LMU KLINIKUM Munich

Walter Brendel Zentrum München

DFG SFB 127

Eurotransplant – Tissue Typing – Annual Meeting 2024

Cardiac xenotransplantation

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I have no conflict of interest to declare

Transregional Collaborative Research Centre (TRR) 127

Biology of xenogeneic cell, tissue and organ transplantation – from bench to bedside

LMU KLINIKUM

Department of Cardiac Surgery

Prof. Dr. B. Reichart

Prof. Dr. P. Brenner

Prof. Dr. M. Schmoeckel

Prof. Dr. S. Michel

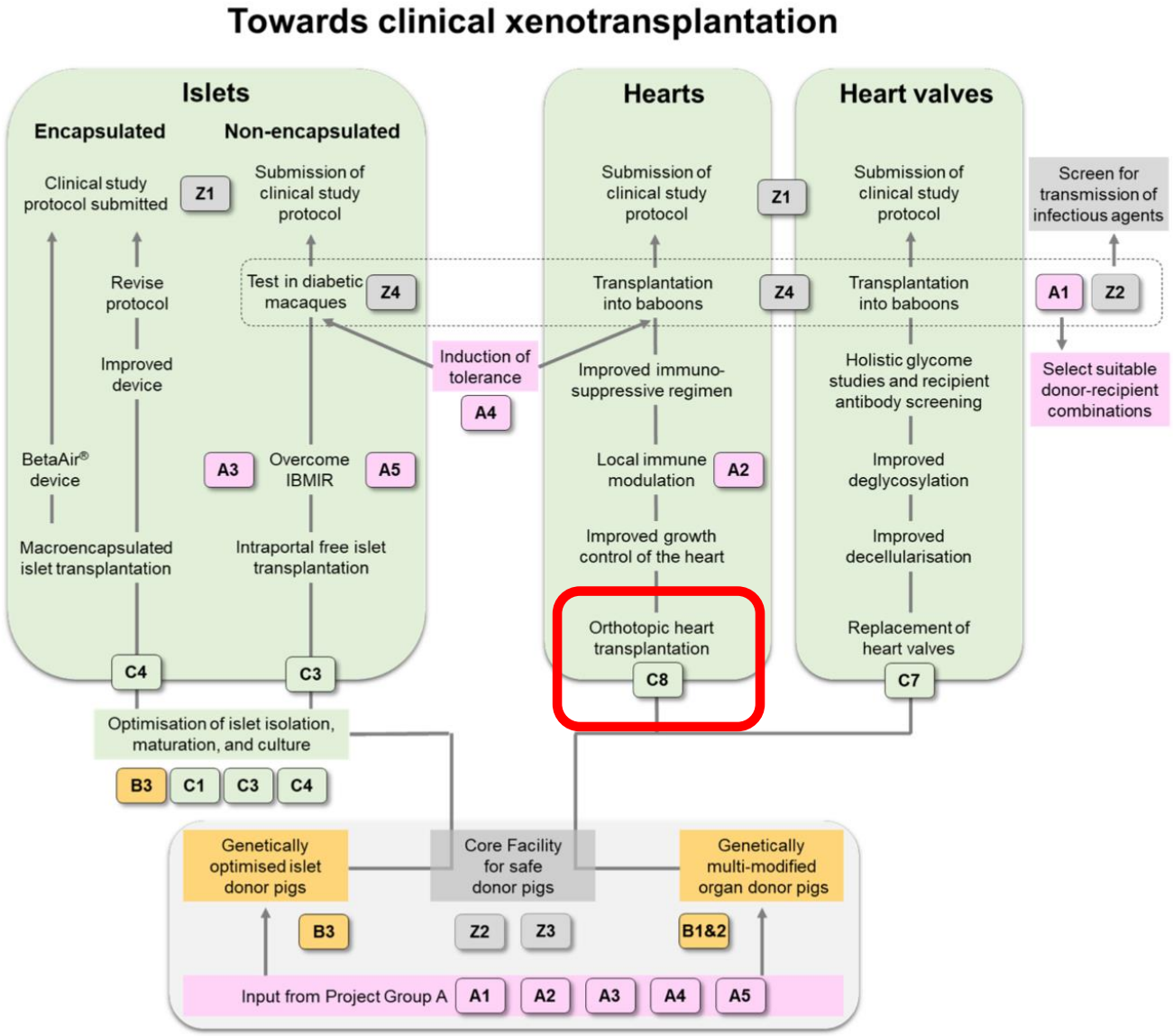
Reinhard Elgaß

Department of Anesthesiology

PD Dr. J.-M. Abicht

PD Dr. M. Längin

Dr. M. Bender



Walter Brendel Centre

Prof. D. Merkus

Dr. vet. M. Shakarami

Doctoral candidates

F. Wall

M. Leuschen

J. Radan

E. Neumann

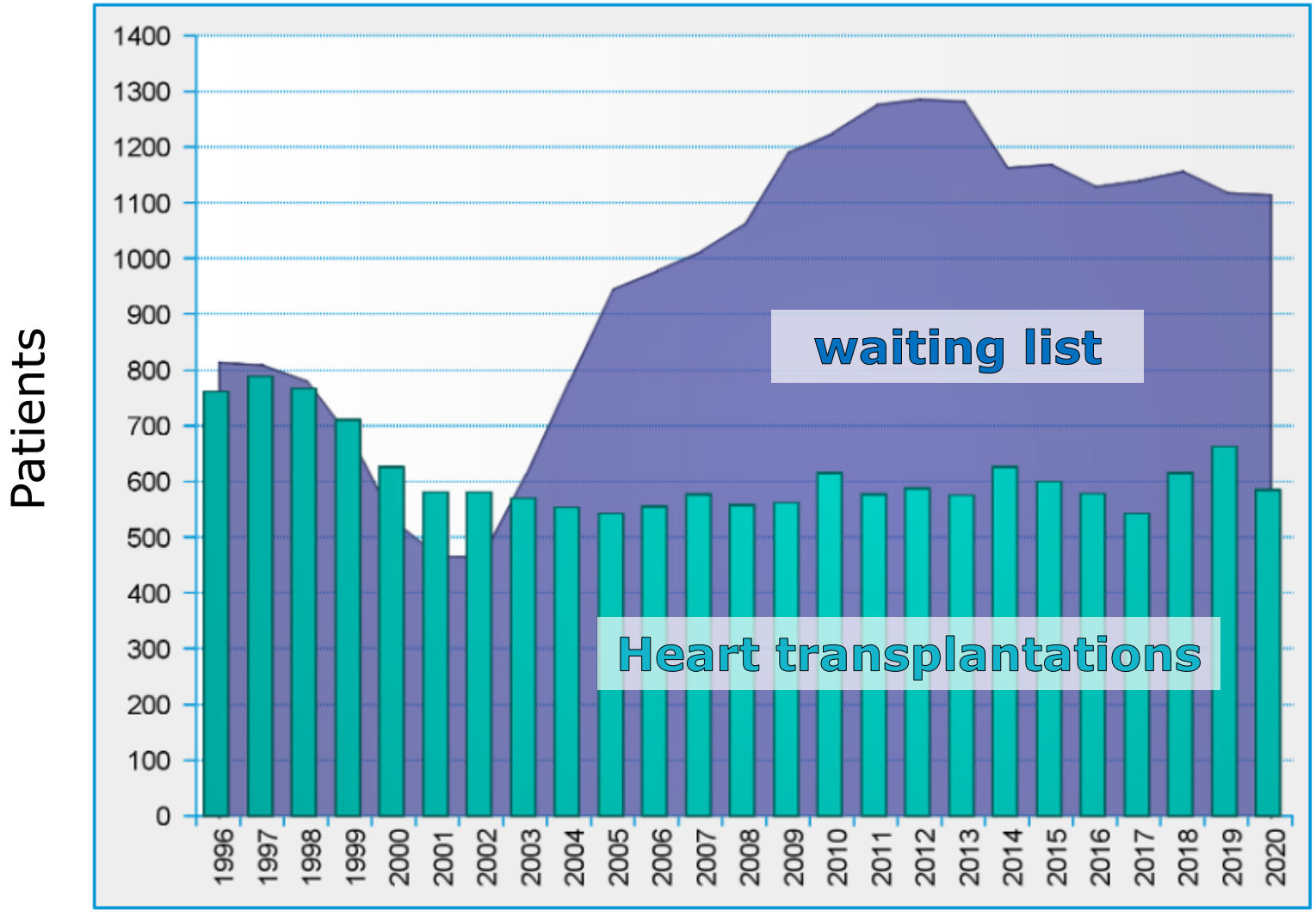
I. Buttgereit

F. Wall

M. Leuschen

Challenge: Organ shortage

Cardiac allotransplantation



Eurotransplant International Foundation 2021

Challenge: Organ shortage

Alternative solution: Xenotransplantation

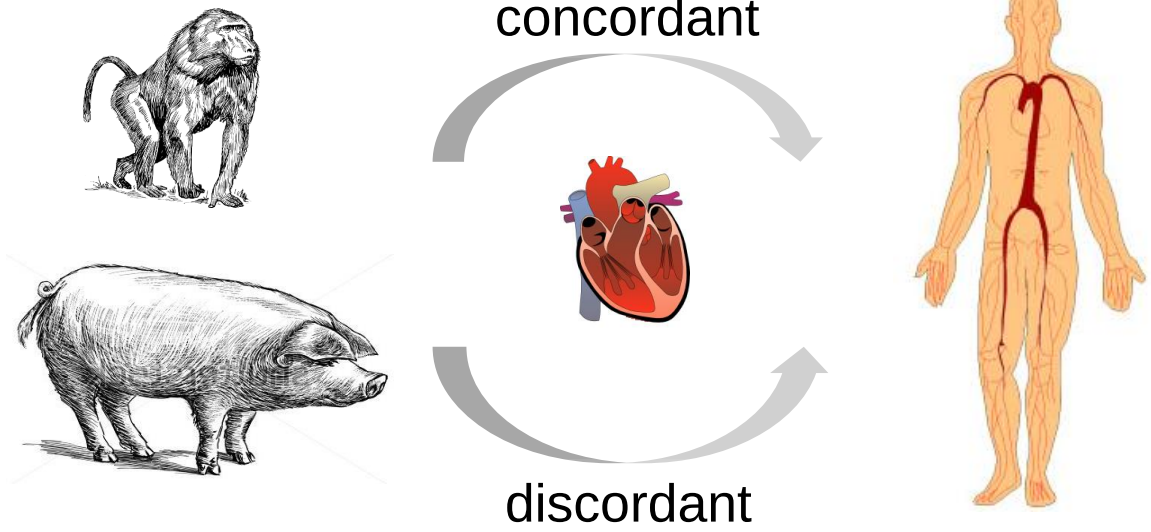
Google xenotransplantation definition

xenotransplantation

/ˌziːnə(ʊ)ˌtranspläːnˈteɪʃ(ə)n/

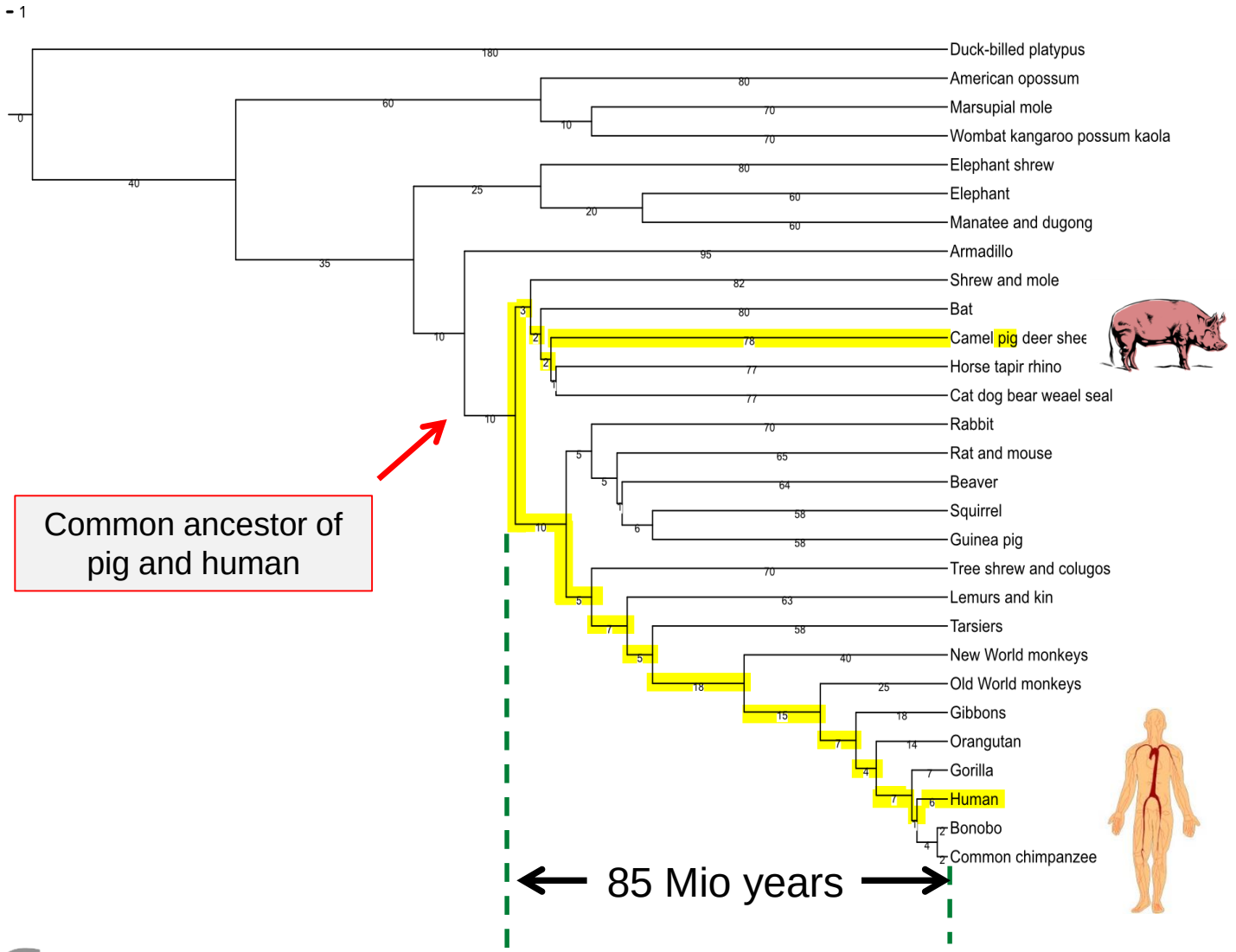
noun

the process of grafting or transplanting organs or tissues between members of different species.



Cardiac xenotransplantation

Which animal as organ donor?



Discordant xenotransplantation:

- between phylogenetically more distant species

Cardiac xenotransplantation

Which animal as organ donor?

