Zoonosenrisiken der Xenotransplantation

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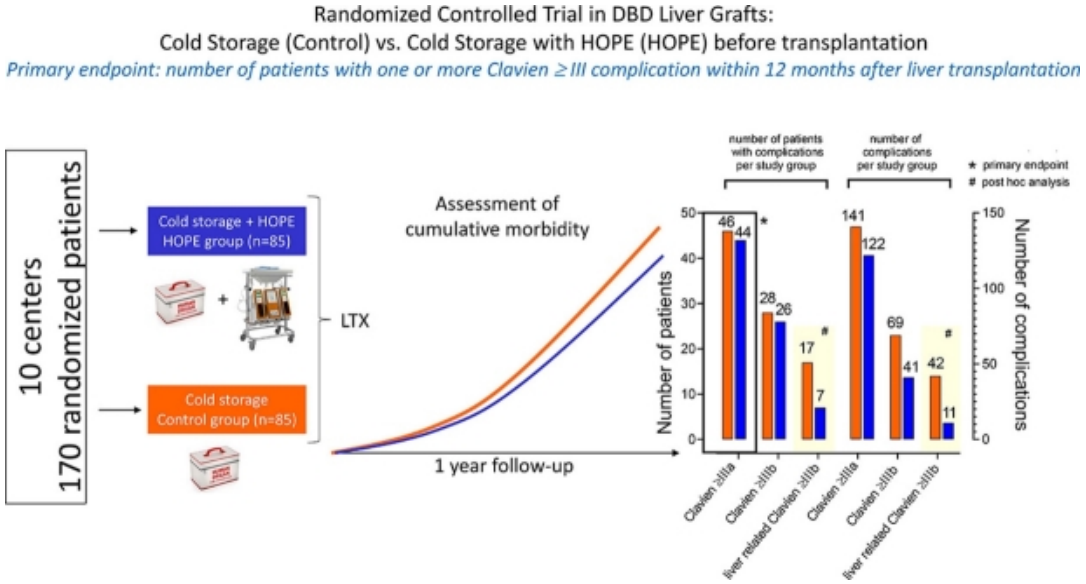
25.08.2023

Advantages of Xenotransplantation

- Unlimited source of organs
- Elective surgery
- Earlier timing of transplantation
- Increased indications for transplantation
- Control over donor/organ “quality”, e.g. *“Designated pathogen free”*

Alternatives to allotransplantation ?

- **Mechanical Assist Devices / Artificial Organs**
  - Dialysis
  - Left ventricular assist-device (LVAD)
    Biventricular assist-device (BVAD)
  - Total artificial heart (TAH)?
  - Artificial liver?
  - Artificial lung?
- ***Ex vivo* regeneration strategies**
- **Xenotransplantation**
- Tissue engineering ?
- Stem cells ?

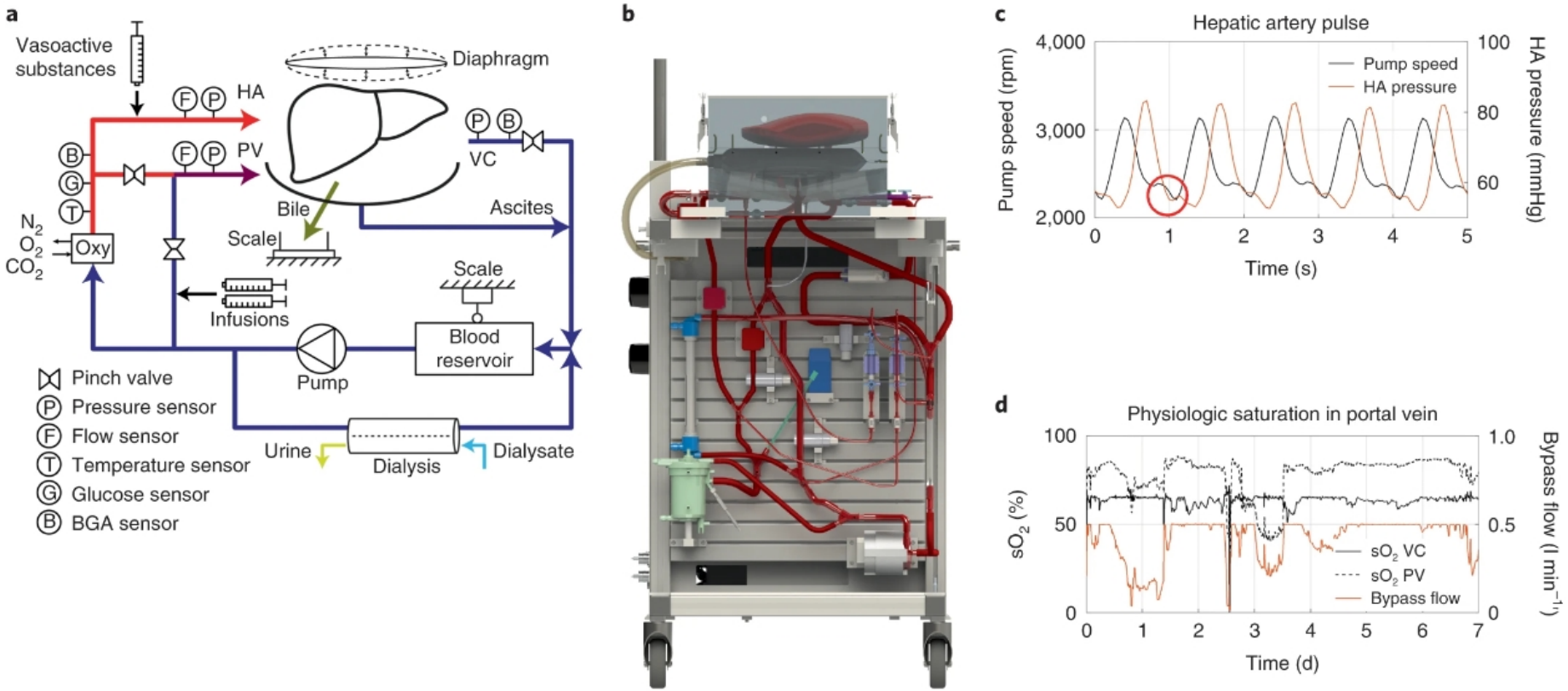


Journal of Hepatology DOI: (10.1016/j.jhep.2022.12.030)

“Alternatives to” = “Expansion of” allotransplantation ?

Ex vivo regeneration strategies

From: An integrated perfusion machine preserves injured human livers for 1 week

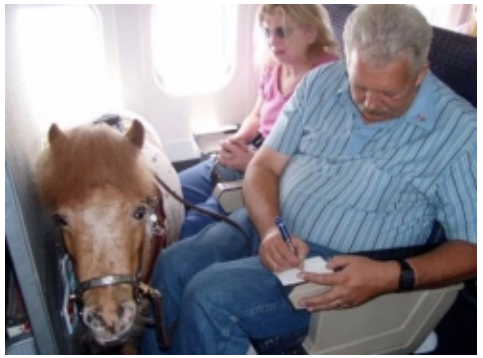


Eshmuminov, D., Becker, D., Bautista Borrego, L. et al. An integrated perfusion machine preserves injured human livers for 1 week. Nat Biotechnol 38, 189–198 (2020). https://doi.org/10.1038/s41587-019-0374-x

Advantages of the pig as organ donor

- Size
- Reproduction
- Worldwide disponibility
- Certain ethical acceptance
- Less infectious risks ?
- Genetic manipulation

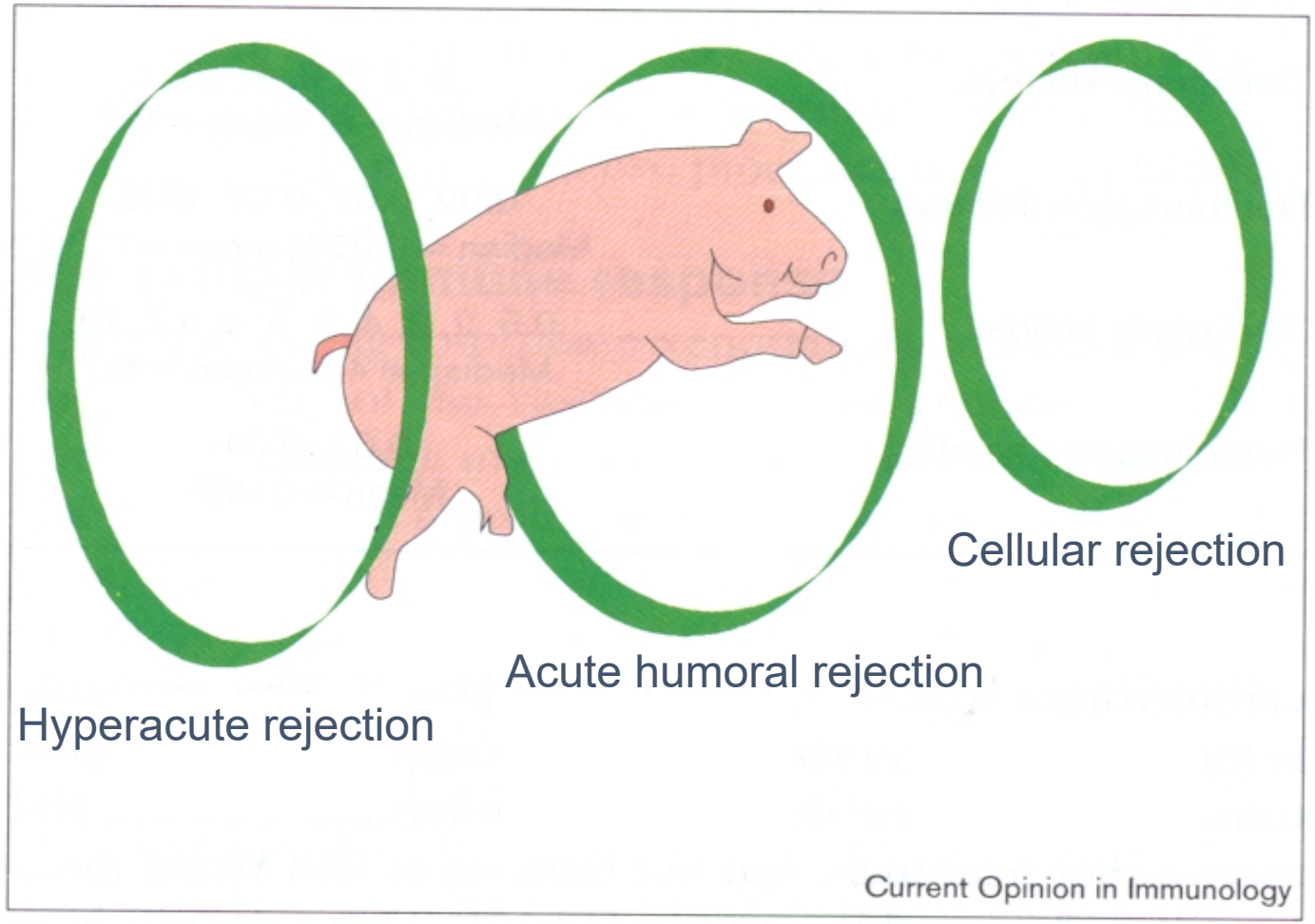
The pig as organ donor



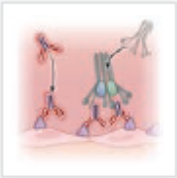
Ethical considerations

- Animal rights
- Religious deliberations
- ESA (=Emotional Support Animal)

Immunological Barriers/Physiological Barriers/Ethical considerations…



Key Concepts



Hyperimmune acute rejection

'hī-pər-i-myün ə-'kyüt ri-'jek-shən

Rejection of a transplanted graft within 48 hours after transplantation, owing to preexisting cytotoxic antibodies in the recipient that bind antigen on the graft, which leads to coagulation, ischemia, and necrosis.

