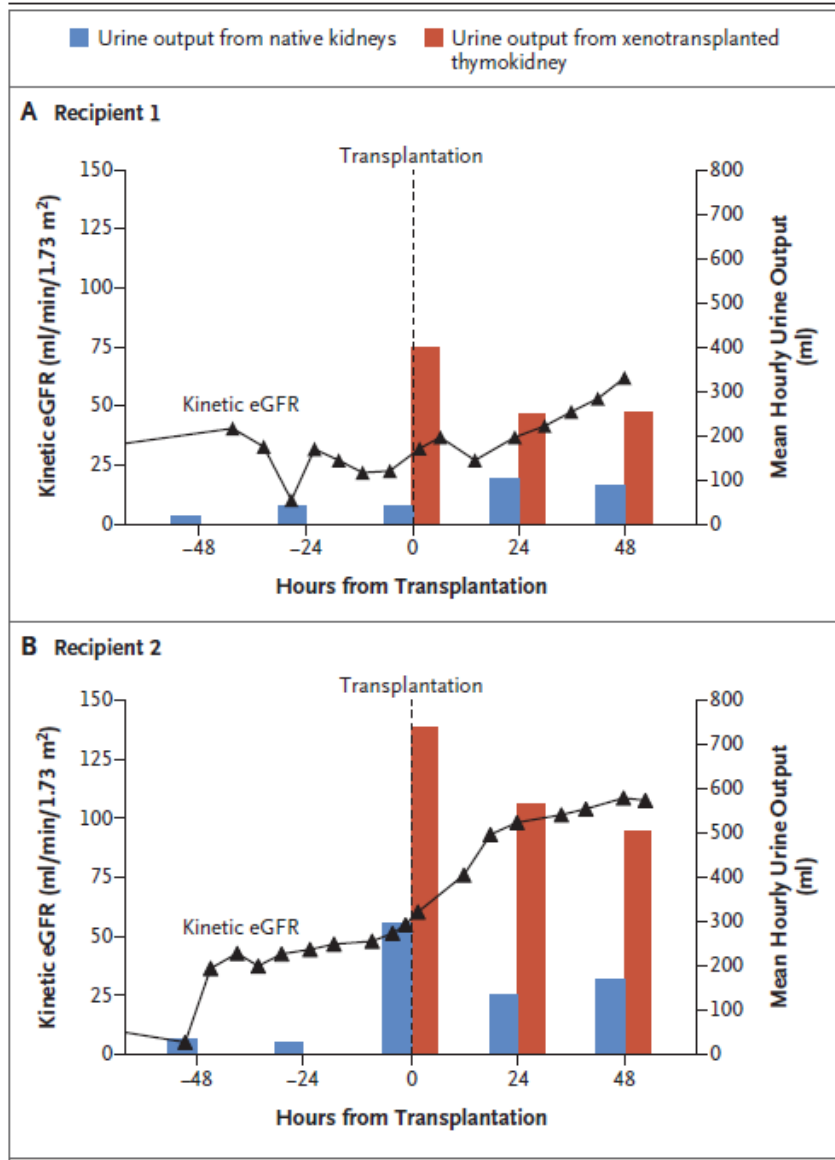
First pig-to-human kidney xenotransplants: immunosuppression

Induction: no induction

Maintenance: MMF
Steroids

[Montgomery et al, n, Nature Med 2022]

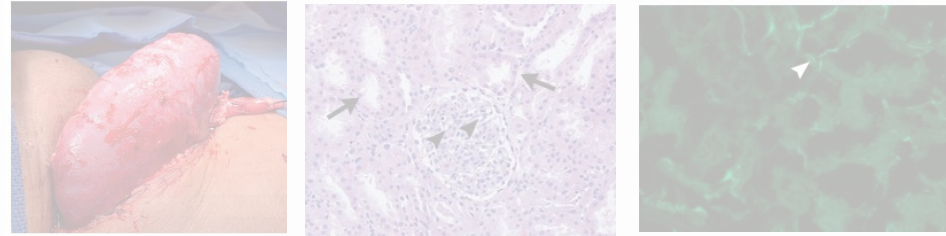
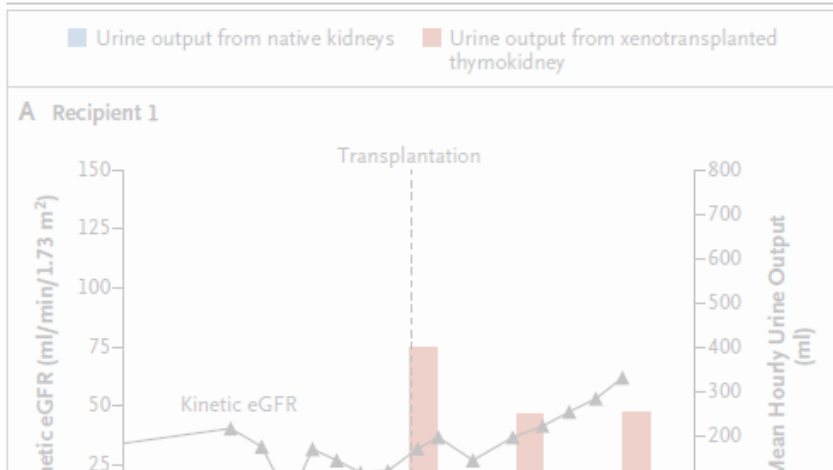
Clinical renal xenotransplantation



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/ml/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)

Clinical renal xenotransplantation

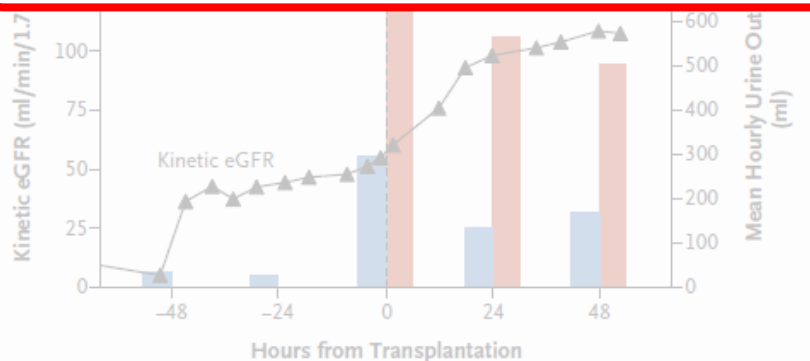


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CONCLUSIONS

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.)



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[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.
- First genetically engineered pig organs have been transplanted into humans and more data is really needed to understand whether xenotransplantation using pig donors is safe may represent a solution to the shortage of human organs and cells