Discordant xenotransplantation:

- between phylogenetically more distant species

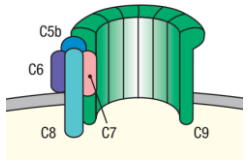
- potential donor: pig

- anatomically + physiologically largely compatible
- short gestational period
- large litters
- fast growth

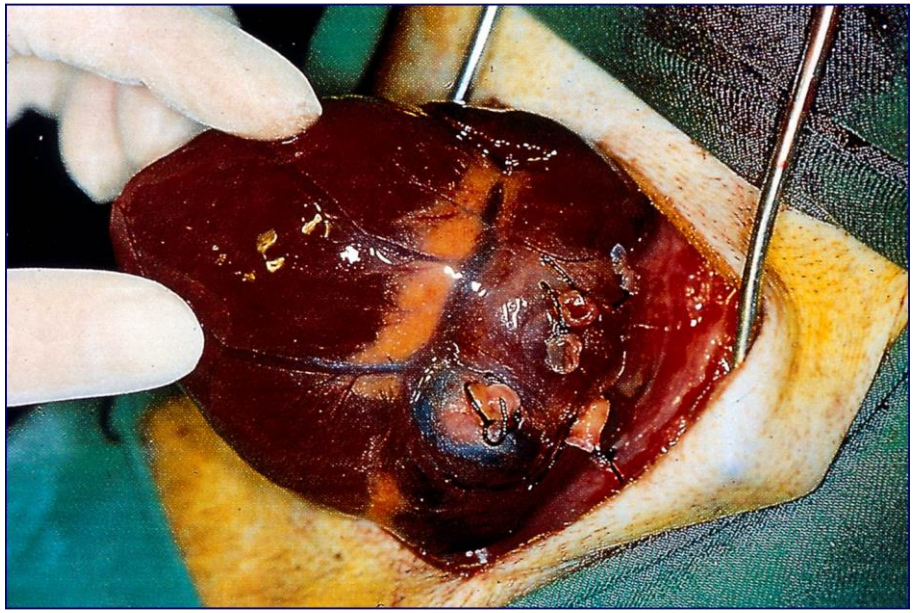
Discordant xenotransplantation

Barriers between donor and recipient

Xenoantigens and preformed antibodies



Complement activation



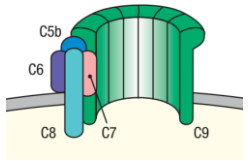
αGAL-KO

Genetic modifications

Discordant xenotransplantation

Barriers between donor and recipient

Xenoantigens and preformed antibodies



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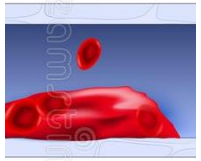
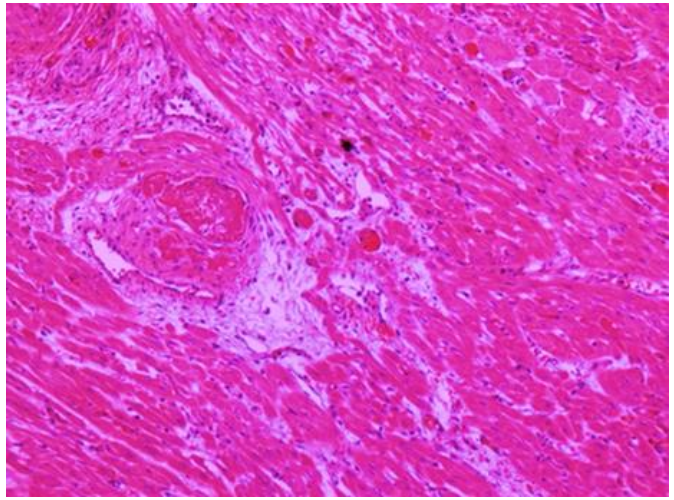


Incompatibility of the coagulation systems



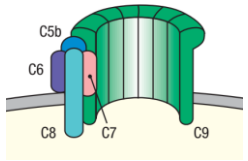
αGAL-KO

Genetic modifications

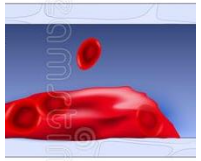
Discordant xenotransplantation

Barriers between donor and recipient

Xenoantigens and preformed antibodies

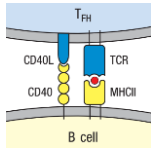


Complement activation



Incompatibility of the coagulation systems

Inflammation/innate immune system



Adaptive immune system

αGAL-KO

Genetic modifications

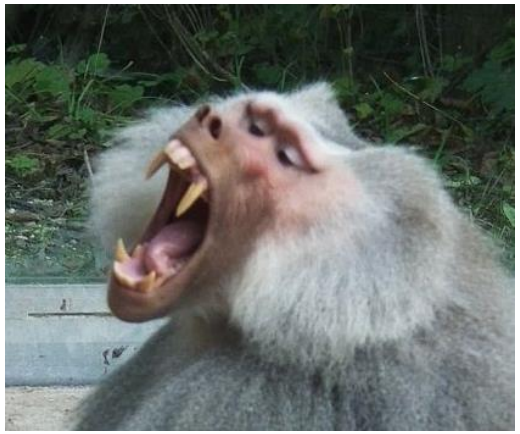
Discordant xenotransplantation

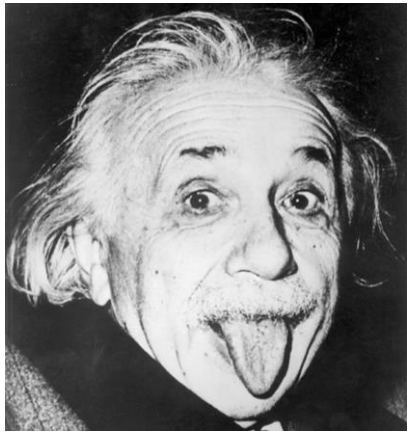
Galactose-alpha-1,3-galactose (αGal)

αGAL:



No αGAL:

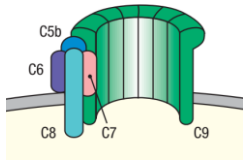




Discordant xenotransplantation

Genetic modifications of the donor pigs

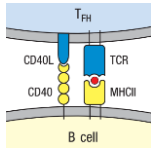
Xenoantigens and preformed antibodies



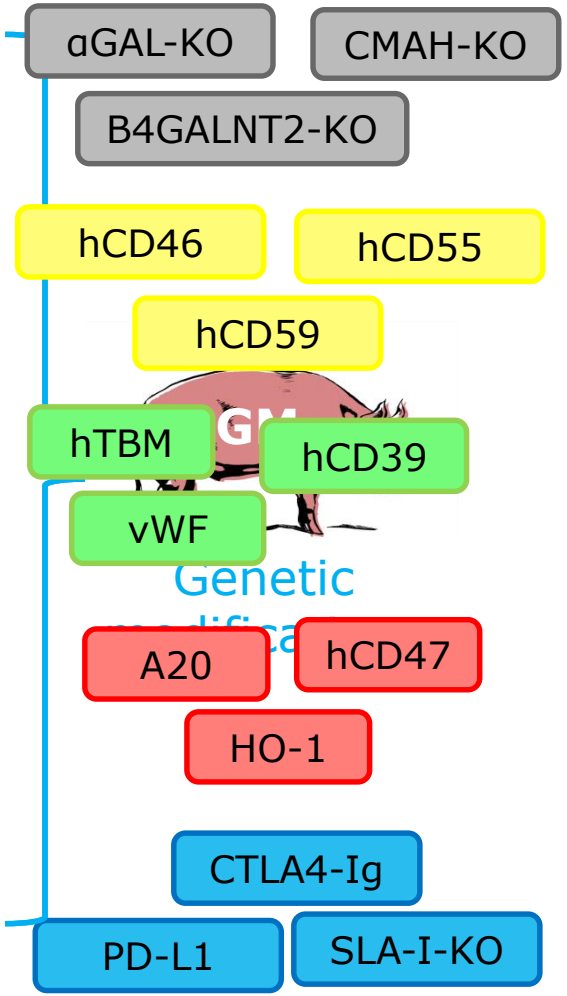
Complement activation

Incompatibility of the coagulation systems

Inflammation/innate immune system



Adaptive immune system

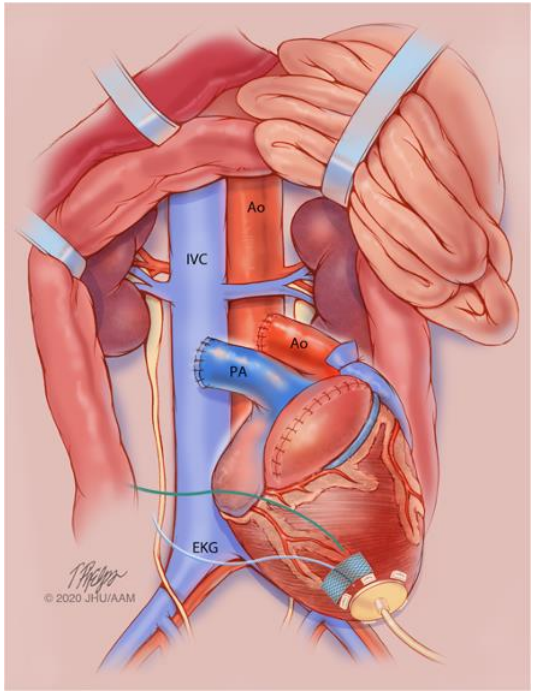


Genetic modifications: D. Ayares, Revivicor, USA und E. Wolf, LMU

Immunosuppression in xenotransplantation

Early studies

Donor	Earlier immune suppression	
	CsA/CyP/steroid	ATG/CD20/tacrolimus/sirolimus
WT	$32^{\S}$ (21 d) [27] $25^{\ddagger}$ (12 d) [28]	n.r.
WT;hCRP	99^{Δ} (26 d) [29] $78^{*\Delta}$ (35 d) [30]	$109^{*\#\dagger}$ (20 d) [31] $137^{*\#\dagger}$ (96 d) [32]
GTKO	n.r.	$128^{\dagger}$ (22 d) [34]
GTKO;hCRP	n.r.	$52^{\nabla\dagger}$ (28 d) [34]
GTKO;hCRP;TBM	n.r.	n.r.

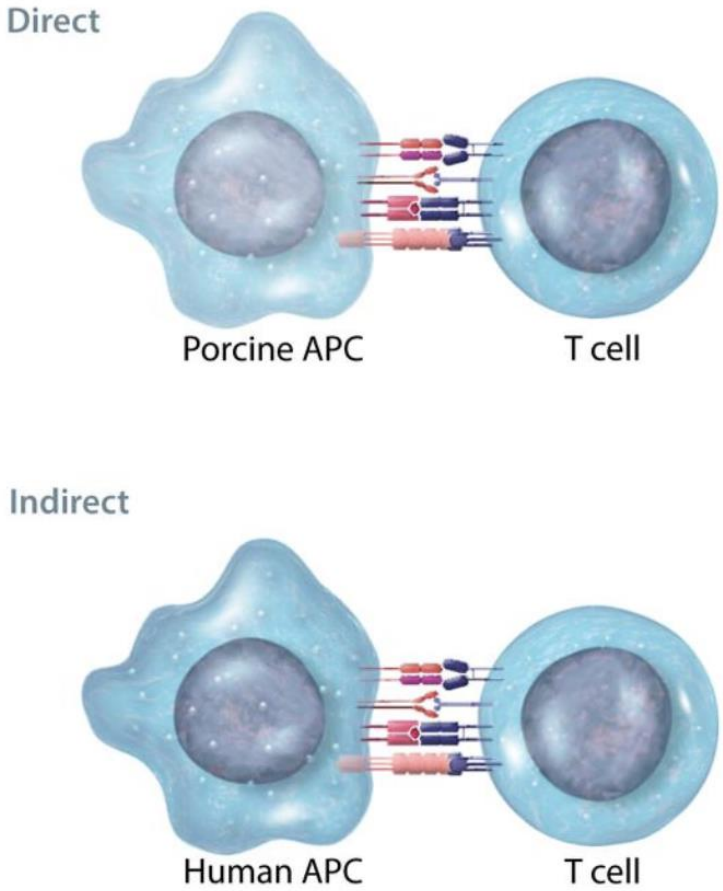


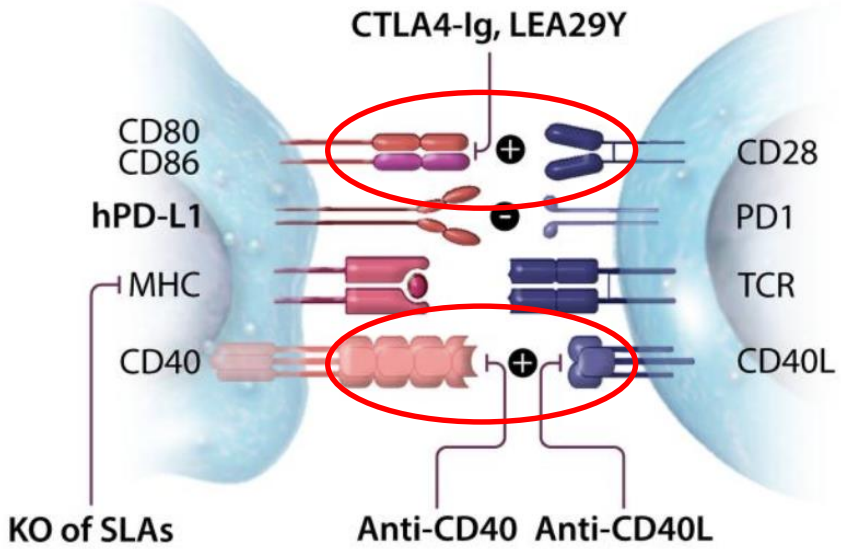
Classical Immunosuppressants are not sufficient to prevent rejection after xenotransplantation

McGregor et al., J Immunol Res 2017:2534653 (2017) (modified)
Abicht et al., Xenotransplantation 22:427-442 (2015) (modified)

Immunosuppression in xenotransplantation

T-cell activation and costimulation





In addition to TCR/MHC interaction, costimulation (e.g. CD28/B7 or CD40/CD40L) is necessary to activate T cells

Reichart et al., Cardiovascular Research 118:3499-3516 (2022)

Immunosuppression in xenotransplantation

Chimeric anti-CD40-Mab 2C10R4

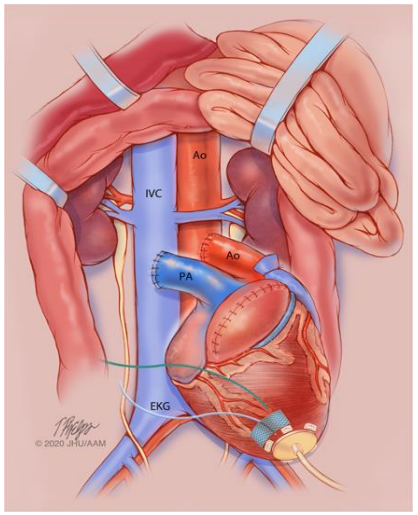
nature COMMUNICATIONS

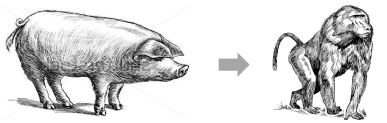
ARTICLE

Received 20 Jan 2016 | Accepted 23 Feb 2016 | Published 5 Apr 2016 DOI: 10.1038/ncomms11138 OPEN