Somatic cell nuclear transfer

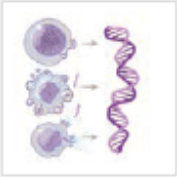
sō-'ma-tik 'sel 'nü-klē-ər trans-'fər

The first step in obtaining a clone of an organism. A somatic cell (or its nucleus) is obtained from the organism and transferred into the cytoplasm of an enucleated oocyte. Subsequent steps include reprogramming of the DNA in the nucleus by oocyte factors (a passive step) and induction of cell division through electric pulses.

Metagenomic sequencing

me-tə-ji-'nō-mik 'sē-kwəns-iŋ

The rapid and simultaneous sequencing of DNA fragments derived from different genomes (of different organisms) in a single sample. Subsequent analysis of the resultant DNA sequences can reveal microbial composition and the presence of pathogens.



Cell-free DNA

'sel-'frē dē-en-'ā

Nonencapsulated nucleic acid fragments found in noncellular components of blood or other bodily fluids. These fragments may be derived from endogenous tissue, including cancerous cells; from pathogens; or from placental or fetal cells. Cell-free DNA can be sequenced, and analysis of DNA sequence reads (a "read" represents the single occurrence of a specific fragment) can be screened for fetal chromosomal trisomies and pathogens. It may be useful in screening for cancer.

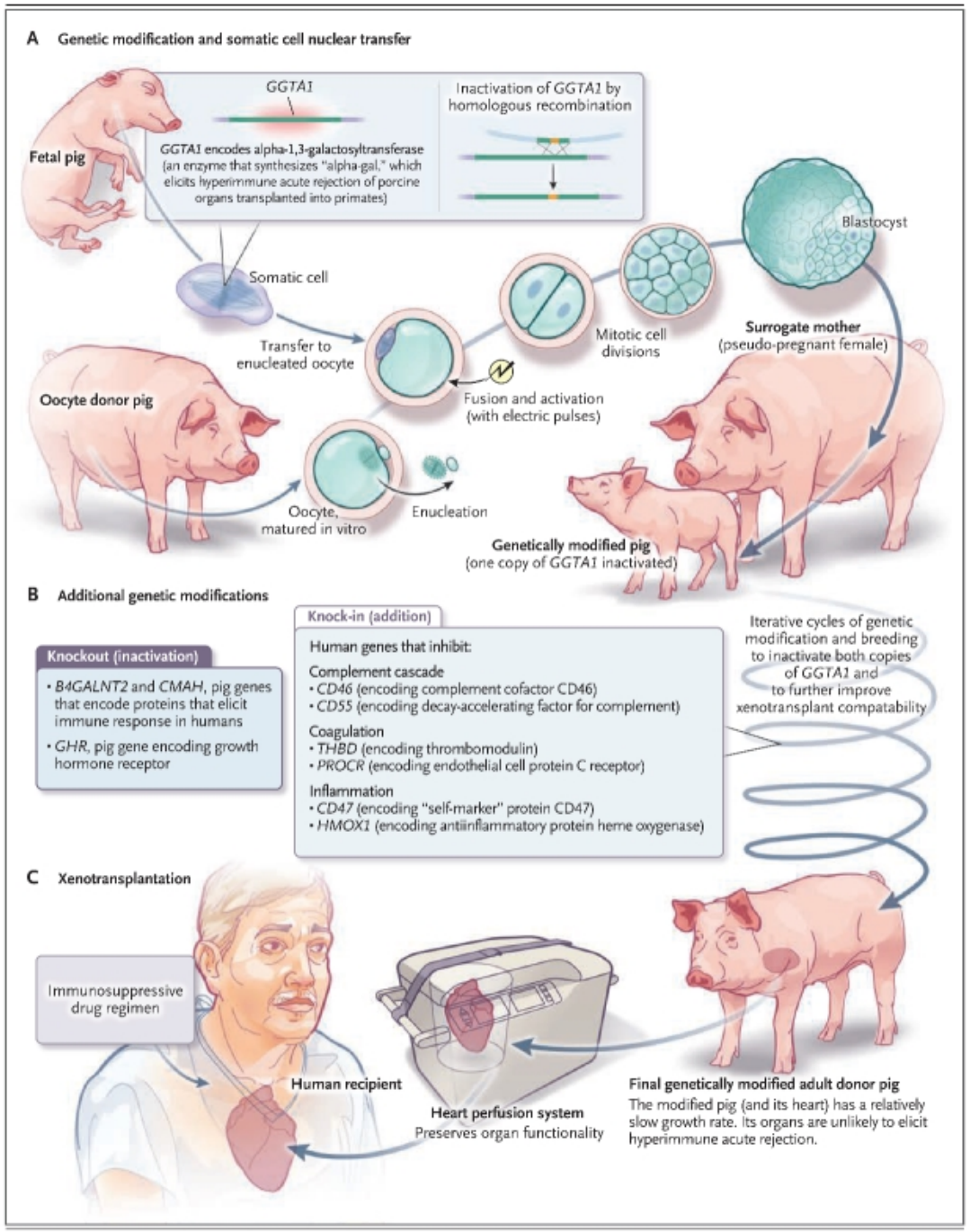
EDITORIALS

SCIENCE BEHIND THE STUDY

Genetic Modification in Pig-to-Human Transplantation

Elizabeth G. Phimister, Ph.D.

N Engl J Med. 2022 Jul 7;387(1):79-82. doi: 10.1056/NEJMe2207422.



Manipulation of the donor

Table S1: Genetic Modifications of Source Animal for Cardiac Xenotransplantation		
Genetic Modification	**Mechanisms**	**Properties**
Xenogeneic Carbohydrate Knockout		
Galactose-α-1,3-galactose KO (GTKO)	Deletion of immunogenic Galactose-α-1,3-galactose (Gal) glycan through knockout of the synthetic enzyme alpha1,3-galactosyltransferase (GT)	Anti-Immunogenic
β1,4-N-acetylgalactosyltransferase KO (B4GalKO)	Deletion of immunogenic blood group SDa antigen through knockout of the synthetic enzyme (B4GalNT2)	
CMP-N-acetylneuraminic acid hydroxylase KO (CMAHKO)	Deletion of immunogenic glycan N-glycolylneuraminic acid (Neu5Gc) through knockout of the synthetic enzyme CMP-N-acetylneuraminic acid hydroxylase (CMAH)	
Growth Hormone Receptor Knockout (GHRKO)	Reduction of downstream insulin growth factor-1 (IGF-1) signaling	Reduce intrinsic graft growth
Human Transgene Expression		
CD46	Suppress human complement activity by mediating cleavage of C3b and C4b complement deposition	Complement Regulation
Decay Accelerating Factor (DAF)	Inhibits C3 and C5 convertase enzymes and downstream complement activation	
Endothelial Cell Protein C Receptor (EPCR)	Activates Protein C	Anti-Coagulation
Thrombomodulin (TBM)	Binds human thrombin, and activates Protein C via activated thrombin	
Hemeoxygenase-1 (HO-1)	Decreases oxidative products	Anti-Inflammatory
CD47	Interacts with macrophage signal regulatory protein (SIRP)α to prevent opsonization and phagocytosis of xenogeneic tissue	

… and infectious diseases questions

Table 1. Approach to Microbiologic Screening of Swine as Xenograft Donors for Humans.*

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

* PCMV denotes porcine cytomegalovirus, PERV porcine endogenous retrovirus, PLHV porcine lymphotropic herpesvirus, and SARS-CoV-2 severe acute respiratory syndrome coronavirus 2.

… and infectious diseases questions

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+ unknown pathogens

Pathogens Not Permitted in Swine with "Designated Pathogen-free" Status.

Table 2. Pathogens Not Permitted in Swine with "Designated Pathogen-free" Status.*

Bacteria

[redacted]

[redacted]

[redacted]

[redacted] (including *Mycobacterium bovis*)

[redacted]

[redacted] (lung transplant)

[redacted] species (*S. enterica* serotype Typhi, serotype Typhimurium, serotype Choleraesuis)

[redacted]

[redacted] (yersinia, campylobacter)

Fungi

[redacted] (colonized or lesions)

[redacted] (lesions)

[redacted]

[redacted]

Parasites (detected in stool or on serologic testing)

[redacted]

[redacted]

[redacted]

Other parasites

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

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

* "Designated pathogen-free" swine must not have infections with the specified pathogens. For xenotransplantation of solid organs and hematopoietic tissues, listed pathogens may be considered in the development of a screening program for source animals.[11,20,103-105]

Two concepts

- Latency
- Genomic origin

Viruses

Adenovirus (swine)

[redacted]

* "Designated pathogen-free" swine must not have infections with the specified pathogens. For xenotransplantation of solid organs and hematopoietic tissues, listed pathogens may be considered in the development of a screening program for source animals.[11,20,103-105]

Latency: After primary infection, a virus becomes part of the virome, and can be re-activated and/or transmitted

As in the human setting, the family of Herpesviridae is the prime contributer

Two concepts

- Latency
- [illegible]

Genomic origin: It is part of your genetic makeup, inherited inevitably to the next generation.