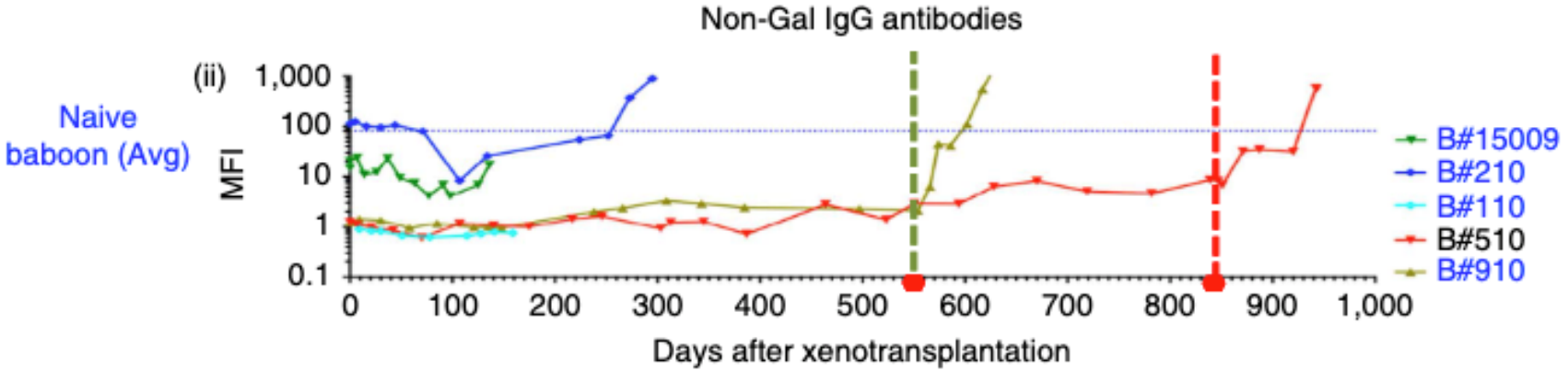
Chimeric 2C10R4 anti-CD40 antibody therapy is critical for long-term survival of *GTKO.hCD46.hTBM* pig-to-primate cardiac xenograft

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]





Heterotopic abdominal model

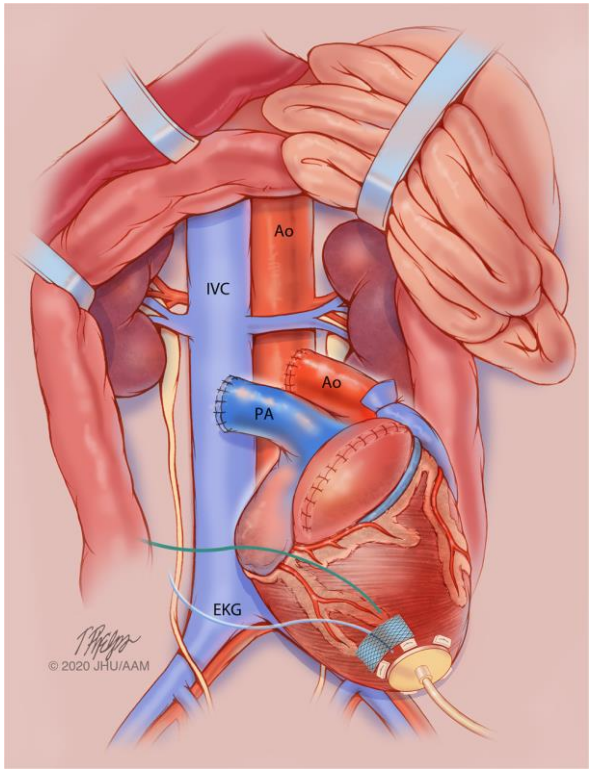


Immunosuppression with CD40/CD40L costimulation blockade prevents rejection after xenotransplantation and enables long-term survival

Mohiuddin et al., Nat Commun 7: 11138 (2016)

Orthotopic cardiac xenotransplantation

Previous results and recommendations

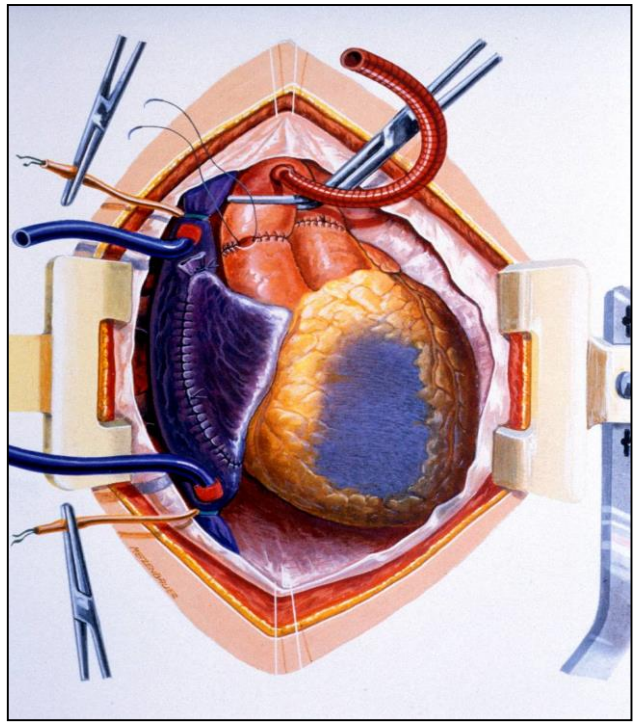


Heterotopic abdominal cardiac xenotransplantation: Not life-supporting

Mohiuddin, Reichart et al., Int J Surg, 2015; Daten aus: McGregor et al., Journal of Immunology Research (2017)
Cooper et al., J Heart Lung Transplant 19;1125-65 (2000)

Orthotopic cardiac xenotransplantation

Previous results and recommendations



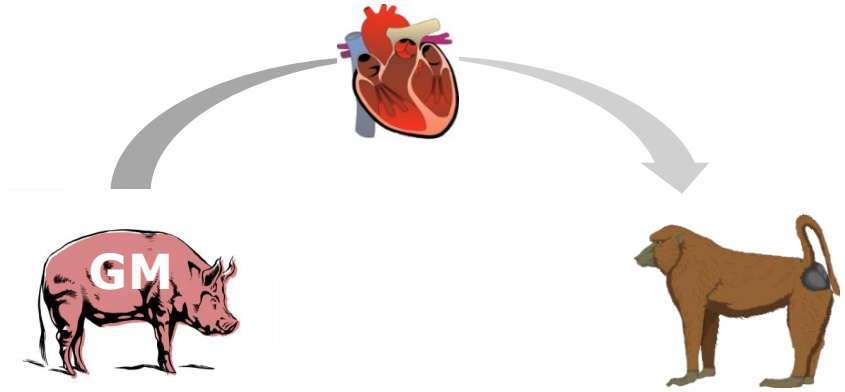
Orthotopic cardiac xenotransplantation: Life-supporting

Recommendations for cardiac xenotransplantation (ISHLT, 2000):

Life-supporting model

At least 3 months survival

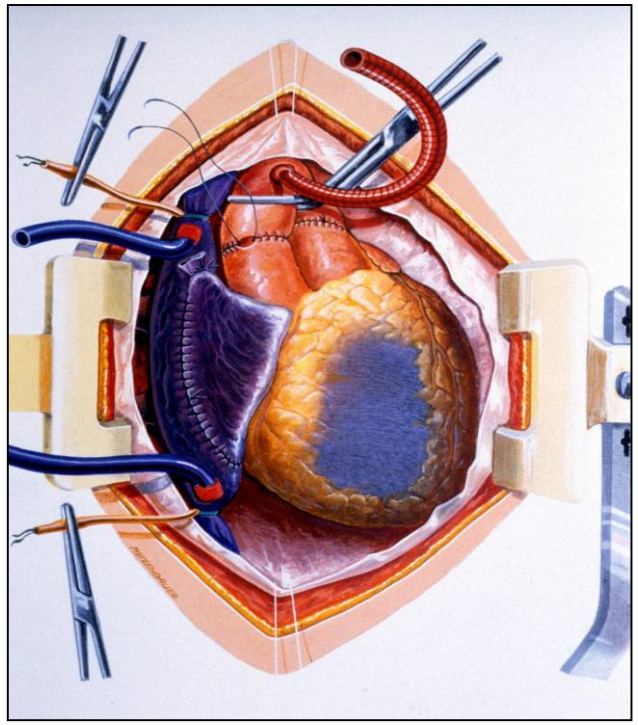
6/10 consecutive experíments



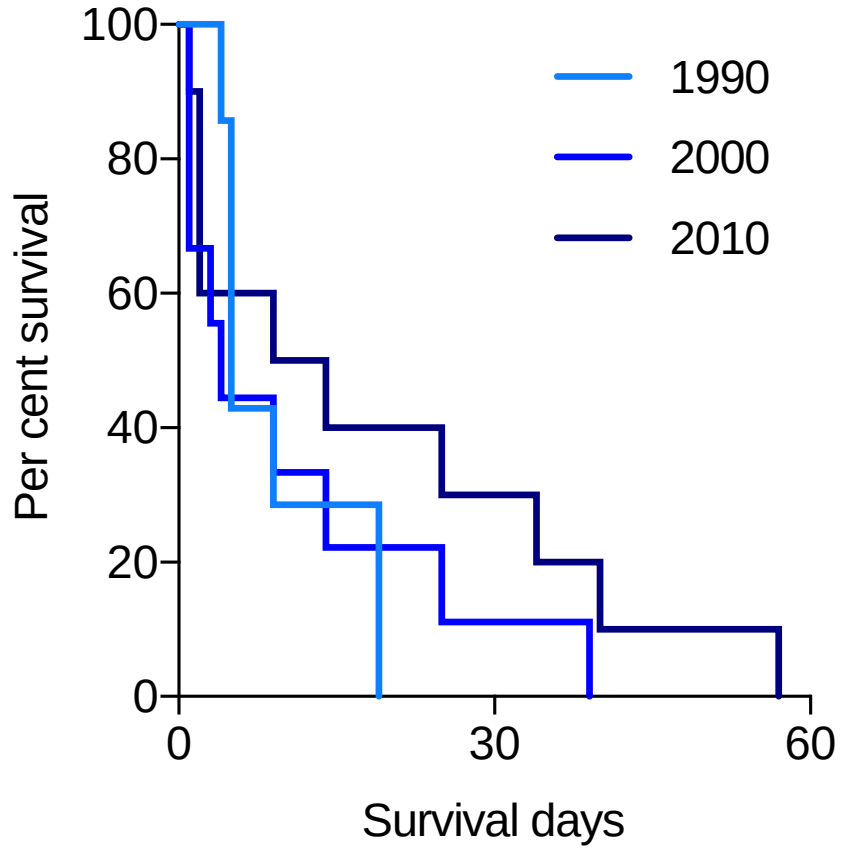
Mohiuddin, Reichart et al., Int J Surg, 2015; Daten aus: McGregor et al., Journal of Immunology Research (2017)
Cooper et al., J Heart Lung Transplant 19;1125-65 (2000)

Orthotopic cardiac xenotransplantation

Previous results and recommendations

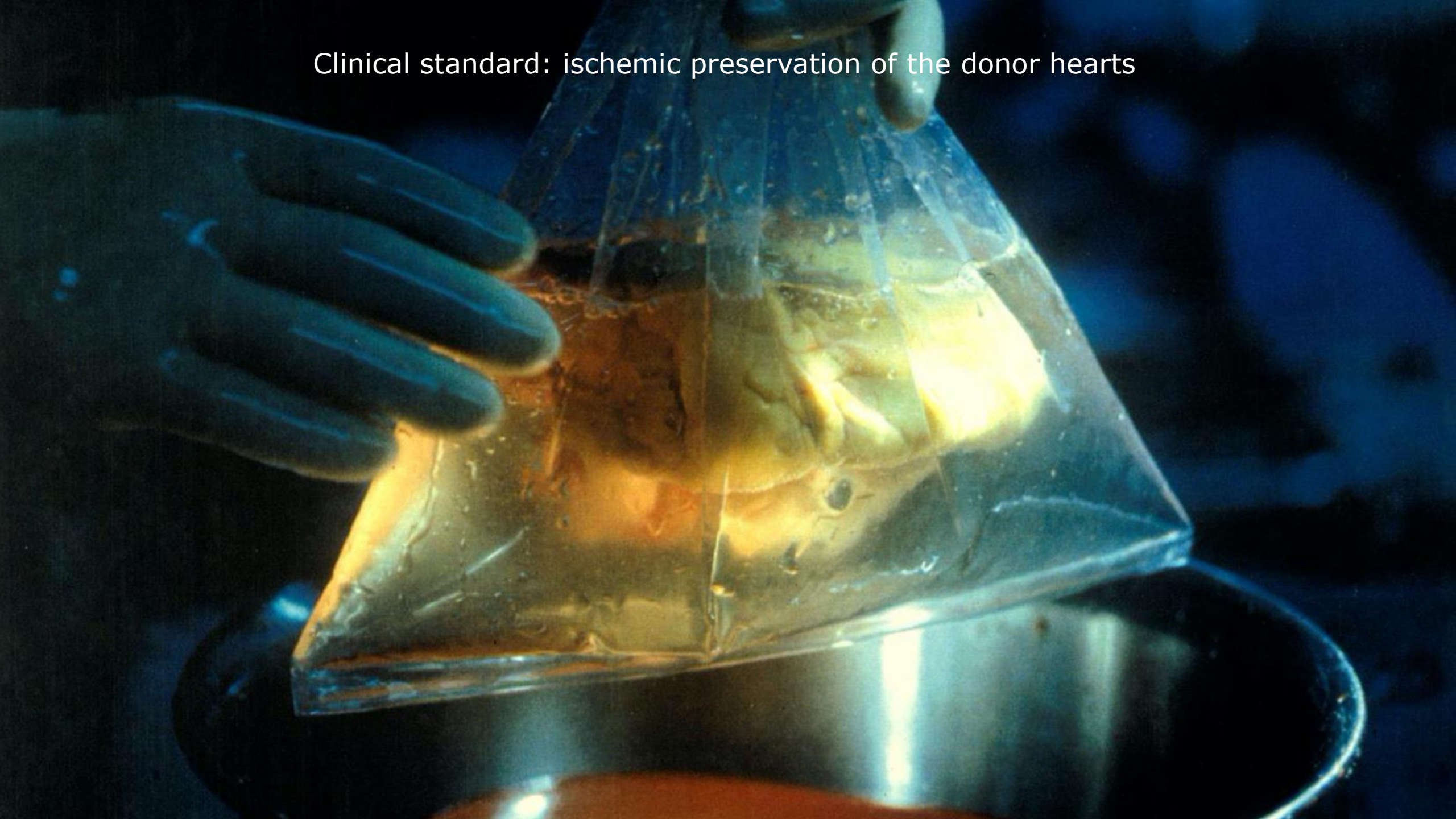


Orthotopic cardiac xenotransplantation: Life-supporting



Mohiuddin, Reichart et al., Int J Surg, 2015; Daten aus: McGregor et al., Journal of Immunology Research (2017)
Cooper et al., J Heart Lung Transplant 19;1125-65 (2000)

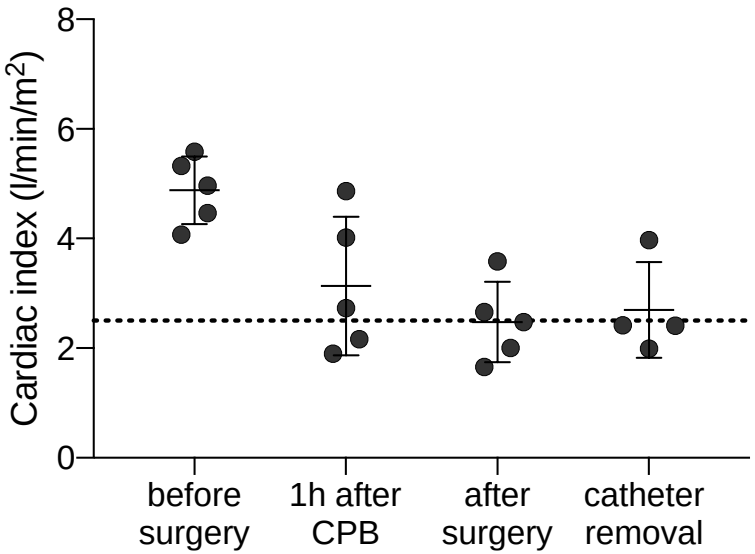
Clinical standard: ischemic preservation of the donor hearts



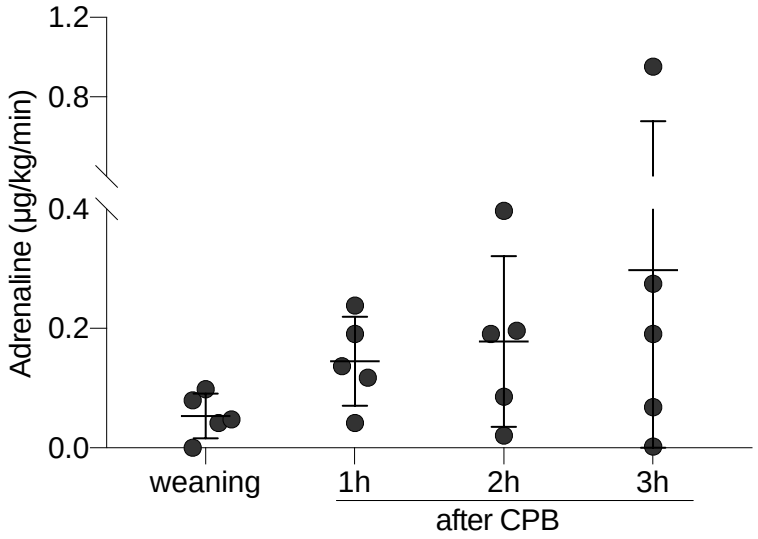
Orthotopic cardiac xenotransplantation

Group I – Graft function

Graft function



Circulatory support

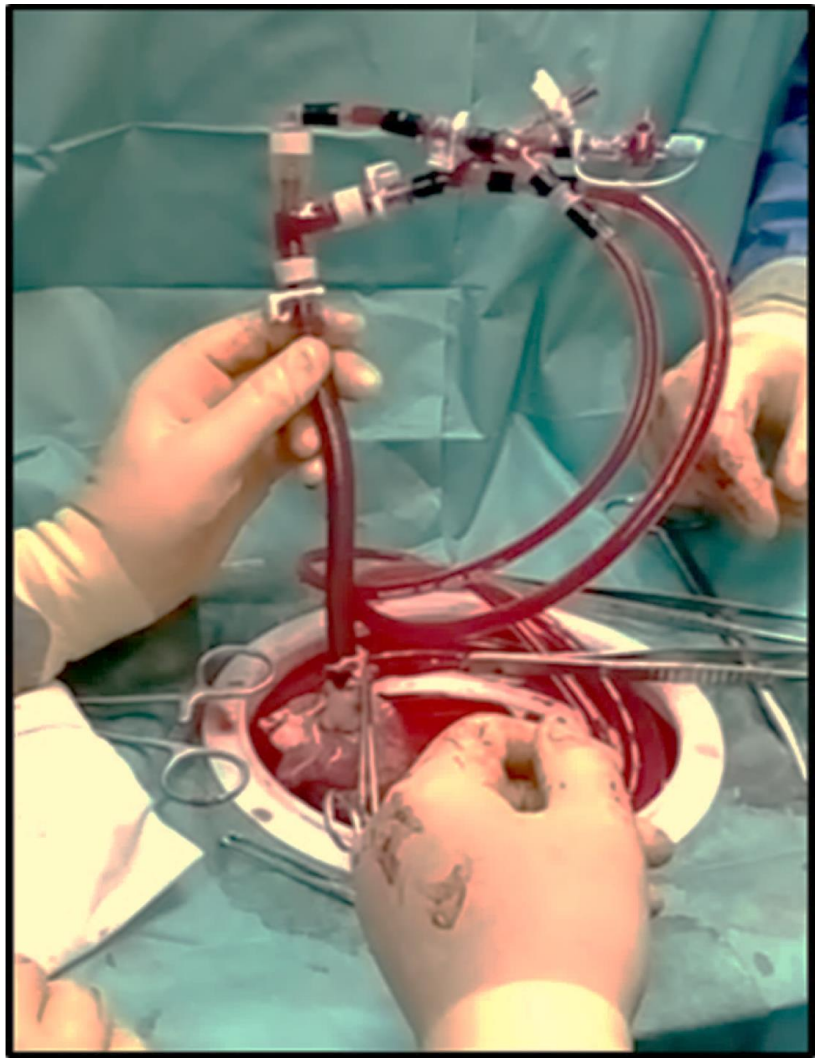


● Group I (n=5)

Is ischemic preservation unsuitable for cardiac xenotransplantation?

Orthotopic cardiac xenotransplantation

Cold non-ischemic preservation



Continious Perfusion

- Pressure- and flow-controlled
- Temperature: 8°C

Preservation solution

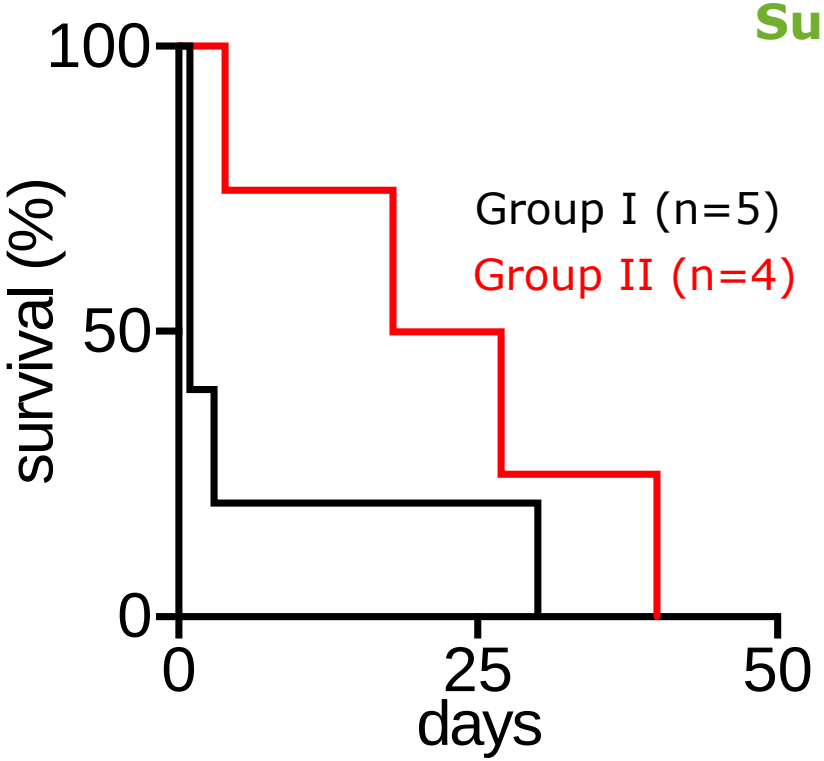
Oxygenated cardioplegic solution containing:

- Albumine
- Erythrocytes
- „Hormone cocktail"

Steen et al. Scand Cardiovasc J 50;193-200 (2016)

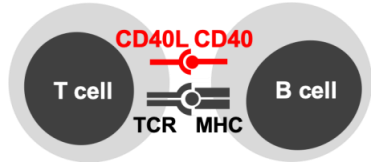
Orthotopic cardiac xenotransplantation

AG Xenotransplantation Munich – Group II



Survival [days]:

	18
	4
	27
	40

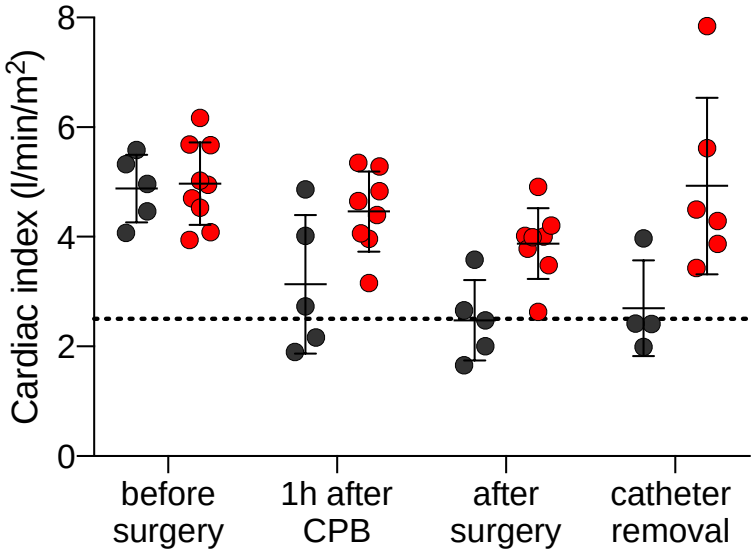


No primary graft failure using
cold non-ischemic preservation

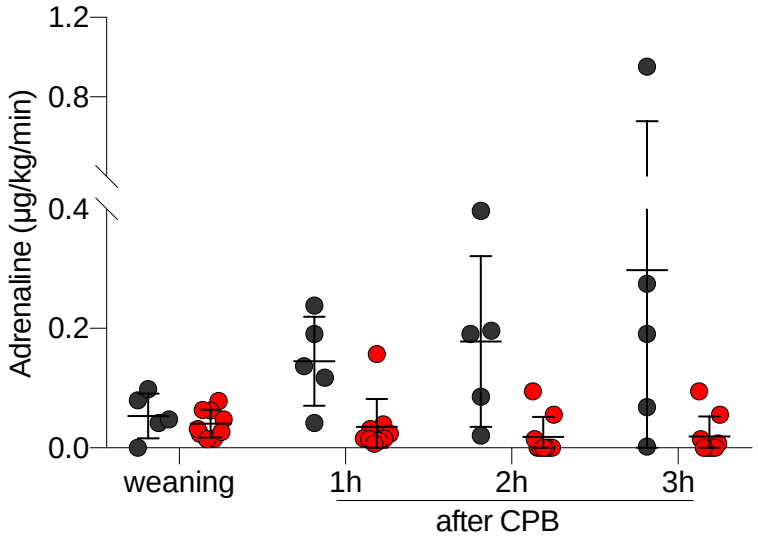
Orthotopic cardiac xenotransplantation

Group II – Graft function

Graft function



Circulatory support



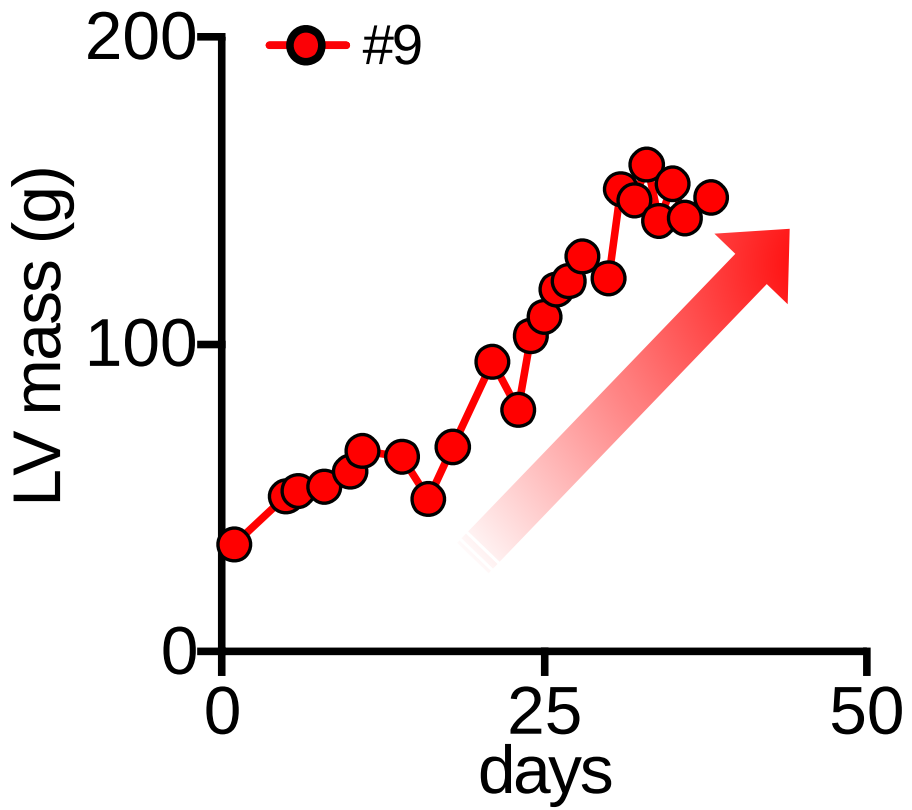
● Group I (n=5)
● Group II (n=4)