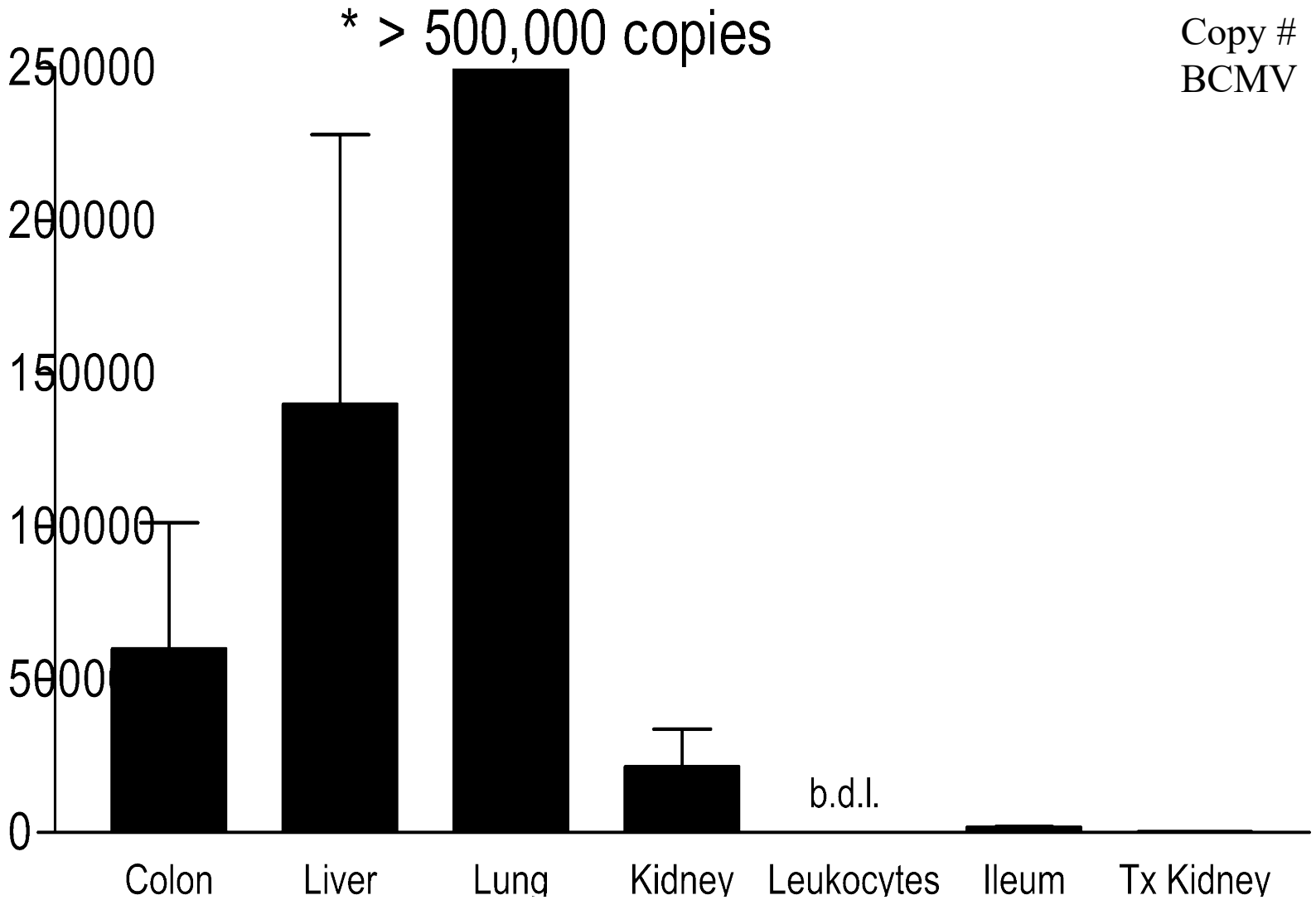
Porcine endogenous retroviruses (same concept does exist in humans)

- No clear role, no role in disease, but may reactivate in a “foreign” environment

Known herpes viruses of baboon and pig

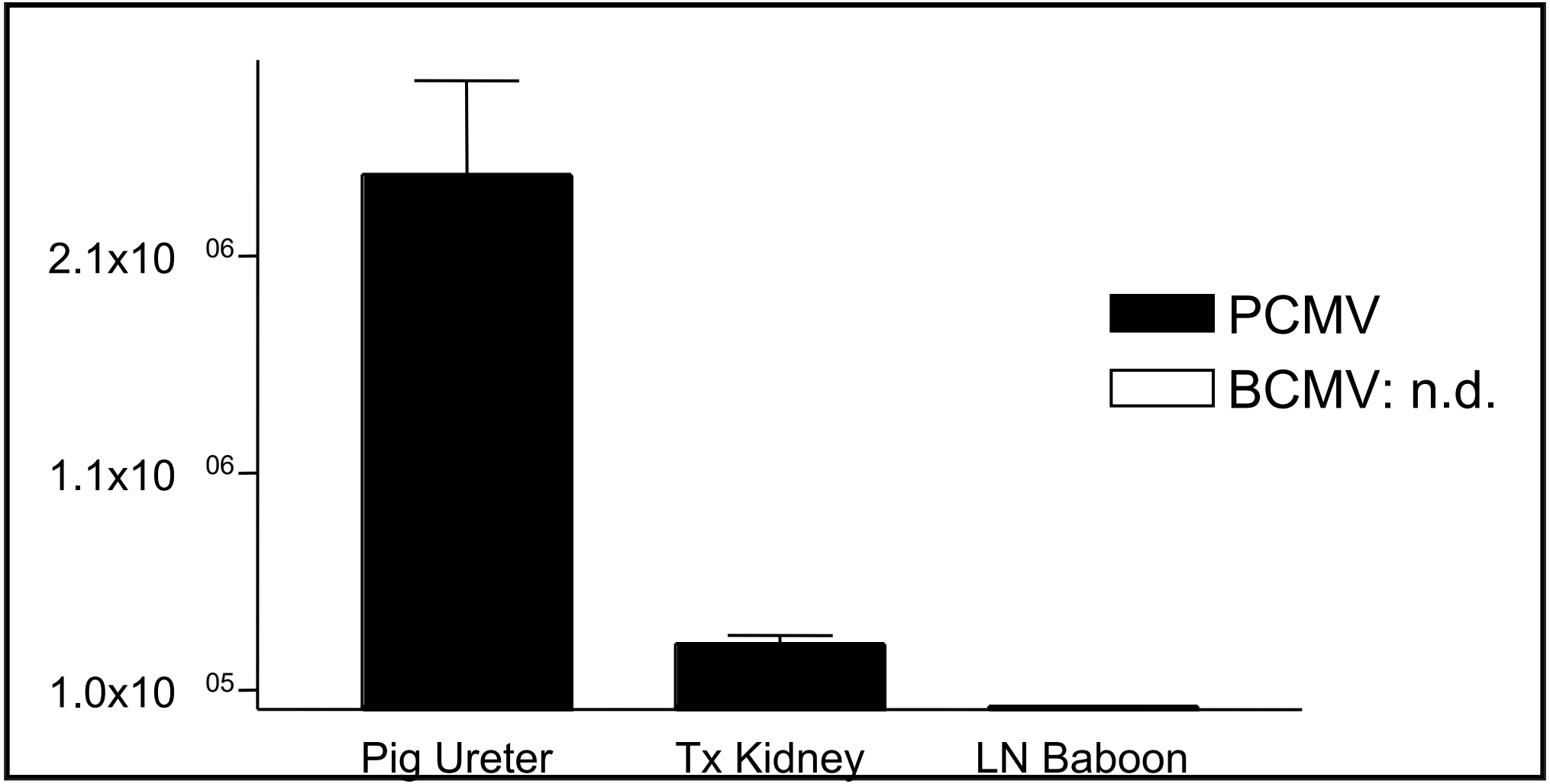
- Porcine cytomegalovirus/porcine roseolavirus (PCMV/PRV): Prevalence of > 90% in commercial herds, biological behaviour very similar to human CMV, genetically closer related to HHV6/7
- Porcine lymphotropic herpesvirus (PLHV) 1-3, similarity to EBV/HHV-8
- Baboon cytomegalovirus (BCMV), very similar to HCMV

- All herpesviruses establish a latent infection



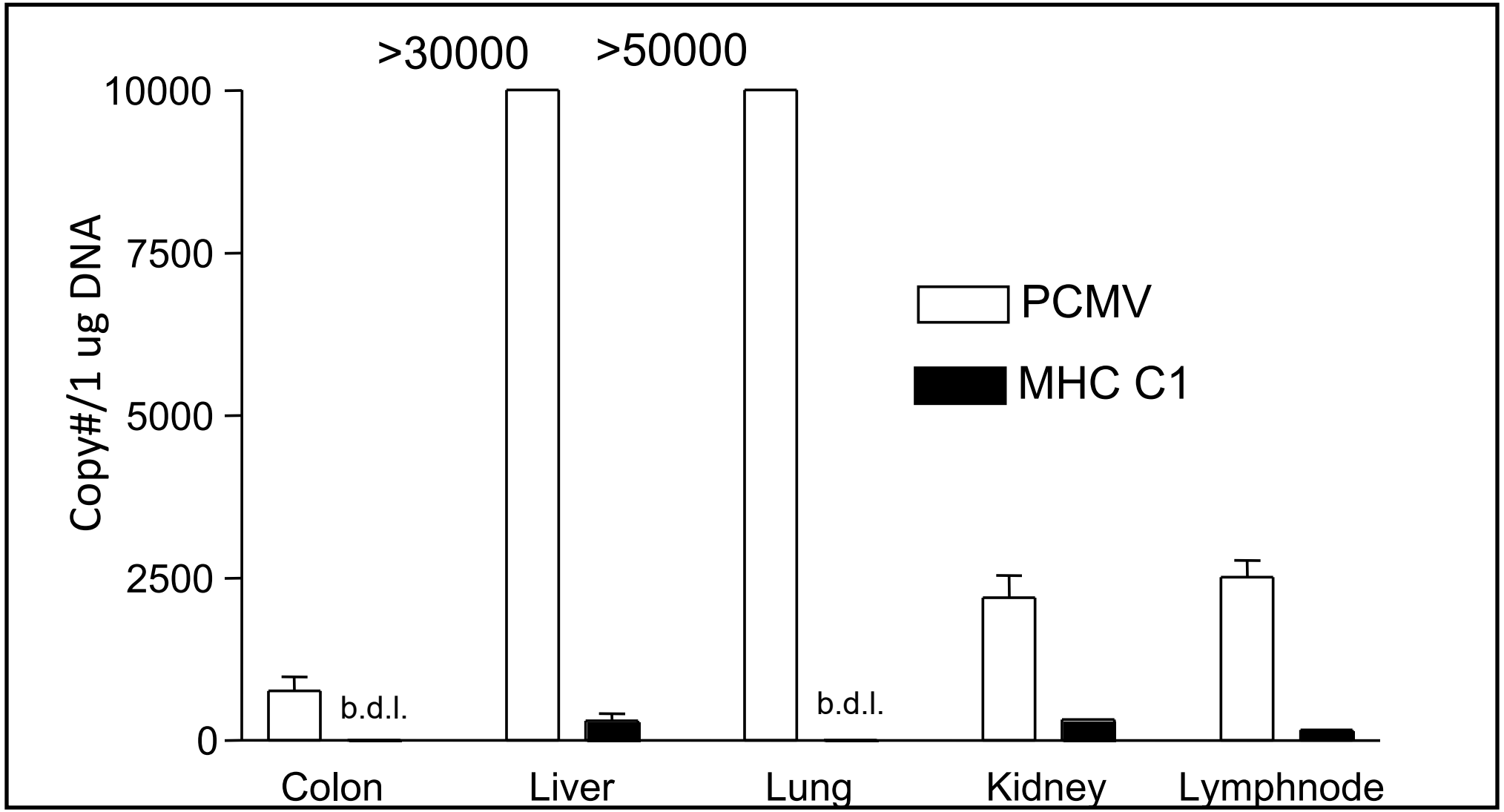
Copies of BCMV in baboon tissues and xenograft at autopsy. BCMV DNA copies in 1ug total DNA. b.d.l.: below quantitative detection limit

J Virol 2002 15;76:4734-4740



Copies of PCMV DNA in porcine xenograft and baboon lymph node biopsies. Lymph node biopsy taken at the time of graftectomy contained small amounts of PCMV. No BCMV was found in any tissue.

J Virol 2002 15;76:4734-4740

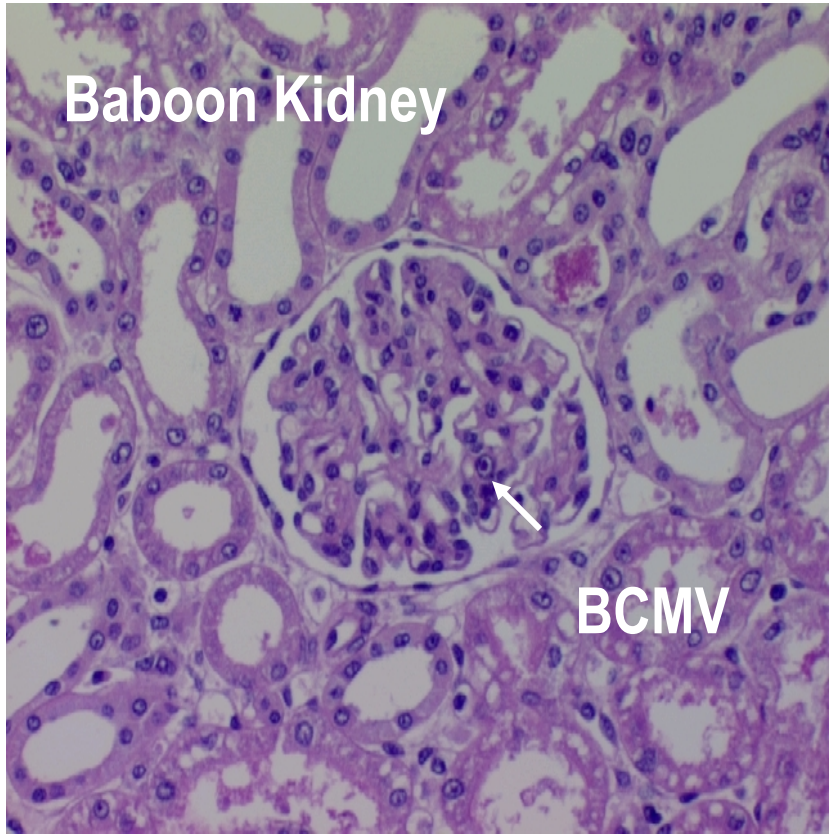


Copies of PCMV and MHCC1 (porcine MHC) DNA in baboon xenograft recipient tissues at autopsy. (b.d.l.: below quantitative detection limit)

J Virol 2002 15;76:4734-4740

- In pig-to-baboon kidney xenotransplants, the DNA copy number of baboon CMV (BCMV) and porcine CMV (PCMV) increased systemically in the majority of recipients and in xenograft tissues during immune suppression.

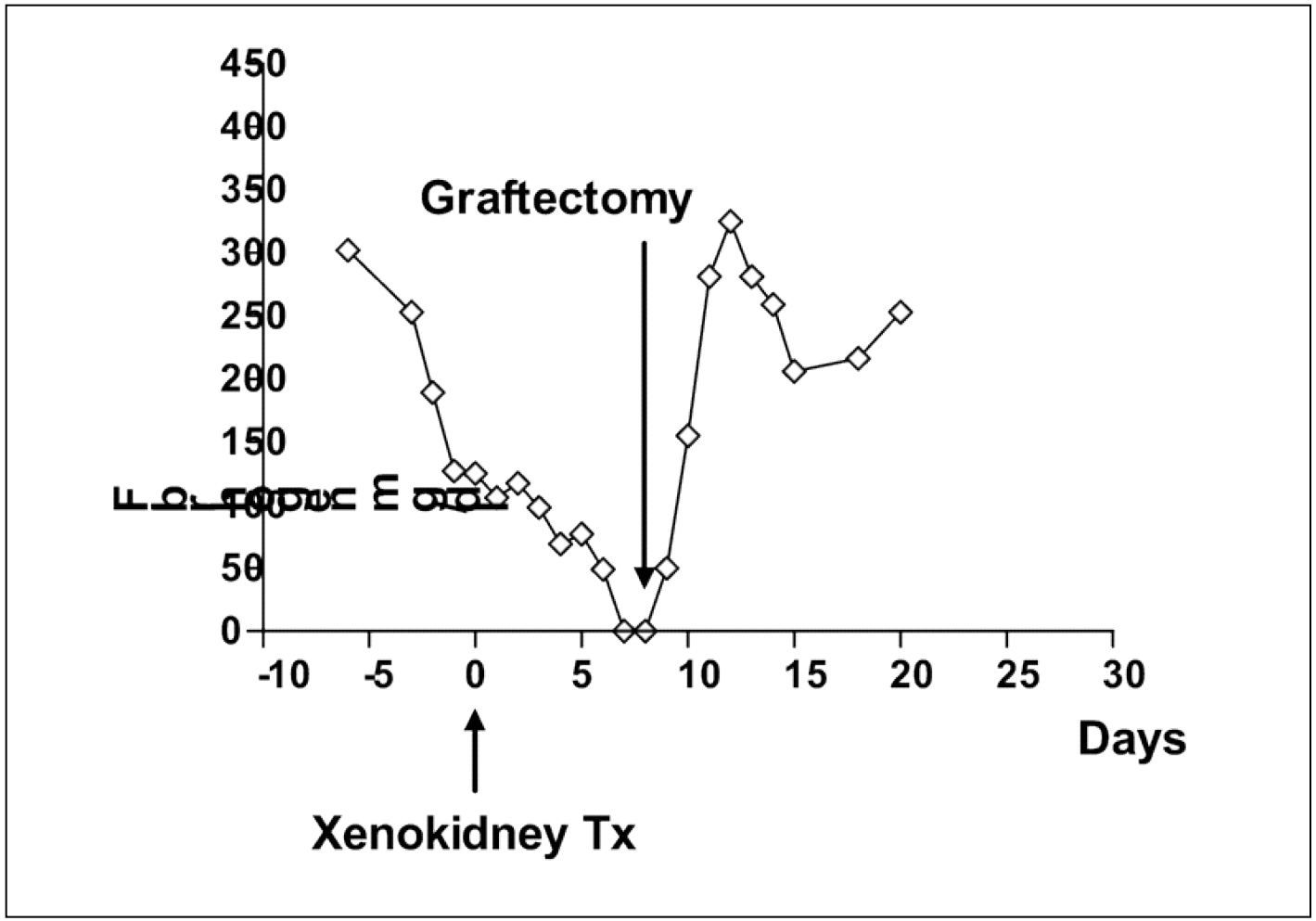
- PCMV was detectable in tissues and blood of the baboon recipient, while BCMV was present in the porcine graft without discernable tissue injury.