Preserved function of the xenografts

Längin et al., Xenotransplantation 2020, modifiziert

Orthotopic cardiac xenotransplantation

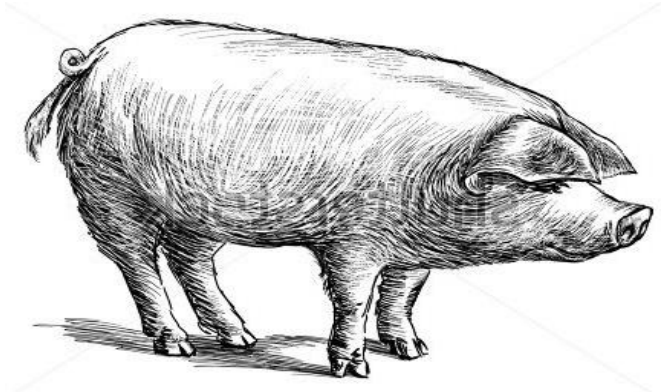
Group II – cardiac overgrowth

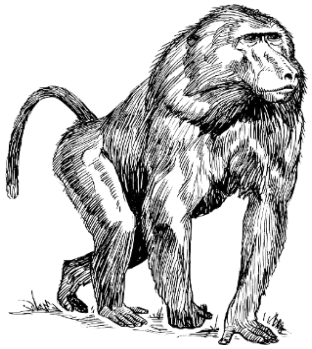


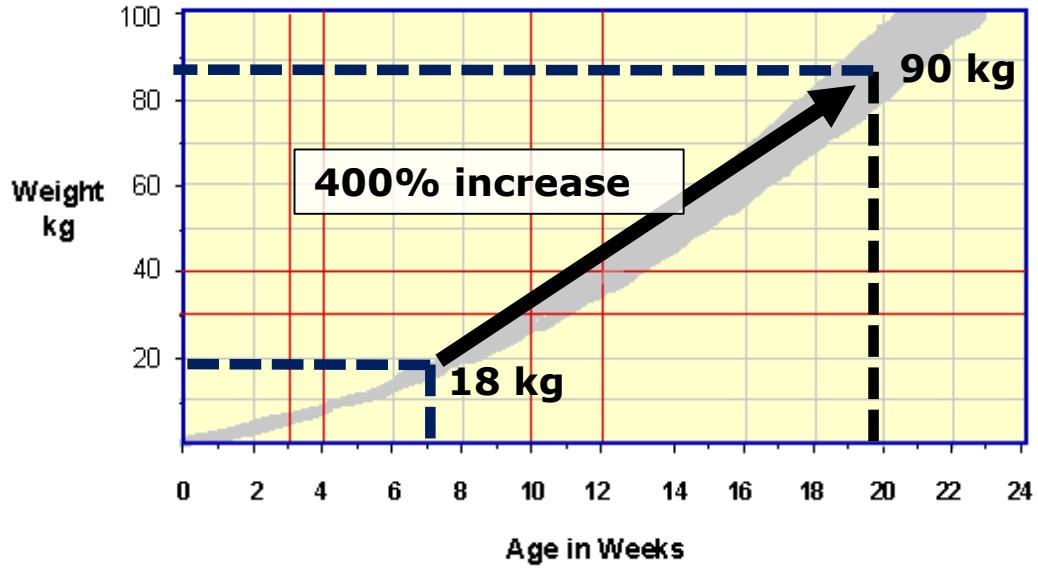
⇒ graft failure

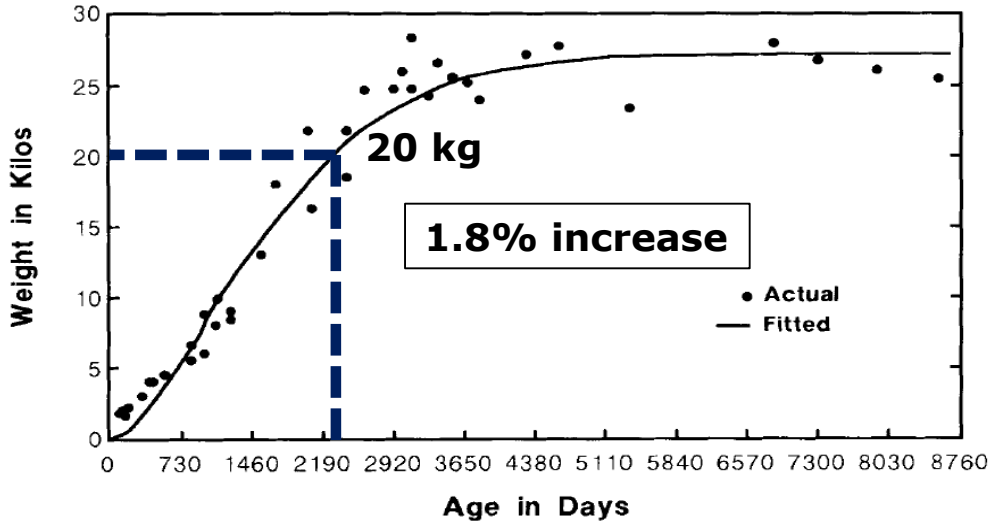
Orthotopic cardiac xenotransplantation

Intrinsic growth





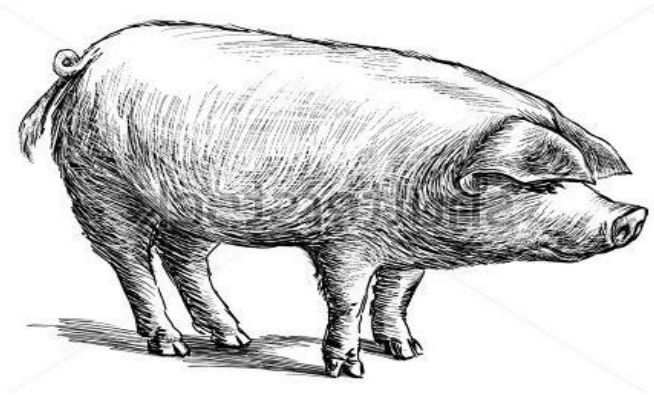




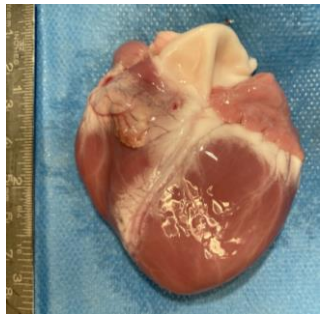
Garth Pig Stockmanship Standards, Carr J; 5m Publishing 1998; Amercan Journal of Primatology 25;219-37 (191)

Orthotopic cardiac xenotransplantation

"Normal" growth of a pig heart

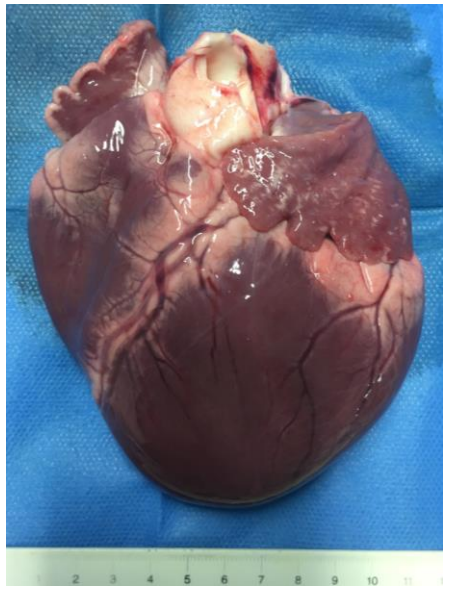


Baboon size

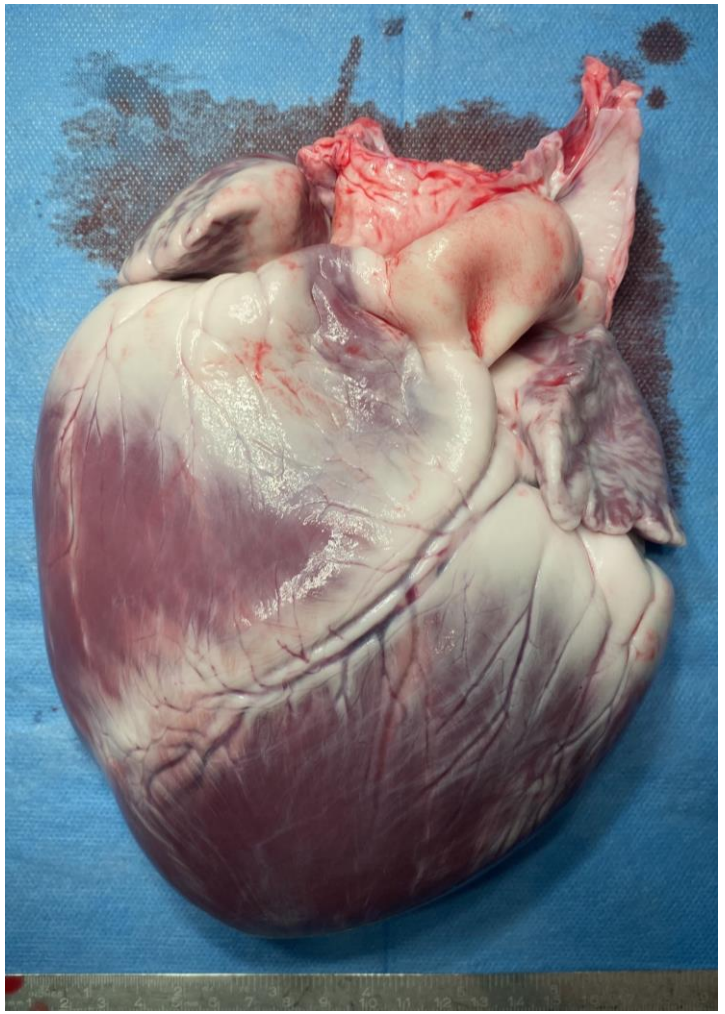


10 kg, about 4 weeks
Heart: 50 g

Human size



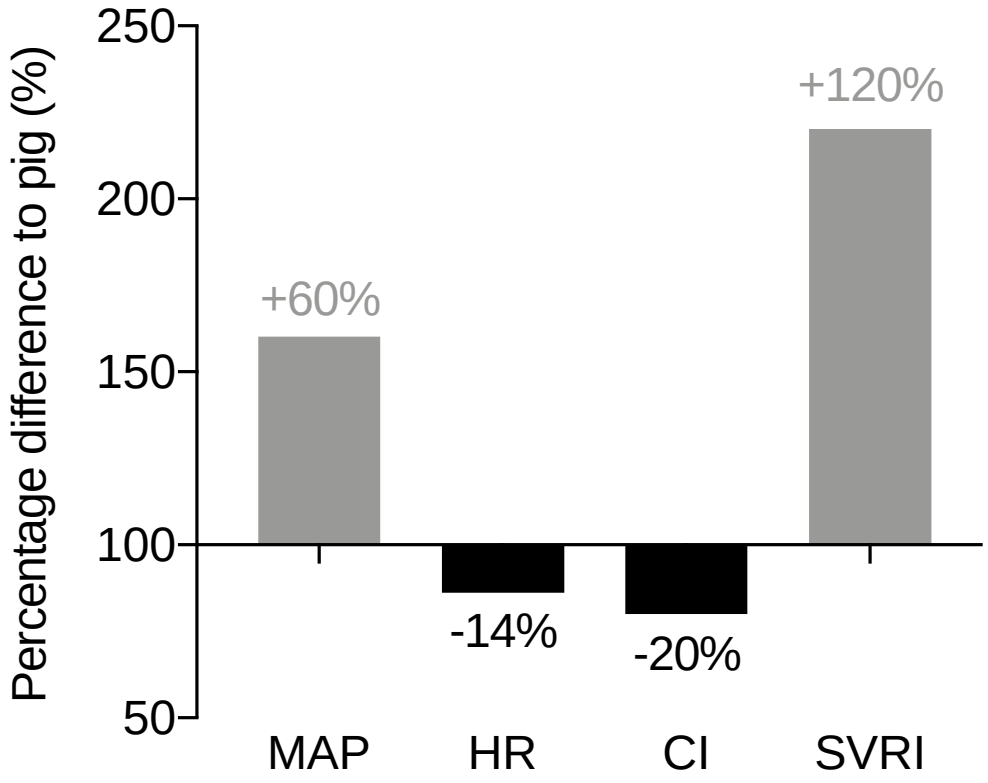
90 kg, about 6 months
Heart: 250 g

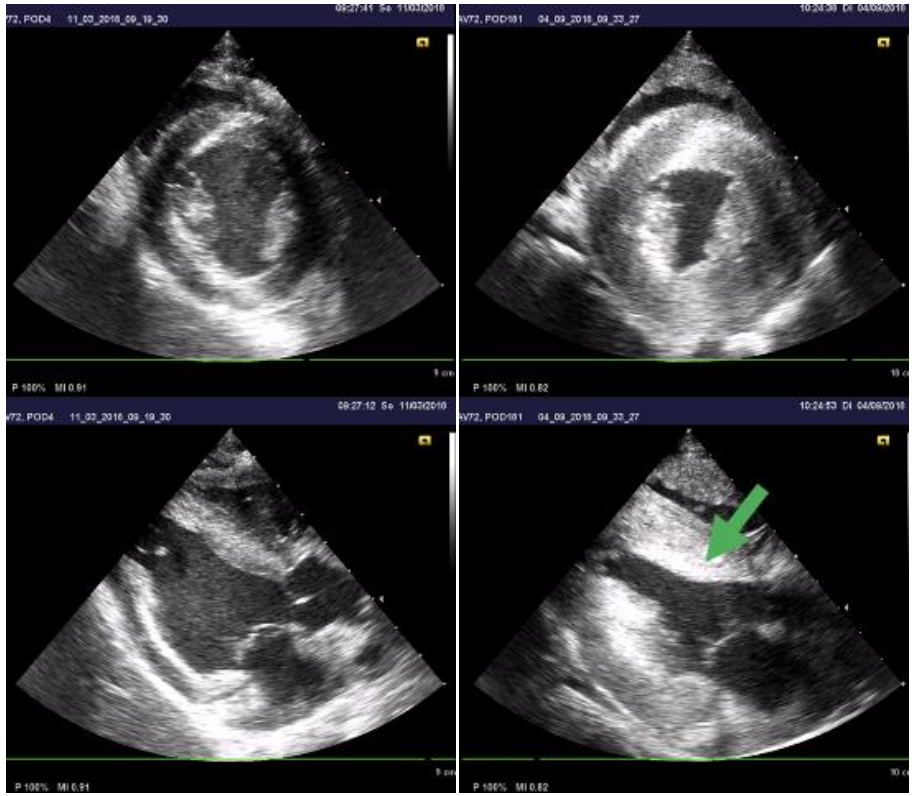


300 kg, about 3 years
Heart: 900 g

Orthotopic cardiac xenotransplantation

Extrinsic cardiac growth





Date of XTx End

⇒ increased afterload induces compensatory myocardial hypertrophy

Längin et al., Xenotransplantation (2019), modified; Längin et al, Transplantation (2023), modified

Orthotopic cardiac xenotransplantation

Drug-based growth inhibition

β-blocker
ACE-inhibitor

- Reduction of cardiac remodelling
- Reduction of left ventricular mass

mTOR-inhibitor
(Rapamycine)

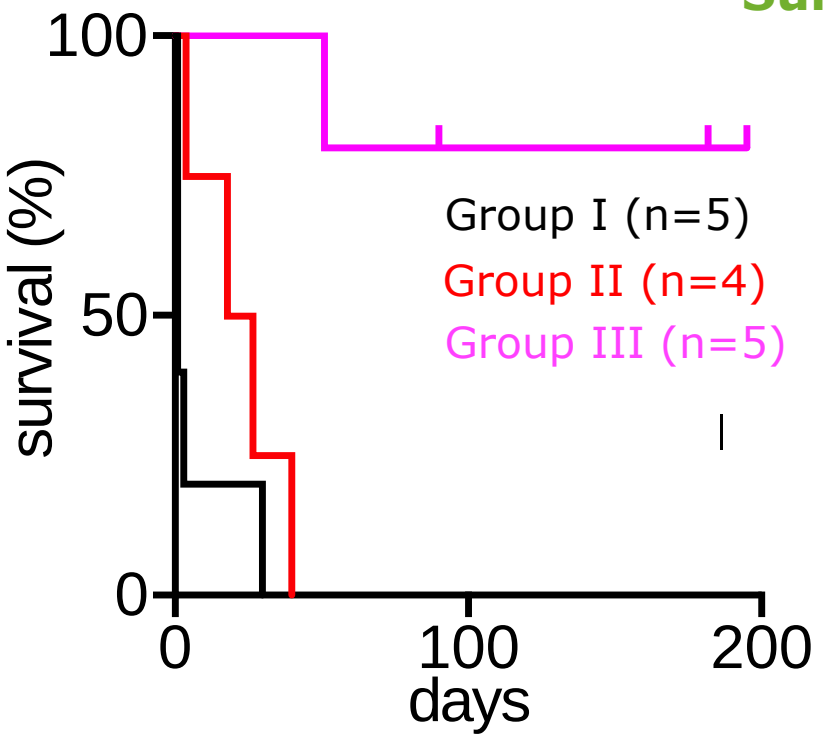
- Inhibition of cell growth and proiliferation
- Reduction/inhibition of cardiac hypertrophy

Reduction of steroid dosage

- High-dose steroids can trigger cardiac hypertrophy in juvenile animals and humans

Orthotopic cardiac xenotransplantation

AG Xenotransplantation Munich – Group III



Survival [days]:

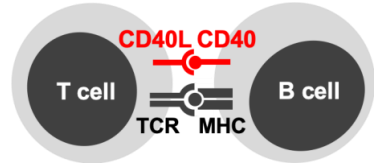
90

51

90

195

182

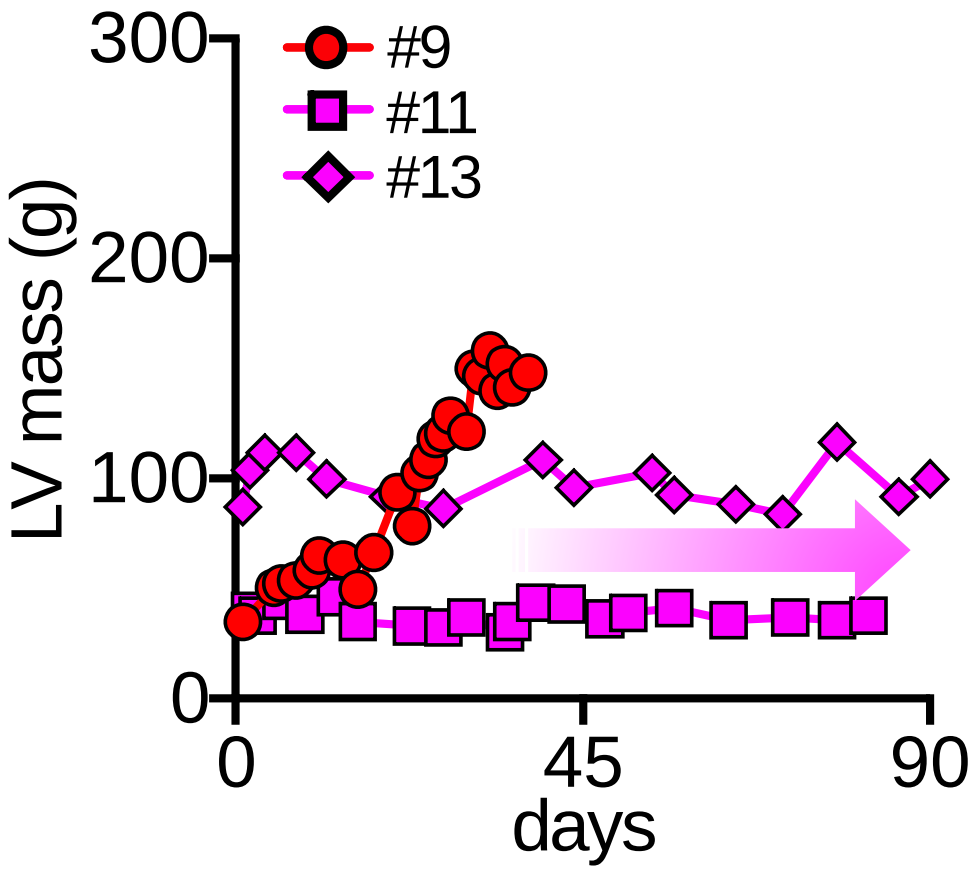


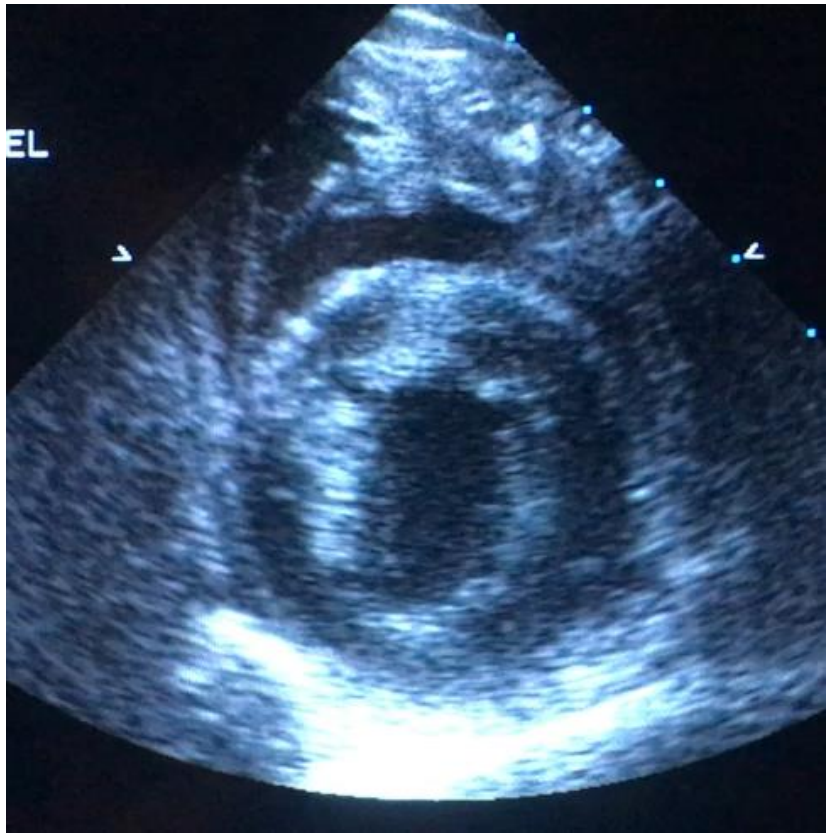
Survival ≥3 months, euthanasia in good general condition

Längin et al. Nature 564;430-3 (2018)

Orthotopic cardiac xenotransplantation

Group III – growth inhibition



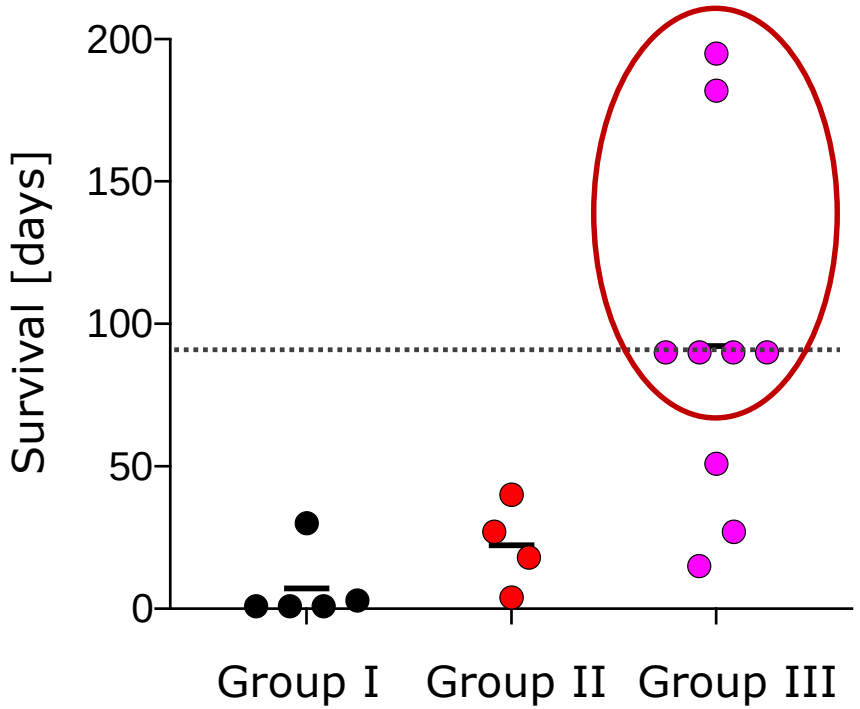


⇒ Preserved cardiac function

Längin et al. Nature 564;430-3 (2018)

Orthotopic cardiac xenotransplantation

Prerequisites for clinical trials



Recommendations for cardiac xenotransplantation (ISHLT, 2000):

- Life-supporting ✓
- At least 3 months survival ✓
- 6/10 consecutive experiments ✓

Basic requirements for clinical pilot study formally fulfilled!

Cooper et al., J Heart Lung Transplant 19;1125-65 (2000); Reichart et al. JHLT 39(8);751-7 (2020)

Orthotopic cardiac xenotransplantation

… when will we finally reach the clinic?!

…we are already there!!!

BRIEF REPORT

Genetically Modified Porcine-to-Human Cardiac Xenotransplantation

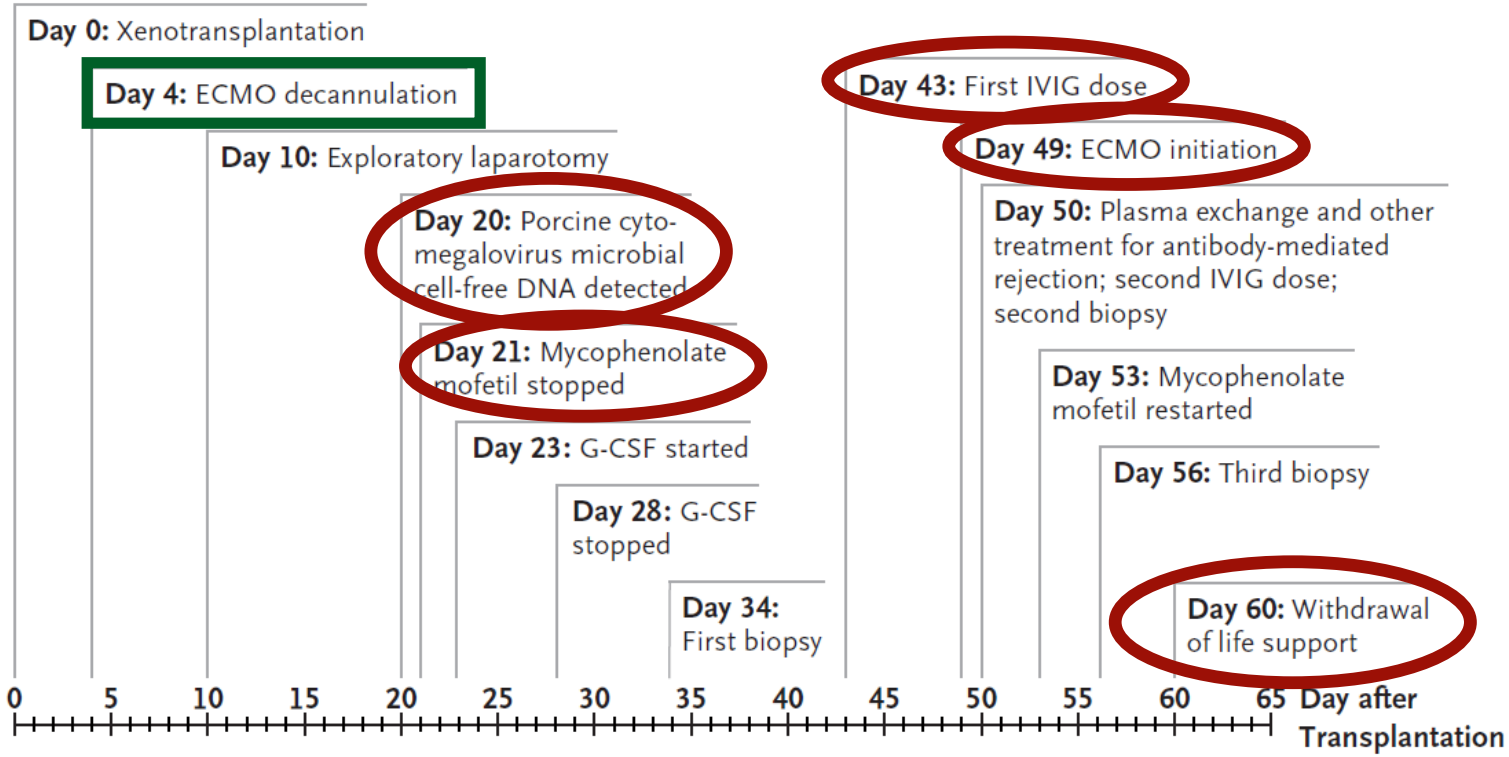
- Male patient, 57 years old, end-stage heart failure because of
  - Non-ischemic cardiomyopathy
  - Reconstructed mitral valve
  - Arterial hypertension
- Low compliance → no allo-Tx, no assist-device

Griffith et al., NEJM (2022)

Orthotopic cardiac xenotransplantation

Compassionate use, January 2022

10-fach genetisch modifiziertes Schweineherz (geklont)
„Compassionate use", IS: humanisierter anti-CD40 Antikörper



Griffith et al., NEJM (2022)
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Orthotopic cardiac xenotransplantation

Compassionate use, January 2022

- Survival: 60 days (first allo-HTx: 18 days)
  - Cause(s)?
    - Insufficient immunosuppression?, IVIG?
    - pCMV?
    - Prior medical history?, pre-/perioperative course?

Griffith et al., NEJM (2022)
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Orthotopic cardiac xenotransplantation

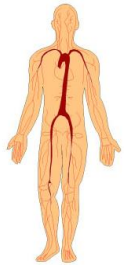
Compassionate use, January 2022
Second patient, September 2023

- Survival: 60 days (first allo-HTx: 18 days)
- Cause(s)?
  - Insufficient immunosuppression?, i.v. Ig?
  - pCMV?
  - Prior medical history?, pre-/perioperative course?
- Male patient, 58 years old, terminal heart disease
- Survival: 40 days
- Cause(s)?
  - No detailed data available yet
  - *"Heart began to show initial signs of rejection"*

Griffith et al., NEJM (2022), https://www.medschool.umaryland.edu
© University of Maryland School of Medicine, 655 W. Baltimore Street, Baltimore MD 21201

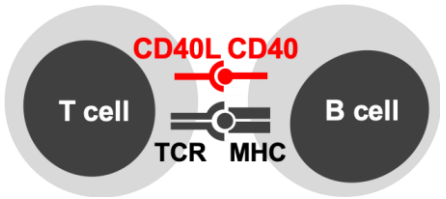
First clinical trial

Challenges



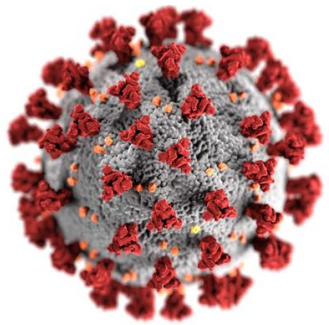
Donor animals

- Genetic modifications?
- Size mismatch/growth?



Immunology

- Antibody? Anti-CD40? Anti-CD40L?
- Xeno cross-matching?