J Virol 2002 15;76:4734-4740

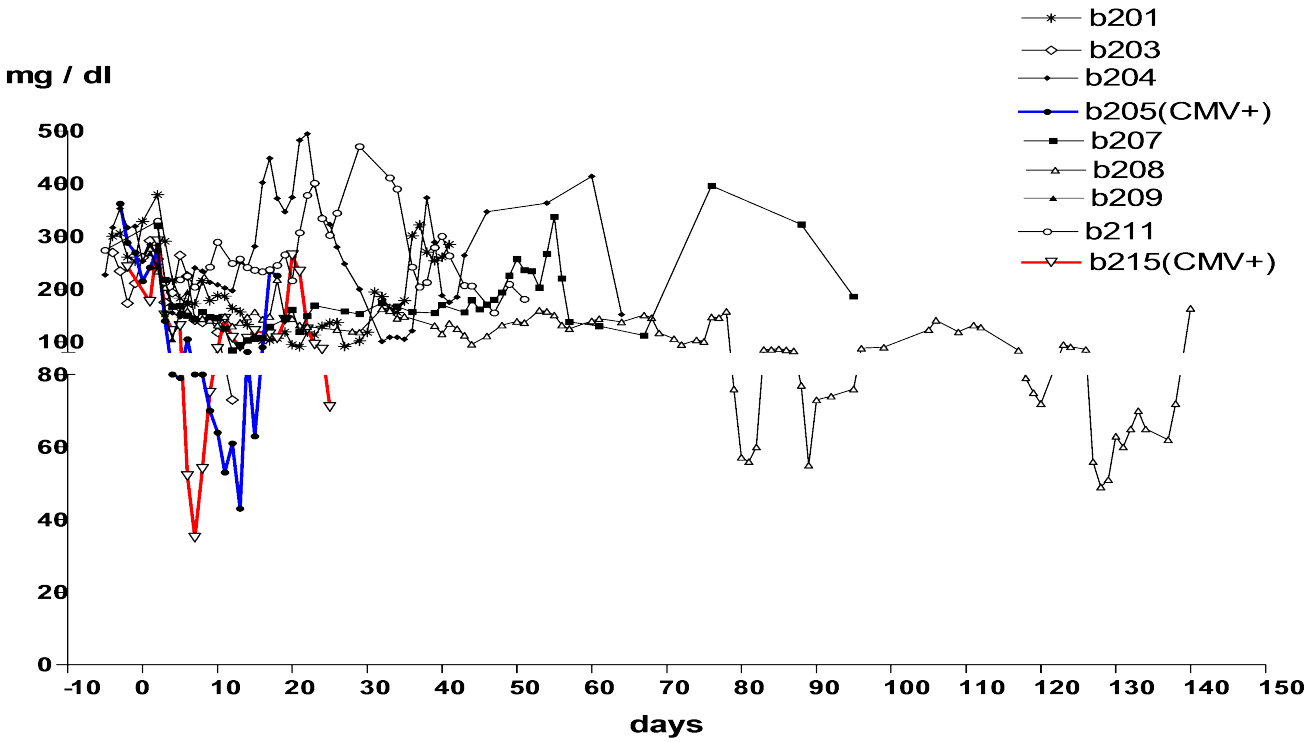
Consumptive coagulopathgy

Kidney xenotransplantation



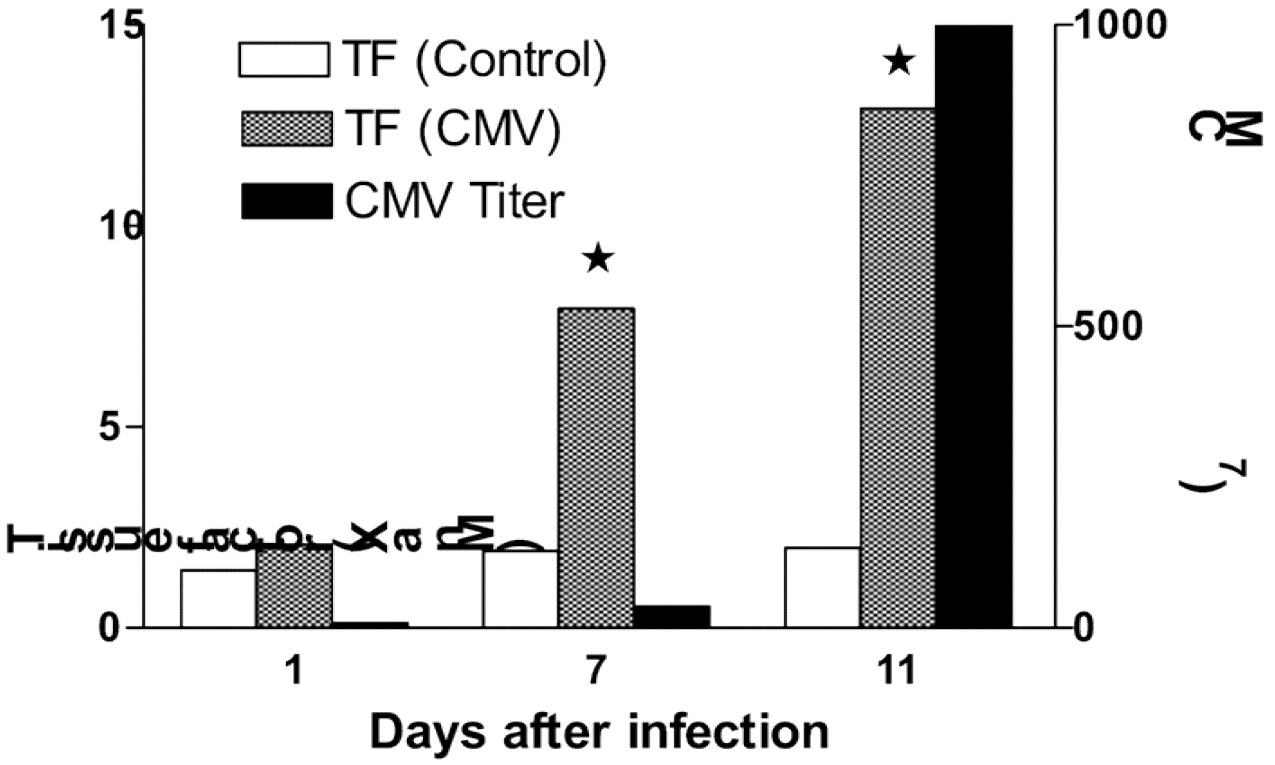
Transplantation 75(11):p 1841-1847, June 15, 2003. | DOI: 10.1097/01.TP.0000065806.90840.C1

Fibrinogen in animals transplanted with a PCMV-free vs PCMV -positive graft



Transplantation 75(11):p 1841-1847, June 15, 2003. | DOI: 10.1097/01.TP.0000065806.90840.C1

Tissue factor activation in primary endothelial porcine cells infected with PCMV

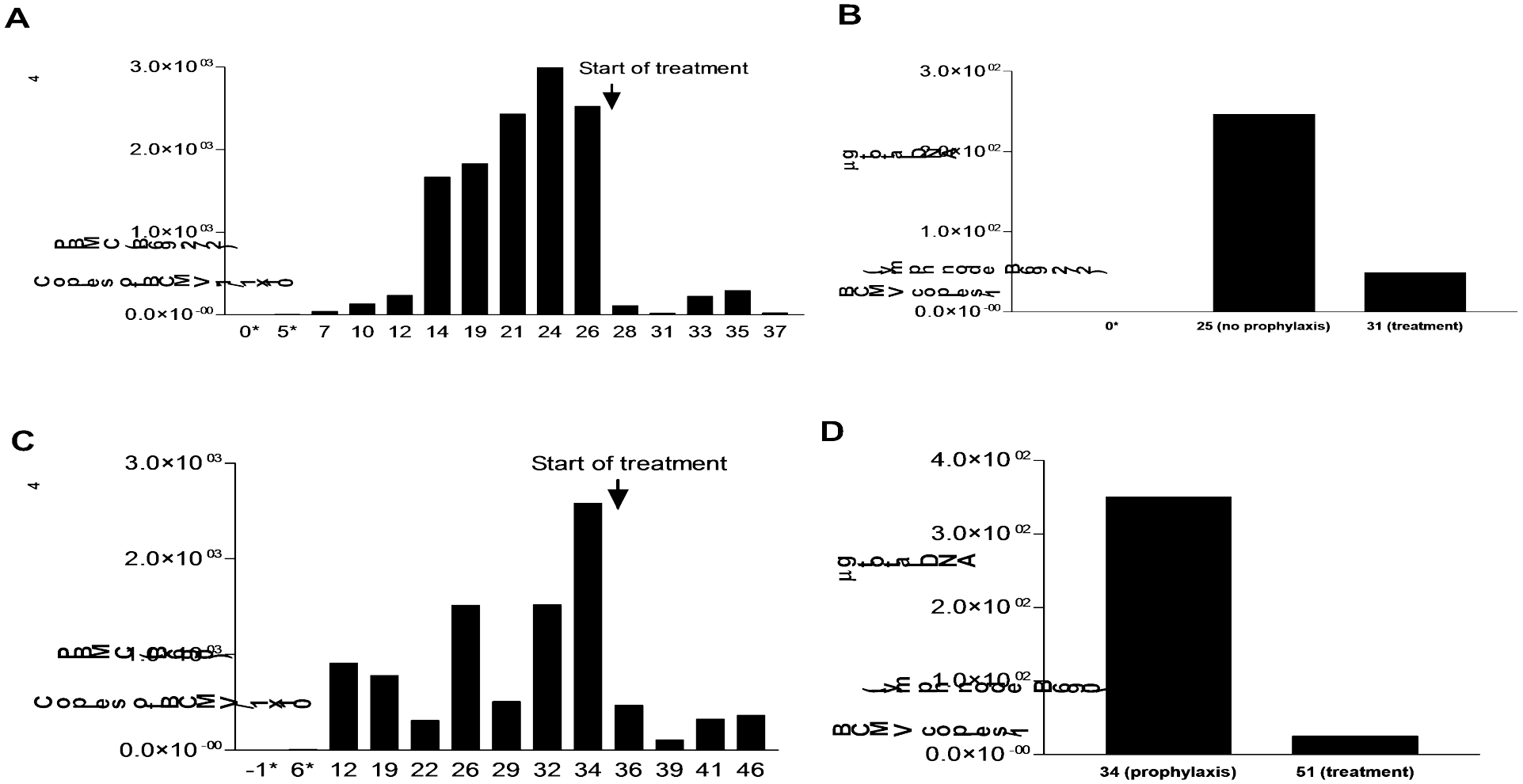


Transplantation 75(11):p 1841-1847, June 15, 2003. | DOI: 10.1097/01.TP.0000065806.90840.C1

Ganciclovir and BCMV

NJ Mueller et al. 'Ganciclovir in Pig-to-Primate Xenotransplantation'

FIGURE 1



Am J Transplant. 2003 Sep;3(9):1057-64. doi: 10.1034/j.1600-6143.2003.00192.x.

Conclusions

- Activation of PCMV in the graft is one of multiple factors which can lead to consumptive coagulopathy in the recipient animal.
- Other co-factors include acute humoral rejection.
- Consumptive coagulopathy can be reversed by early graftectomy in some animals.
- PCMV infection of primary endothelial porcine cells results in tissue factor activation, an early step in the coagulation cascade.

Conclusions (cont.)

- Similar to the human setting, activation of PCMV can result in direct (tissue damage) and indirect effects (coagulation disorders, rejection (?))
- Treatment of porcine viruses with all known drugs was not successful

Exclusion of pathogens

- Early weaning was tested as a strategy to eliminate porcine herpesviral infections (and possibly others).

- Piglets were separated shortly after birth and hand-raised, no other contact than to littermates was allowed

- Pigs were kept in isolation till date of transplantation.

Xenotransplantation. 2005 Jan;12(1):59-62. doi: 10.1111/j.1399-3089.2004.00196.x.

Exclusion of pathogens

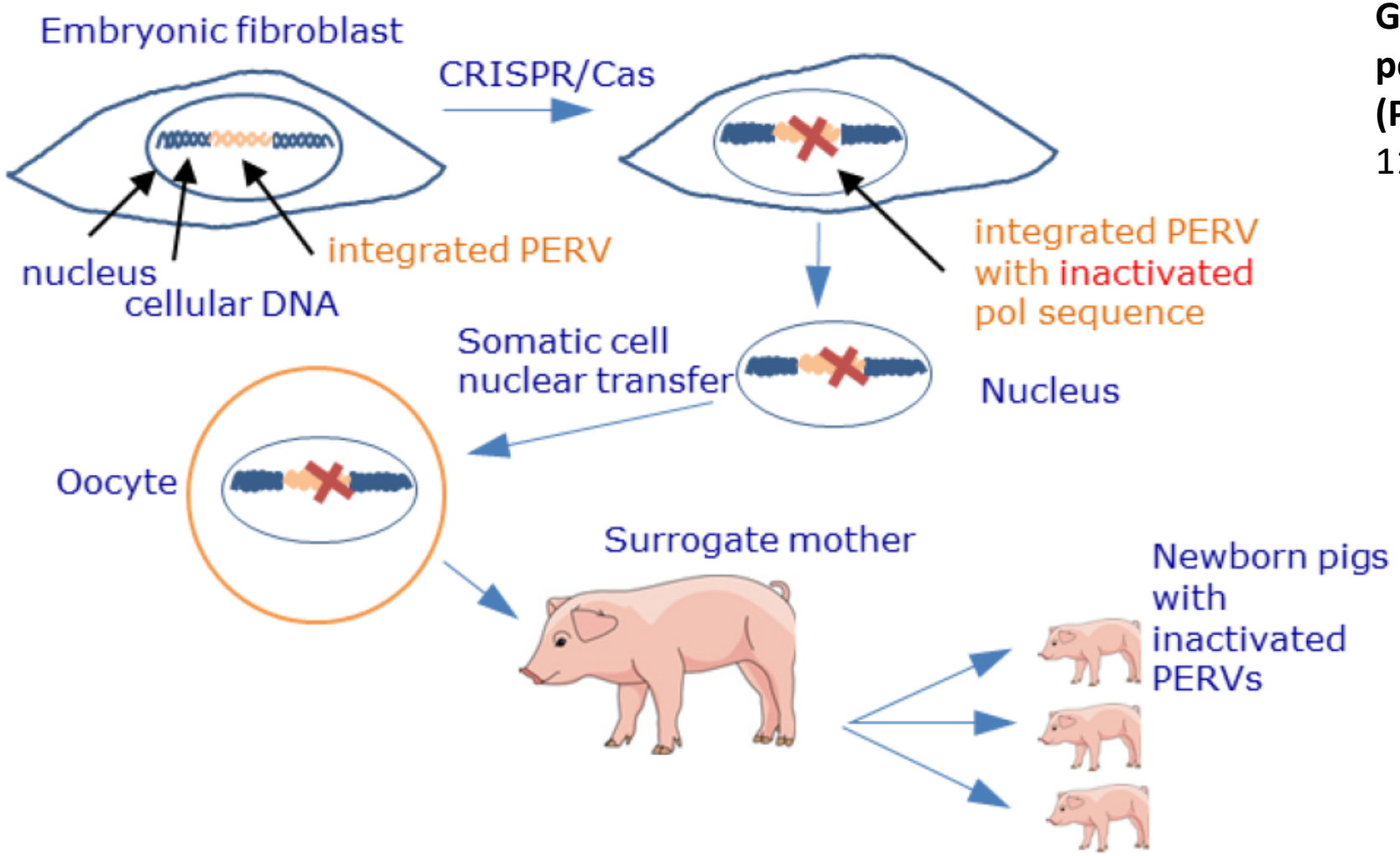
- One big exception are porcine endogenous retroviruses, as they are built into the genome

- Various strategies have been applied
- Silencing (genome editing)
- Selection of suitable pigs
- *Antiretroviral drugs*
- *Vaccination*

Xenotransplantation. 2005 Jan;12(1):59-62. doi: 10.1111/j.1399-3089.2004.00196.x.

Exclusion of pathogens
Systematic silencing via CRISPR-CAS

Yang, L.; Güell, M.; Niu, D.; George, H.; Lesha, E.; Grishin, D.; Aach, J.; Shrock, E.; Xu, W.; Poci, J.; et al. **Genome-wide inactivation of porcine endogenous retroviruses (PERVs). Science** 2015, 350, 1101–1104



Porcine Endogenous Retroviruses and Xenotransplantation, 2021, Denner J. *Viruses* **2021**, *13*(11), 2156; https://doi.org/10.3390/v13112156

Why do we care about PERV?

- Contrary to the other pathogens, which pose a risk primarily for the recipient, PERV could theoretically recombine and give rise to a potentially dangerous pathogen
- Therefore the risk must be weighed for the individual receiving a life-saving organ on the one hand and the general public on the other hand.

Porcine Endogenous Retroviruses and Xenotransplantation, 2021, Denner J. *Viruses* **2021**, *13*(11), 2156; https://doi.org/10.3390/v13112156

What do we know about PERV?

- Despite the *in vivo* infection potential of PERV into human cells, no infection was observed in all human recipients of porcine tissue

Porcine Endogenous Retroviruses and Xenotransplantation, 2021, Denner J. *Viruses* **2021**, *13*(11), 2156; https://doi.org/10.3390/v13112156

Real-life

Original Article: Brief Report

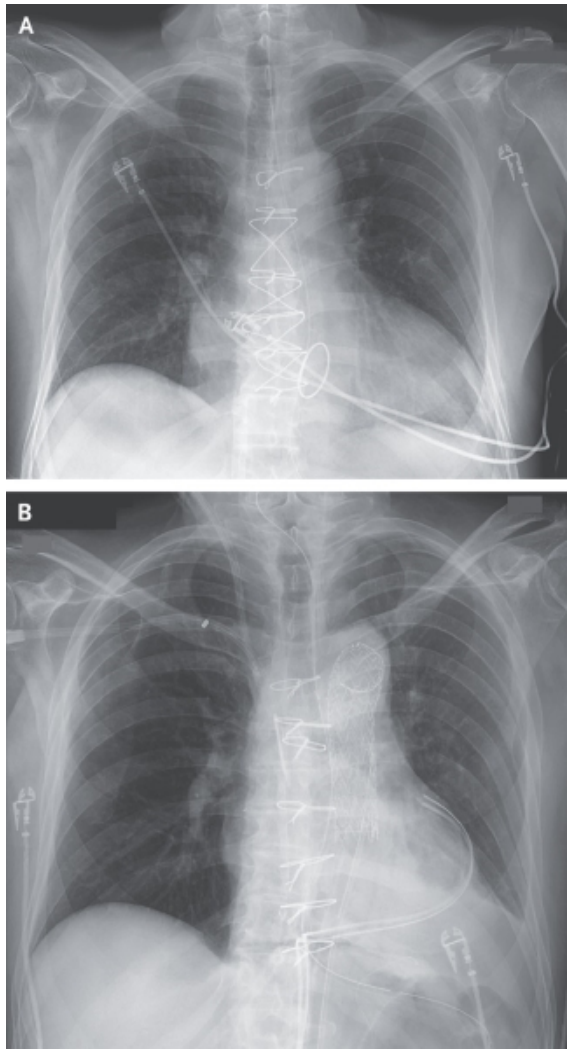
Genetically Modified Porcine-to-Human Cardiac Xenotransplantation

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.

N Engl J Med
Volume 387(1):35-44
July 7, 2022

The NEW ENGLAND JOURNAL of MEDICINE

57, m, on ECMO, non-ischemic cardiodmyopathy, LVEF 10%, poor adherence, not a candidate for allotransplantation. Single organ failure. Decision for xenotransplantation.