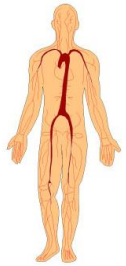


Infections/ Zoonoses

- Pathogen-free donor animals

First clinical trial

Challenges



Donor animals

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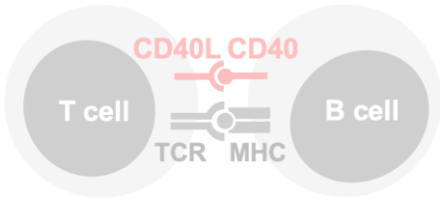

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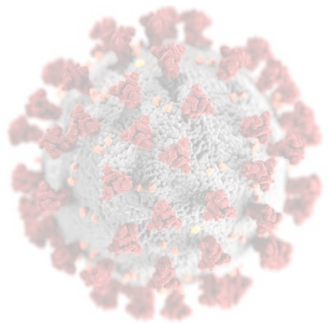
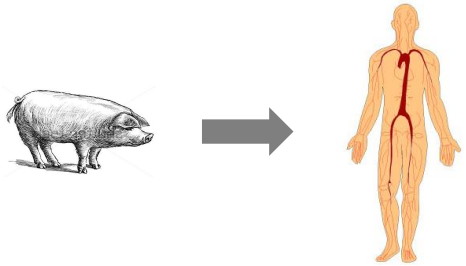


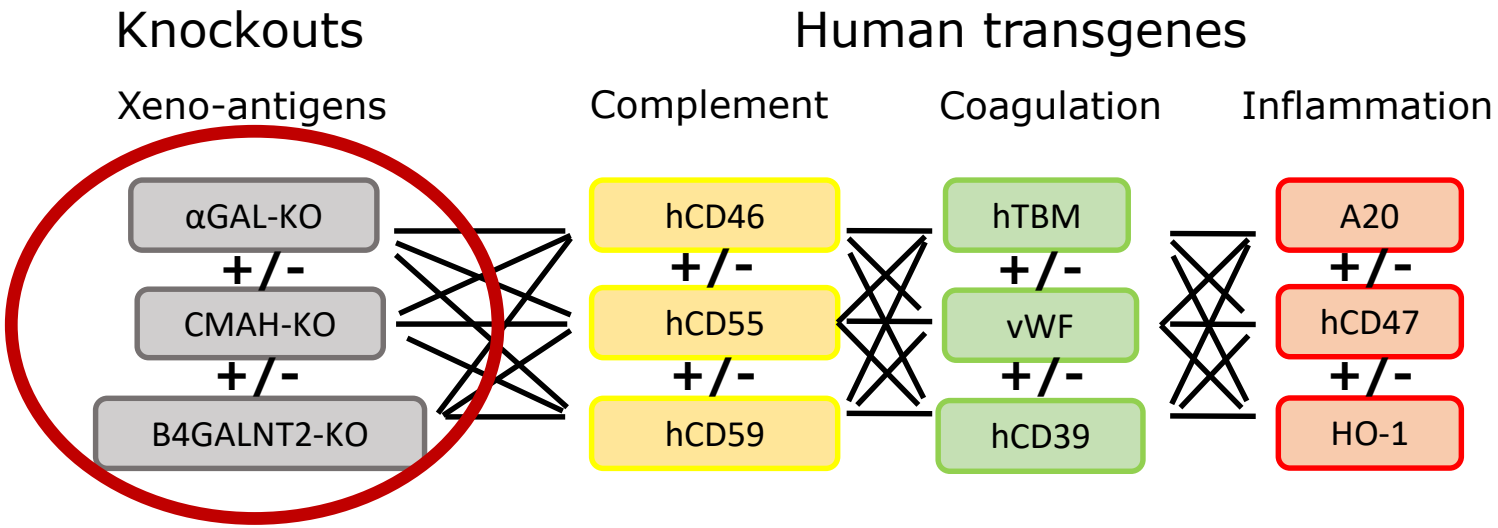
Infections/ Zoonoses

- Pathogen-free donor animals

Ideal donor pigs

Genetic Modifications

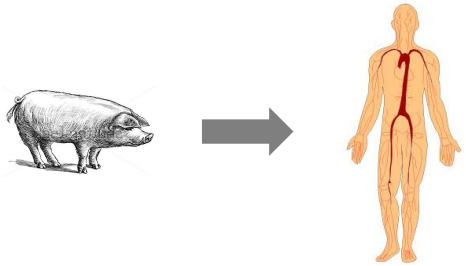


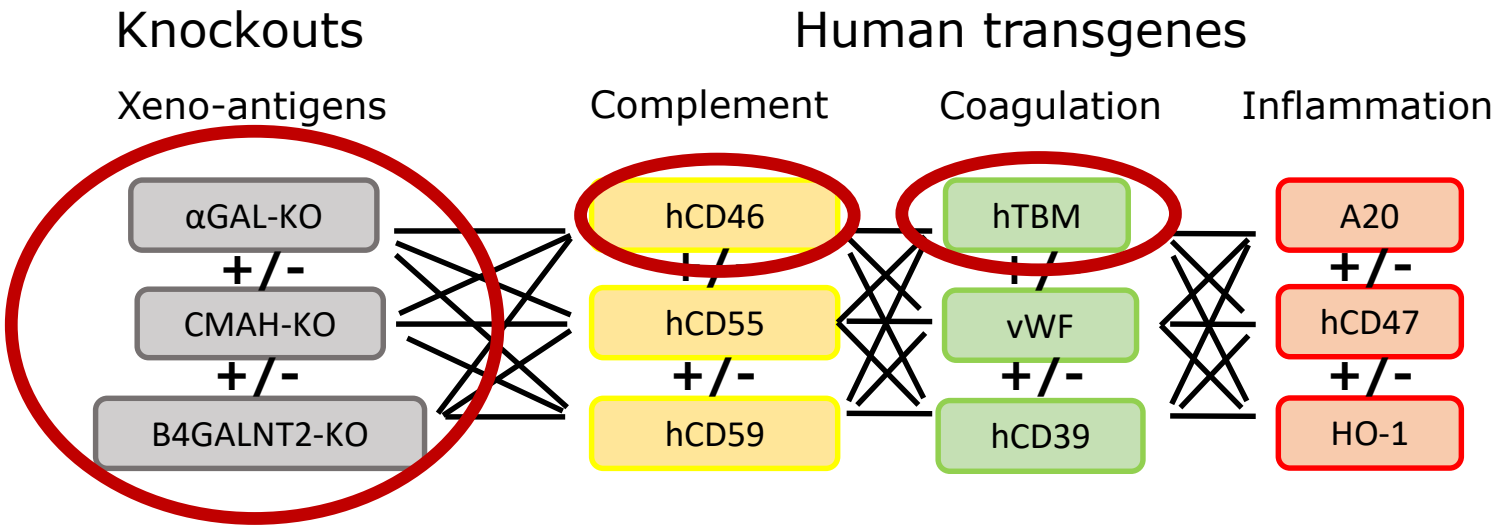


- Three knockouts
- Human Transgenes
  - As much as possible or as few as necessary?
  - Cloning or breeding?

Ideal donor pigs

Genetic Modifications

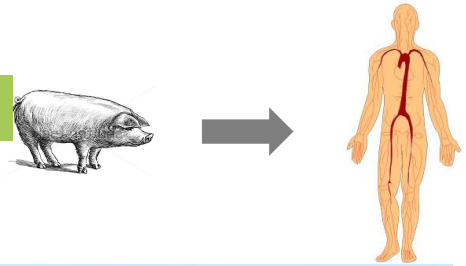




- Three knockouts
- Human Transgenes
  - As much as possible or **as few as necessary!**
  - Cloning or **breeding!**

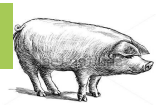
Ideal donor pigs

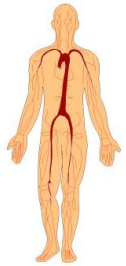
Growth – Auckland Island pigs



Ideal donor pigs

Growth – Auckland Island pigs





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www.lmu.de/cimm/
CENTER FOR INNOVATIVE MEDICAL MODELS

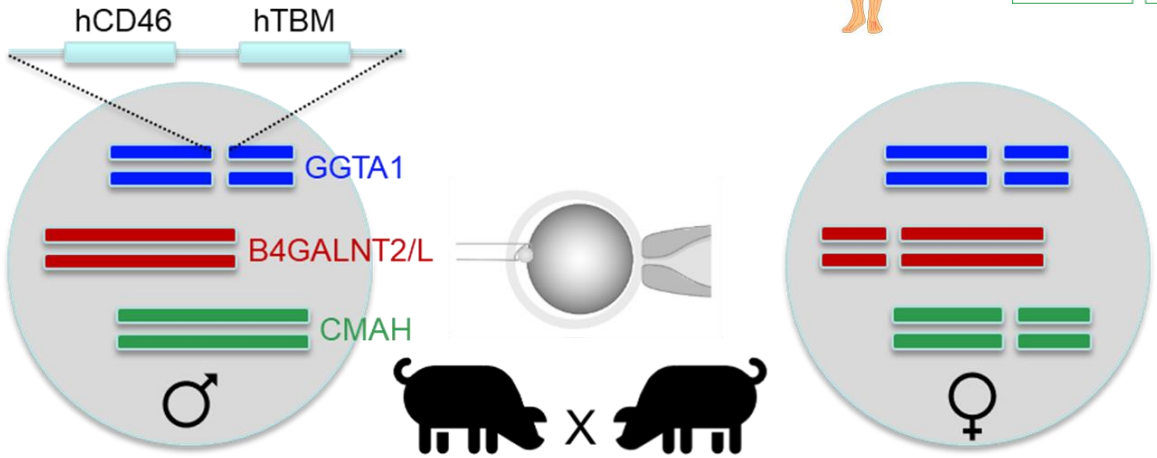
CiMM

Line A:

GGTA1-KO

hCD46-tg

hTBM-tg



Line B:

GGTA1-KO

CMAH-KO

B4GALNT2-KO

F1: GGTA1$^{-/-}$, CMAH$^{+/-}$, B4GALNT2/L$^{+/-}$, hCD46, hTBM → baboon

F2: GGTA1$^{-/-}$, CMAH$^{-/-}$, B4GALNT2/L$^{-/-}$, hCD46, hTBM → human

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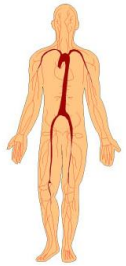
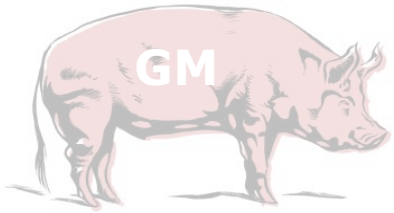
Walter Brendel Zentrum München

First clinical trial

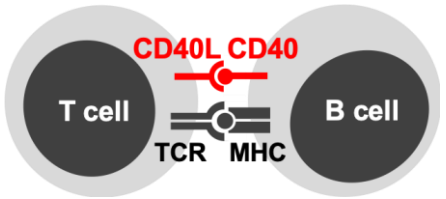
Challenges





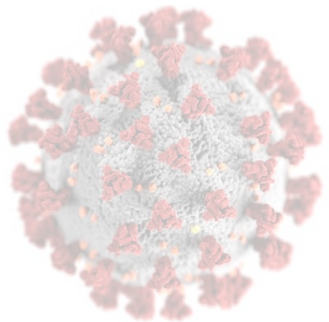
Donor animals

- Genetic modifications?
- Size mismatch/growth?



Immunology

- Antibody? Anti-CD40? Anti-CD40L?
- Xeno cross-matching?



Infections/ Zoonoses

- Pathogen-free donor animals

First clinical trial

Which antibody?

TABLE 1.

Anti-CD154 antibodies targeting the CD40/CD40L pathway in the pipeline

Name of agent	Experiment
Anti-CD154	
Ruplizumab (hu5C8)	Cardiac xenotra
Toralizumab (IDEC-131)	Cardiac xenotra
ABI793	Cardiac xenotra
	Islet xenotransp
H106	Islet xenotransp
Fc receptor-modified CD154 antibody	
CDP7657 (Dapirolizumab Pegol)	SLE[39,40]
Tegoprubart (AT-1501)	Kidney transpla
Letolizumab (BMS-986004)	Hematopoietic s transplantati
Dazodalibep (HZN-4920/VIB4920)	Kidney transpla
TNX-1500	Kidney transpla
	Cardiac transpl

SLE, systemic lupus erythematosus.

TABLE 2.

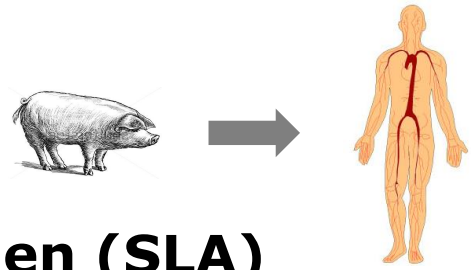
Anti-CD40 antibodies targeting the CD40/CD40L pathway in the pipeline

Name of agent	Experiment	Outcome	Clinical trial status
Anti-CD40			
ch5D12	Kidney allotransplantation[48]	Prevented rejection and extended kidney allograft survival up to 217 d in rhesus monkey	
3A8 (3A8R1)	Skin and islet transplantation[49] Cardiac xenotransplantation[50]	Prolonged alloislet transplantation model up to 298 d in NHP; demonstrated cardiac xenotransplantation up to 27 d in NHPs	
Chi220	Islet transplantation[13,14,51]	Suppressed the primary immune response to cytomegalovirus and modestly prolonged renal and islet allograft survival; prolonged survival of porcine neonatal islet in rhesus macaques	
2C10R4	Cardiac, liver, kidney xenotransplantation[12,52-56]	Prolonged cardiac xenotransplantation up to 945 d in heterotopic and 264 d in life-supporting orthotopic model; prolonged liver xenotransplantation up to 31 d	
Bleselumab 4D11 (ASKP1240)	Kidney transplantation[57-60] Islet allotransplantation[61]	Prevents renal allografts rejection in cynomolgus monkeys and suppresses antidonor antibodies	
Iscalimab (CFZ533)	Kidney transplantation[62] Liver transplantation[63]	Prevented allograft rejection in renal (phase 1) and liver transplant recipients	Phase 1
KPL-404	Rheumatoid arthritis[64,65]	Suppresses the T-cell–dependent antibody response (TDAR); has been used in first porcine–to-human cardiac xenotransplantation	Phase 1
Oligonucleotide (RNAi) for anti-CD40			
NJA-730a	Healthy volunteer[66]	Prolongs the engraftment of bone marrow in murine recipients (up to 45 d)	Phase 1

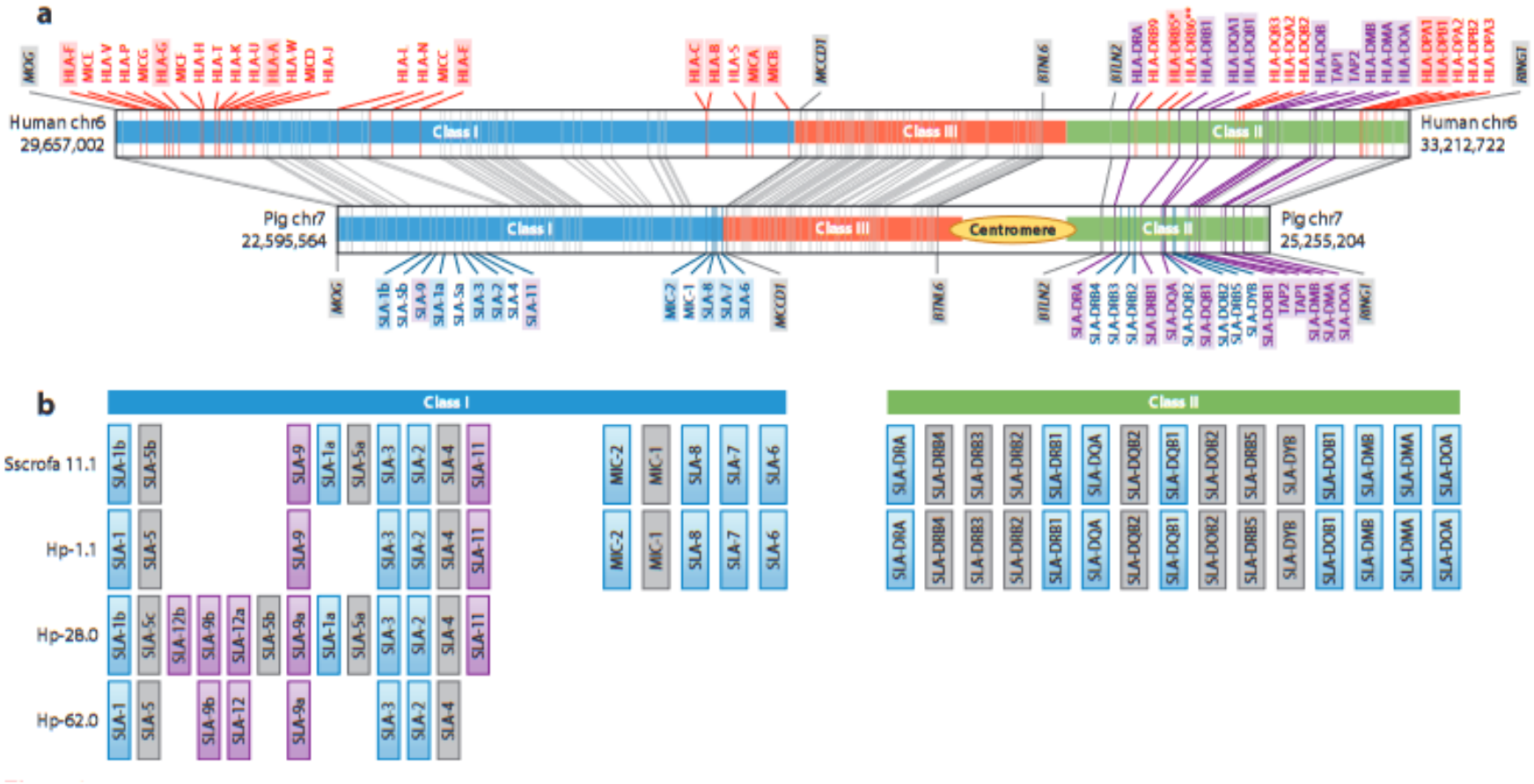
Singh et al., Transplantation (2023)

First clinical trial

Immunology – Xeno cross-matching?