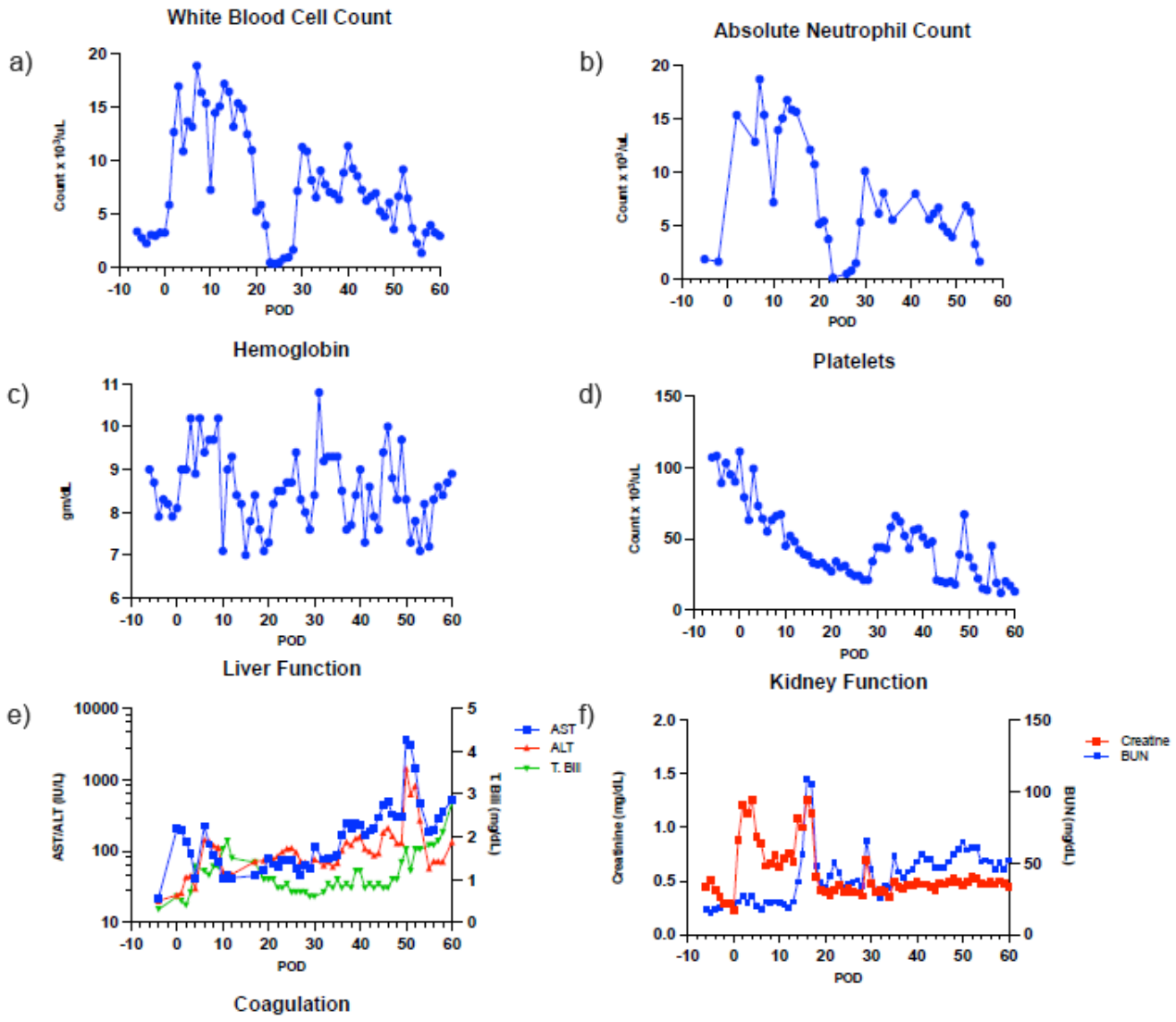
Before

After

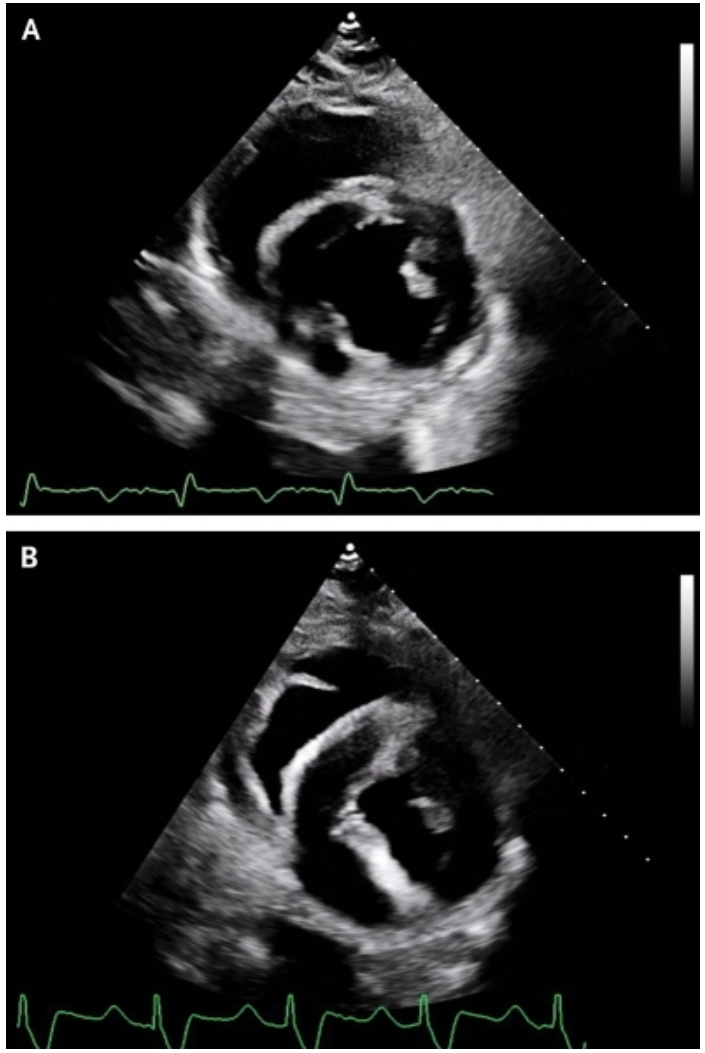
Revivicor donor pig

Table S3: Results of Commercial Pathogen Screening of Source Pig				
Test	**Sample**	**Assay**	**Result**	**Date**
Hepatitis E	Feces	Real-time PCR	Negative	1/7/22
Herpes virus gamma	Buffy coat	PCR	Negative	1/7/22
Influenza A	Nasal swab	Real-time PCR	Negative	1/7/22
Mycoplasma hyopneumoniae	Nasal swab	Real-time PCR	Negative	1/7/22
Porcine cytomegalovirus	Nasal swab	Real-time PCR	Negative	1/7/22
Porcine circovirus type 2	Serum	Real-time PCR	Negative	1/7/22
Porcine circovirus 3	Serum	Real-time PCR	Suspect [Ct 39]	1/7/22
Porcine Epidemic Diarrhea virus (S gene)	Feces	Real-time PCR	Negative	1/7/22
Porcine deltacoronavirus	Feces	Real-time PCR	Negative	1/7/22
Transmissible Gastroenteritis virus	Feces	Real-time PCR	Negative	1/7/22
Porcine reproductive and respiratory syndrome virus (PRRSV)	Serum	Real-time PCR	Negative	1/7/22
Porcine endogenous retrovirus A	Buffy coat	PCR	Positive [Ct 20]	12/21/21
Porcine endogenous retrovirus B	Buffy coat	PCR	Positive [Ct 22]	12/21/21
Porcine endogenous retrovirus C	Buffy coat	PCR	Negative	12/21/21

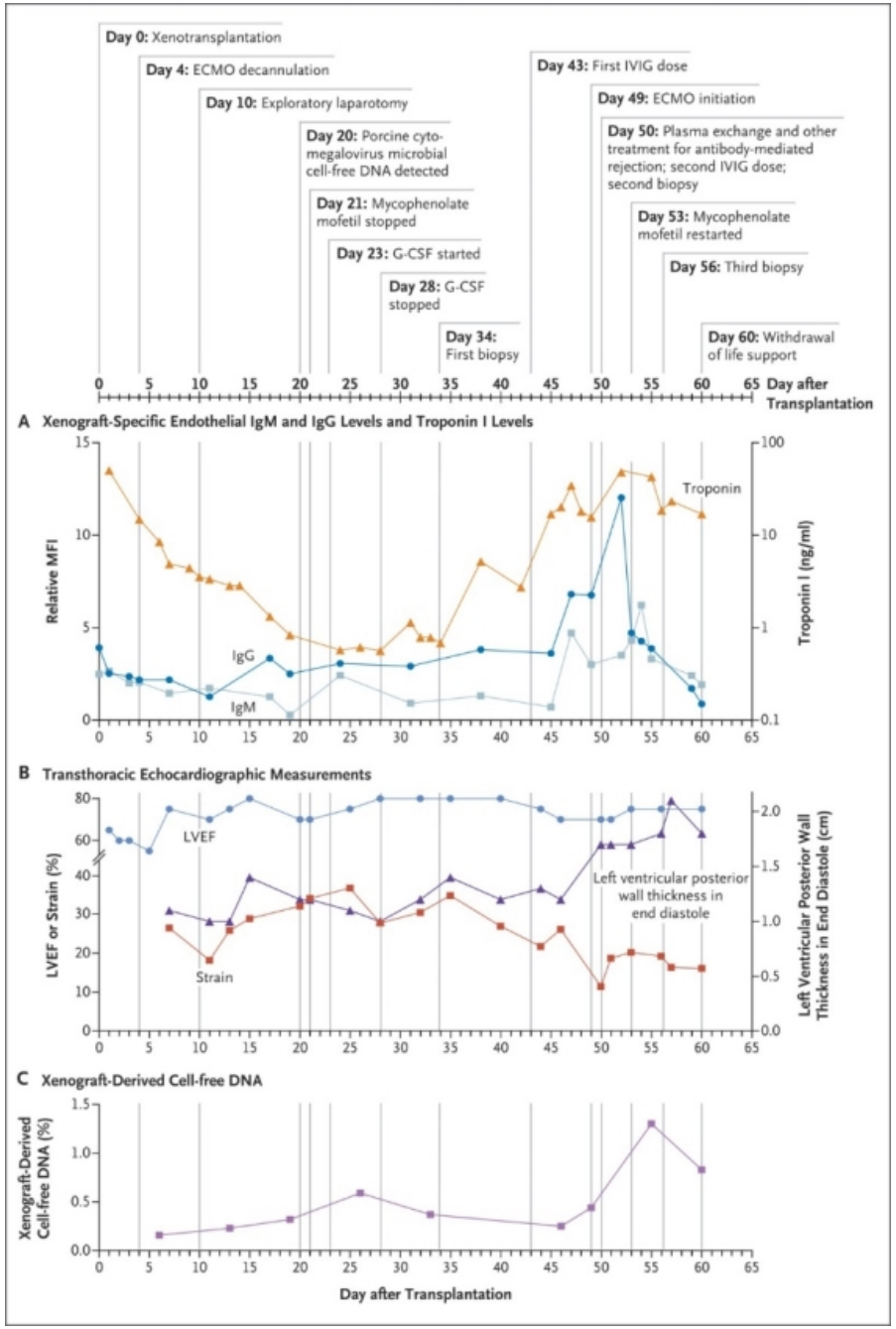
Very intense immunosuppression



Transthoracic Echocardiography.



https://www.nejm.org/do/10.1056/NEJMdo006500/full/?requestType=popUp&relatedArticle=10.1056%2FNEJMoa2201422



Clinical Details and Test Results during the Postoperative Course.

Unbiased testing for human pathogens with the use of a plasma microbial cell-free DNA (mcfDNA) test (KT, Karius) Testing for mcfDNA was also performed under a research-use-only protocol for surveillance of a limited number of porcine viral pathogens (Table S4);

Table S4: KT® Research Use Only Pathogen screening by mcfDNA	
Virus	Sequences**
Porcine type-C oncovirus	1
Suid herpesvirus 2 (porcine CMV)	1
Porcine parvovirus*	1
Porcine circovirus 1 (PCV1)*	64
Porcine circovirus 2 (PCV2)*	2423
Porcine circovirus 3 (PCV3)*	251
Porcine circovirus 4 (PCV4)*	1
African swine fever virus	133
Suid alphaherpesvirus 1	3
Porcine lymphotropic herpesvirus 1	1
Porcine lymphotropic herpesvirus 2	1
Porcine lymphotropic herpesvirus 3	1
*single-stranded DNA virus **source: National Center for Biotechnology Innovation (NCBI) RefSeq and Genbank	

Figure S2:

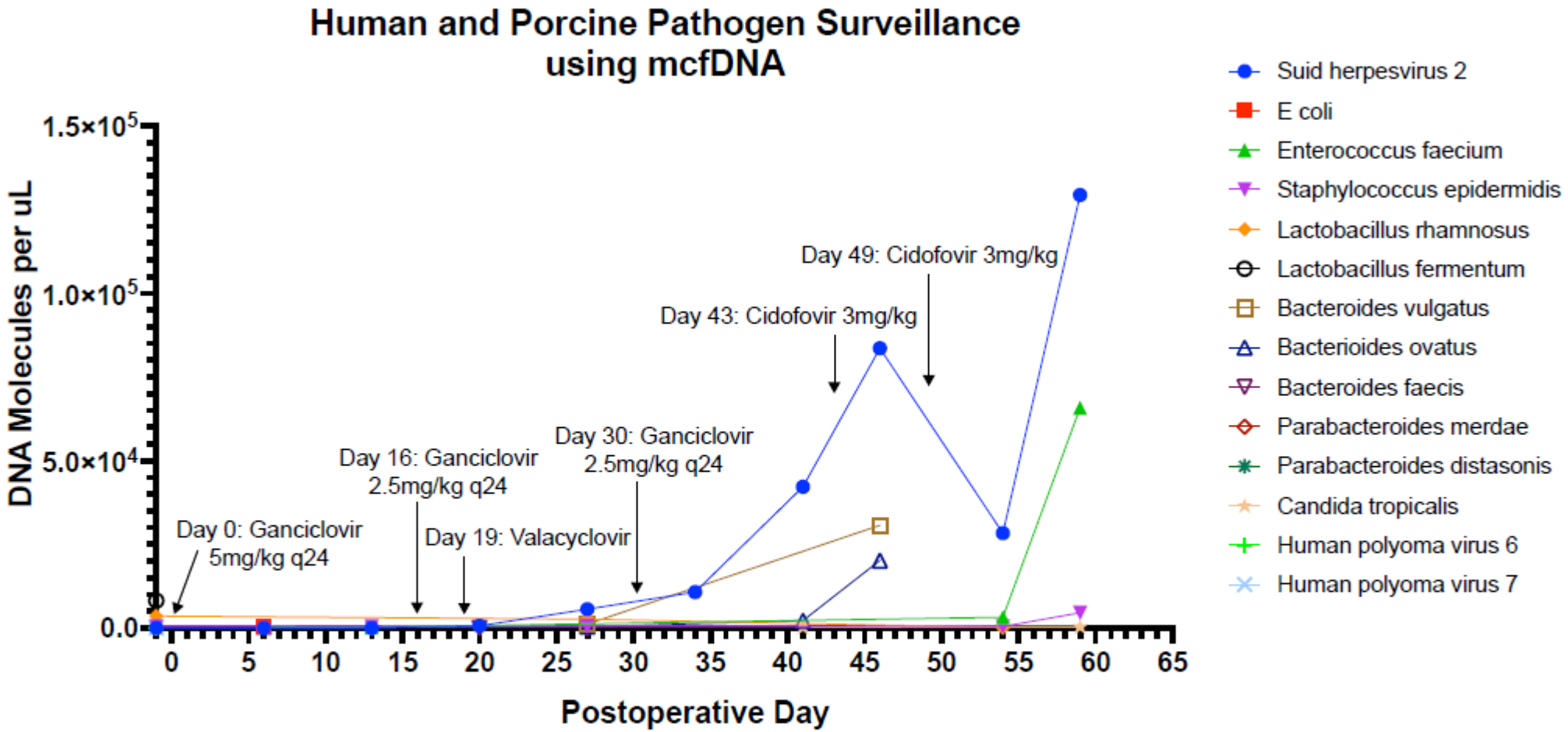
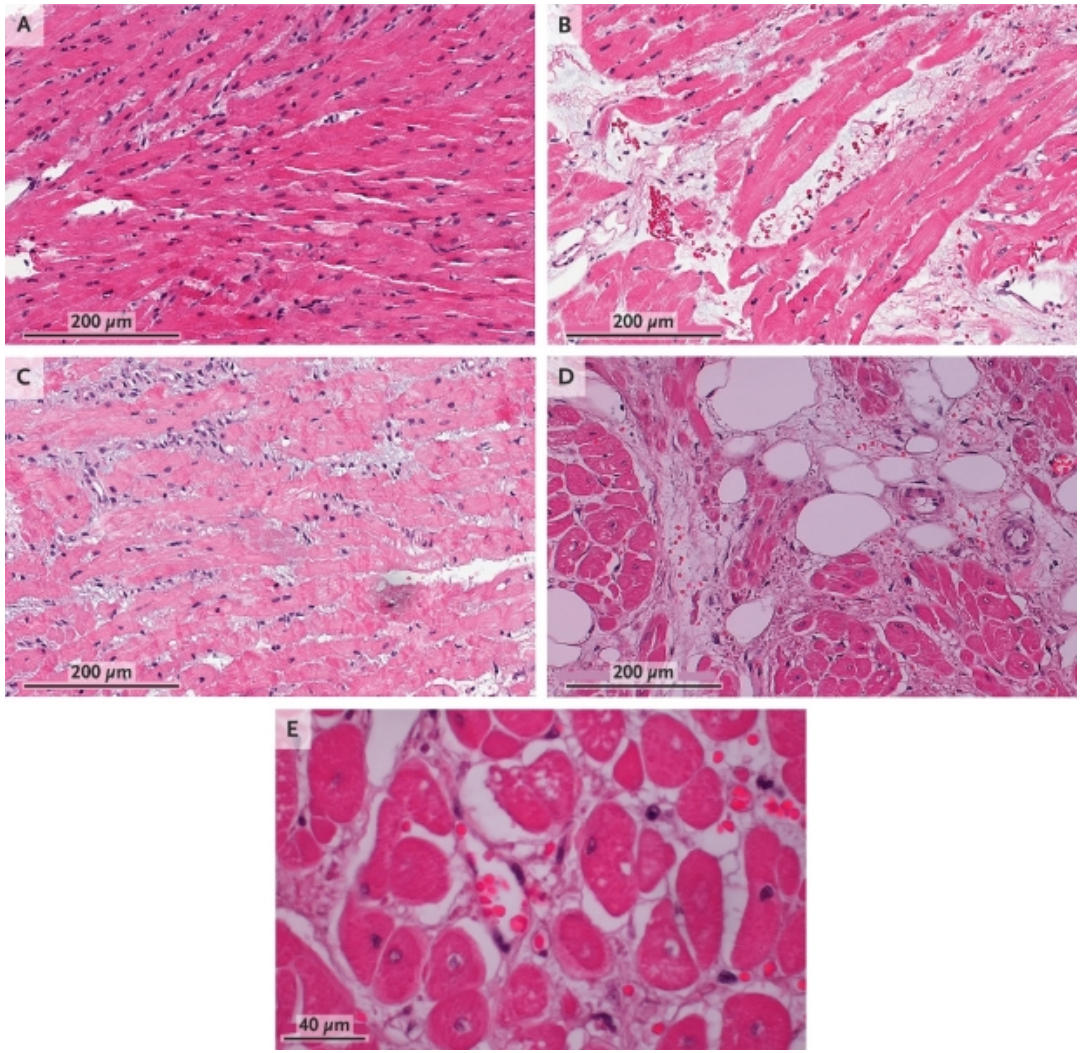


Figure S2: Unbiased longitudinal surveillance of recipient plasma by mcfDNA revealed presence of suid herpesvirus 2 (porcine cytomegalovirus, pCMV). Superimposed treatment for pCMV is indicated by arrows. There was no detection of latent human DNA viruses following xenotransplantation.

Histologic Assessments



The first endomyocardial biopsy (Panel A, day 34 after transplantation) showed normal histologic characteristics.

The second endomyocardial biopsy (Panel B, day 50) showed interstitial red-cell extravasation, fragmentation, and edema without cellular infiltrate or intravascular thrombosis.

The third endomyocardial biopsy (Panel C, day 56) showed some resolution of interstitial edema and red-cell extravasation but also showed evidence of necrotic myocytes.

The septum at autopsy (Panels D and E, day 60) showed interstitial edema and red-cell extravasation, myocardial necrosis, scant fibrosis, and central nuclei.

How should we proceed?

- Careful protocols must be in place for
  - regular surveillance of recipients
  - Optimal diagnostic procedures if an infection is suspected
  - Surveillance for next of kin

Review Article

Risks of Infectious Disease in Xenotransplantation

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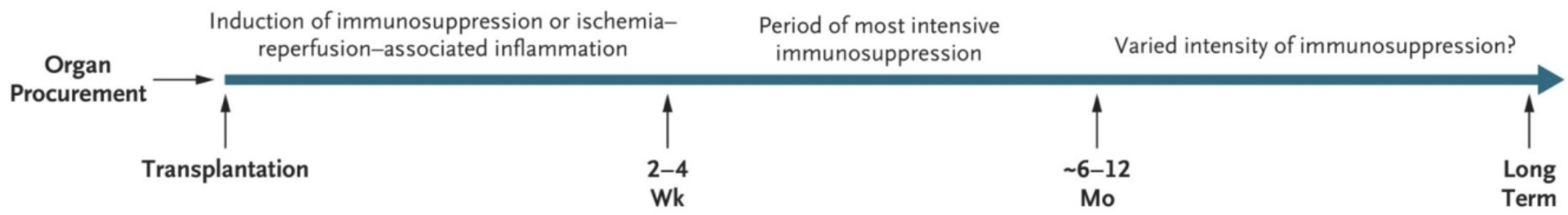
Timeline of Infections after Xenotransplantation.



	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

Approach to Microbiologic Screening of Swine as Xenograft Donors for Humans.

Table 1. Approach to Microbiologic Screening of Swine as Xenograft Donors for Humans.*

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

* PCMV denotes porcine cytomegalovirus, PERV porcine endogenous retrovirus, PLHV porcine lymphotropic herpesvirus, and SARS-CoV-2 severe acute respiratory syndrome coronavirus 2.

Routine Testing of Xenograft Recipients.

Table 4. 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]

* Additional testing is needed for persons with infectious syndromes. EBV denotes Epstein–Barr virus, HCMV human cytomegalovirus, PBMC peripheral-blood mononuclear cell, PLHV-2 PLHV type 2, P-MHC porcine major histocompatibility complex, p-mtCOII porcine mitochondrial cytochrome *c* oxidase subunit II, and QNAT quantitative NAT.

† QNAT for viruses must be normalized against chimerism studies to correct for the number of circulating pig cells in each blood sample.

Summary

- Elimination of all (so far known) porcine viruses is possible, with the (partial) exception of PERV
- By genetic manipulation/donor selection, the danger of PERV transmission can be minimized, but never entirely prevented
- Generation of a “pandemic” virus is highly unlikely (and will happen via other routes)

Summary

- Xenotransplantation must be performed

- Under strict protocols for recipients and next of kin

From a purely infectious diseases point of view, the risk seems acceptable both for the individual and the public

The three key ethical questions -my personal view

- Infectious disease risk for community
- Animal rights considerations
- Planning of trials: who should be included?