Swine Leukocyte Antigen (SLA)

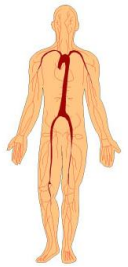


structurally very similar to HLA

Hammer et al., Annu. Rev. Anim. Biosci. (2020)

First clinical trial

Immunology – Xeno cross-matching?



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SLA and HLA – relevant?

Yes

HHS Public Access
Author manuscript
Transplantation. Author manuscript; available in PMC 2019 February 01.

Published in final edited form as:
Transplantation. 2018 February ; 102(2): 249–254. doi:10.1097/TP.0000000000001924.

Swine leukocyte antigen (SLA) class II is a xenoantigen

Joseph M. Ladowski, MS[1], Luz M. Reyes, PhD[1], Gregory R. Martens, MD[1], Jam MD[2], Zheng-Yu Wang, PhD[1], Devin E. Eckhoff[1], Matt Tector, PhD[1], and A. Josej MD, PhD[1]

[1]Department of Surgery, University of Alabama at Birmingham, Birmingham AL, US.
[2]Department of Surgery, Indiana University School of Medicine, Indianapolis, IN, US

J Am Soc Nephrol 14: 2677–2683, 2003

Cross-Reactivity between Swine Leukocyte Antigen and Human Anti–HLA-Specific Antibodies in Sensitized Patients Awaiting Renal Transplantation

IMMUNOBIOLOGY

HLA ANTIBODIES PRESENT IN THE SERA OF SENSITIZED PATIENTS AWAITING RENAL TRANSPLANT ARE ALSO REACTIVE TO SWINE LEUKOCYTE ANTIGENS[1,2]

Naziruddin, Bashoo[3]; Durriya, Syedah[3]; Phelan, Donna[3]; Duffy, Brian F.[3]; Olack, Barbara[3]; Smith, Douglas[4]; Howard, Todd[3]; Mohanakumar, T.[3,5]

Author Information

Transplantation 66(8):p 1074-1080, October 27, 1998.

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NO CORTÉS,‡ CLARA ALONSO BLANCO,† and
DÉS CAÑEDO§

'e la Salud, Universidad de A Coruña, A Coruña, Spain; and Departments of ental Surgery, and ‡Nephrology, Juan Canalejo Hospital, A Coruña, Spain

Allosensitization Does Not Increase the Risk of Xenoreactivity to α1,3-Galactosyltransferase Gene-Knockout Miniature Swine in Patients on Transplantation Waiting Lists

ny S. Wong, Kazuhiko Yamada, Masayoshi Okumi, Joshua Weiner, Patı -Lin Tseng, Frank J. M. F. Dor, David K. C. Cooper, Susan L. Saidman, . and David H. Sachs

HHS Public Access
Author manuscript
Transplantation. Author manuscript; available in PMC 2019 May 01.

Published in final edited form as:
Transplantation. 2018 May ; 102(5): e195–e204. doi:10.1097/TP.0000000000002060.

IMMUNE RESPONSES OF HLA-HIGHLY-SENSITIZED AND NONSENSITIZED PATIENTS TO GENETICALLY-ENGINEERED PIG CELLS

Xenotransplantation 2006: 13: 357–363
Printed in Singapore. All rights reserved
doi: 10.1111/j.1399-3089.2006.00319.x

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XENOTRANSPLANTATION

taka Hara, MD, PhD[1,], Cassandra Long[1], Iwase Hayato, MD, milla Macedo, MD[1], Massimo Mangiola, PhD[2], Adriana ib, MD[1], David Ayares, PhD[4], David K. C. Cooper, MD, PhD, MD[1]*

Allosensitized humans are at no greater risk of humoral rejection of GT-KO pig organs than other humans

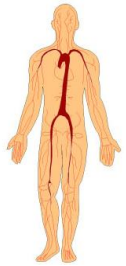
- Cooperation with:
  - Andrea Dick, Laboratory for Immunogenetics and Molecular Diagnostics, LMU Munich
  - Teresa Kauke, Division for Thoracic Surgery, LMU Munich
- Retrospective analyses of the preclinical pig-to-baboon experiments
- „Prospective" pig-to-human analyses

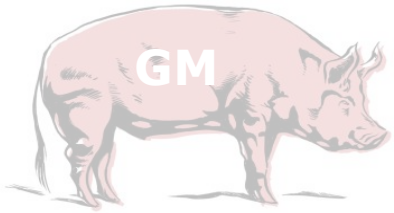
DFG SFB 127

Walter Brendel Zentrum München

First clinical trial

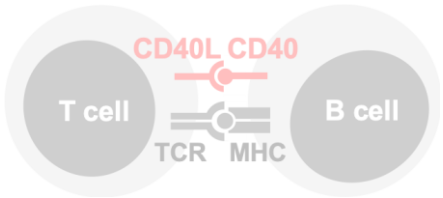
Challenges





Donor animals

- Genetic modifications?
- Size mismatch/growth?



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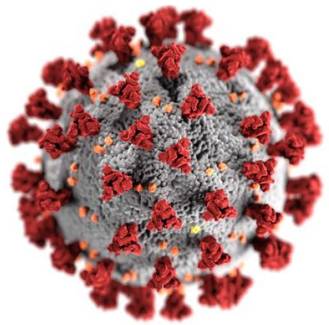


Infections/ Zoonoses

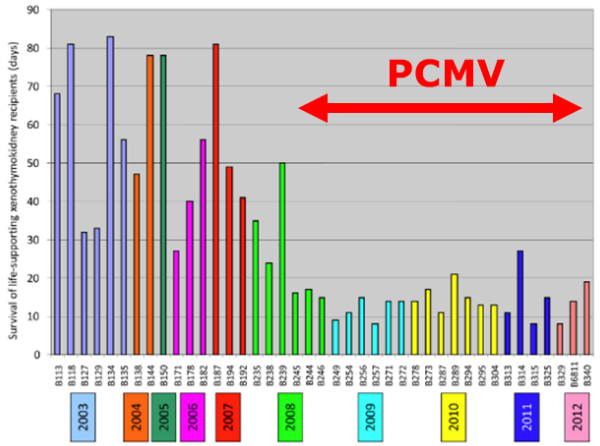
- Pathogen-free donor animals

First clinical trial
Infections/Zoonoses

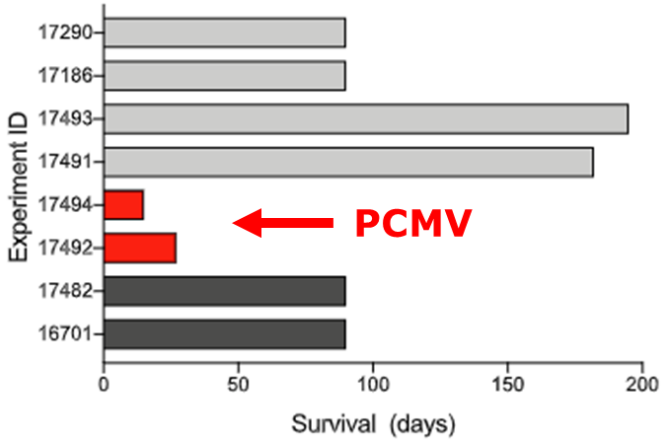
- **P**orcine **C**yto**M**egalo**V**irus = porcine roseolovirus (similar to HHV6)

- No pathogenic significance in pigs, BUT
- Strong evidence for reduced xenograft survival (also detected in Mr. Benett´s xenograft)

Kidneys



Hearts

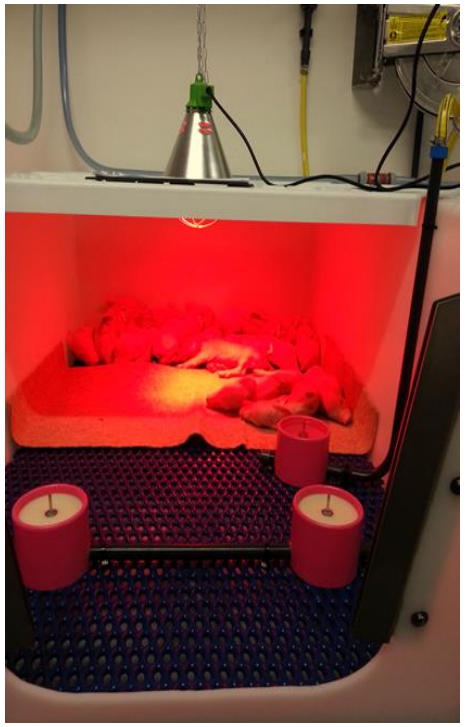


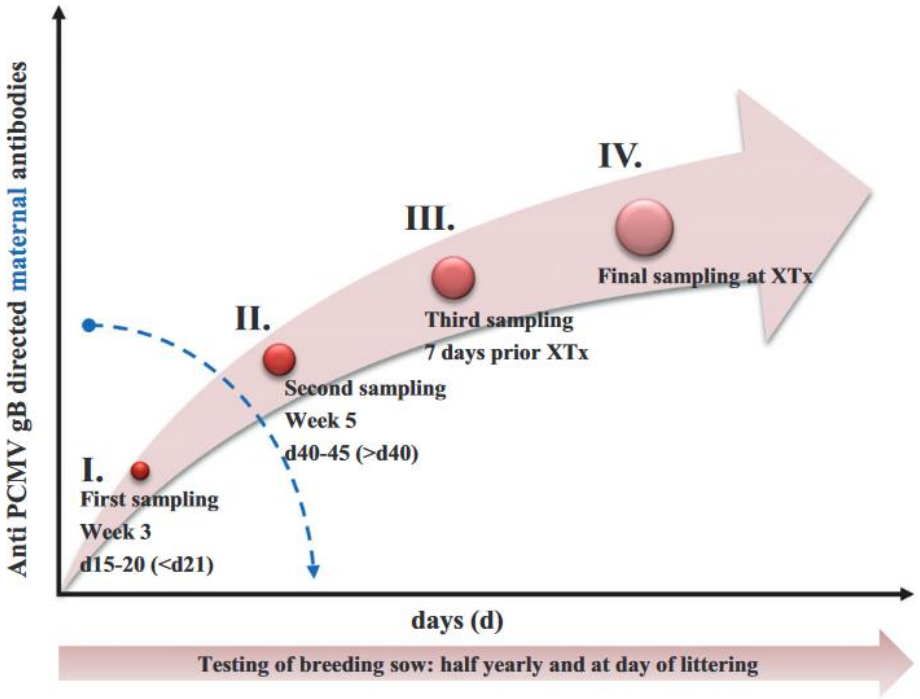
- No causal treament or vaccination

Denner et al., Scientific Reports (2020)
Yamada et al., Transplantation (2014)
Reichart et al., JHLT (2020)

First clinical trial
Infections/Zoonoses

- Early weaning (day 1-3) of the donor animals prevents PCMV-transmission/-infection





Godehardt et al., Xenotransplantation (2023)
Egerer et al., Xenotransplantation (2018)
Pictures from https://www.schulzebremer.com/1800

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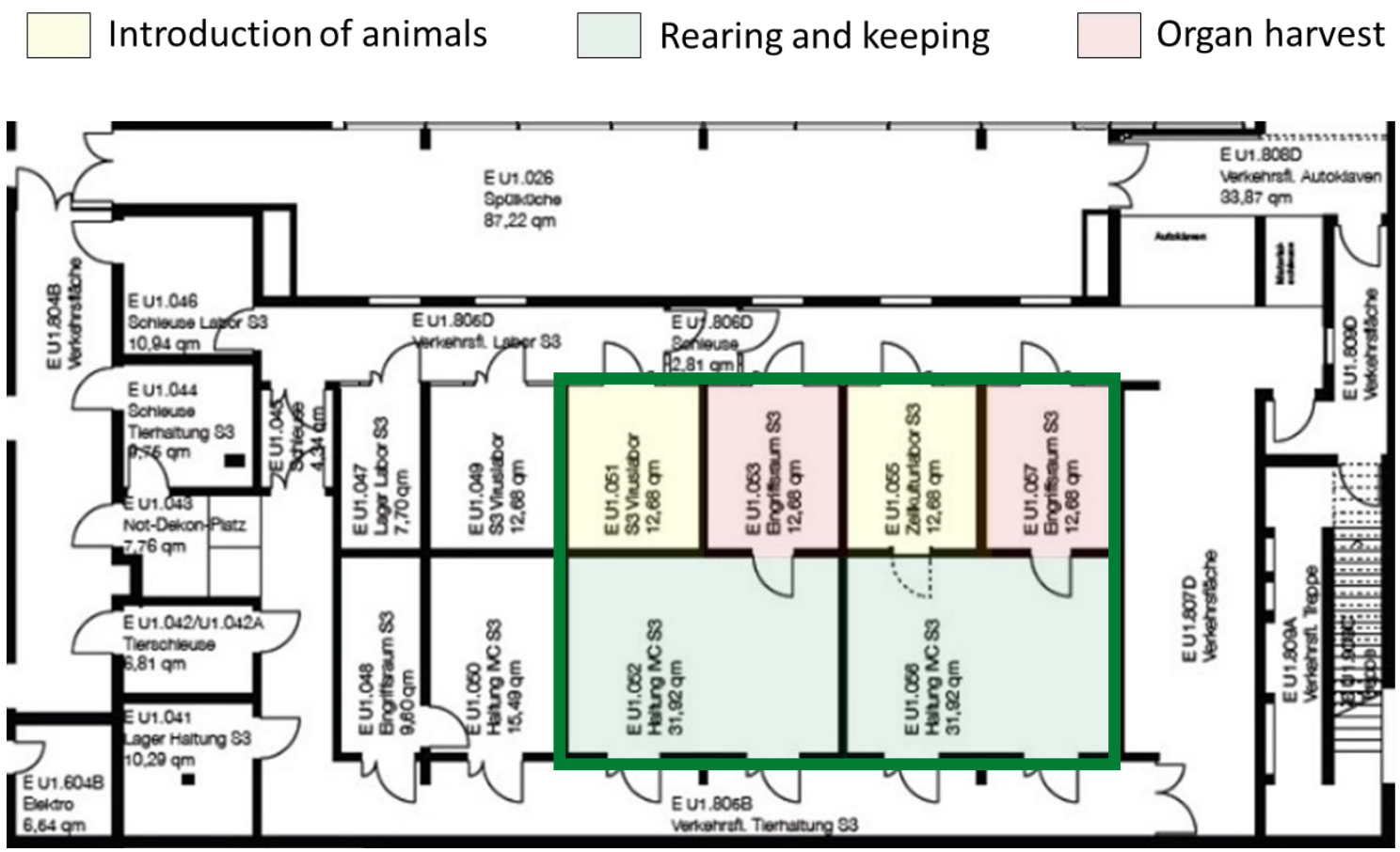

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Thank you for your attention!

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