Cellular, Tissue, and Gene Therapies Advisory Committee Meeting

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HARVARD MEDICAL SCHOOL
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Xenotransplantation: Immunosuppression and Prospects for Tolerance

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No significant financial conflicts to declare (Moderna consultant: allo)
NIH grant support for research in heart, liver Xenotransplantation
Industry support (eGenesis, Revivicor, Tonix) for sponsored research in Xeno
Chair, IXA Ethics Committee

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Xenotransplantation

What is the opportunity?

- **Normal organs from healthy genetically engineered pigs**
  - *Defined quality: no brain death, transmitted disease*
- **Sourced from SPF facilities**
  - *Minimize infectious risks*
- **Pigs: short gestation, rapid growth, multiparous**
  - *Supply potentially unlimited, available when needed*
- **Potential to condition donor and recipient**
  - *Reduce IS burden; "tolerance" induction*

Xenotransplantation

What is the risk?

- **Results uncertain**
  - *Preclinical models imperfect, translation largely untested*
- **Endogenous retrovirus, 'unknown unknown' infections**
  - *Risk could extend to caregivers, and beyond*
- **Equitable access**
  - *Will cost be a barrier?*
  - *Once successful, will supply meet demand?*

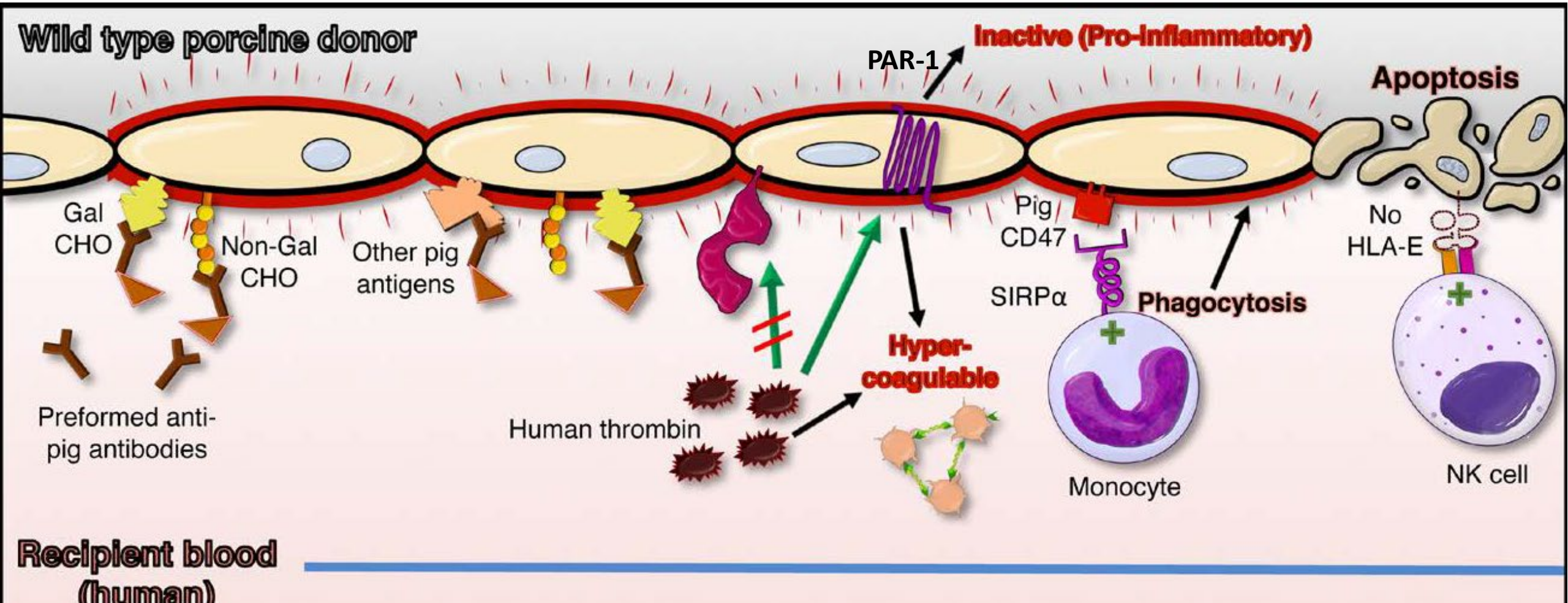
Xenotransplantation

Keys to recent progress

- **Pigs with mechanism-directed gene modifications**
  - Carbohydrate target removal (Gal: GTKO; Neu5Gc: CMAHKO; Sd: β4GKO)
  - Complement regulatory proteins (human CD46, CD55, CD59)
  - Coagulation regulation (human TBM, EPCR, TFPI, CD39)
  - 'Self-recognition' (HLA-E, human CD47)
- **Definition of an effective, safe immunosuppression regime**
  - CD40/CD154 Costimulation blockade; *uncertain whether 'conventional' IS helps*
- **Organ preservation advances (especially for heart)**
  - Ischemia minimization to prevent 'PCXD'
- **PERV 'low-risk' pigs (KO or lacking PERV-C)**

Xenotransplantation

Rationale: Mechanism-directed Gene Modifications



Goals to Enable Xenotransplantation

Control known mechanisms of GalTKO.hCPRP Heart Injury

-Preformed or elicited non-gal antibody, complement

-Consumptive coagulopathy (recip), thrombotic microangiopathy (graft)

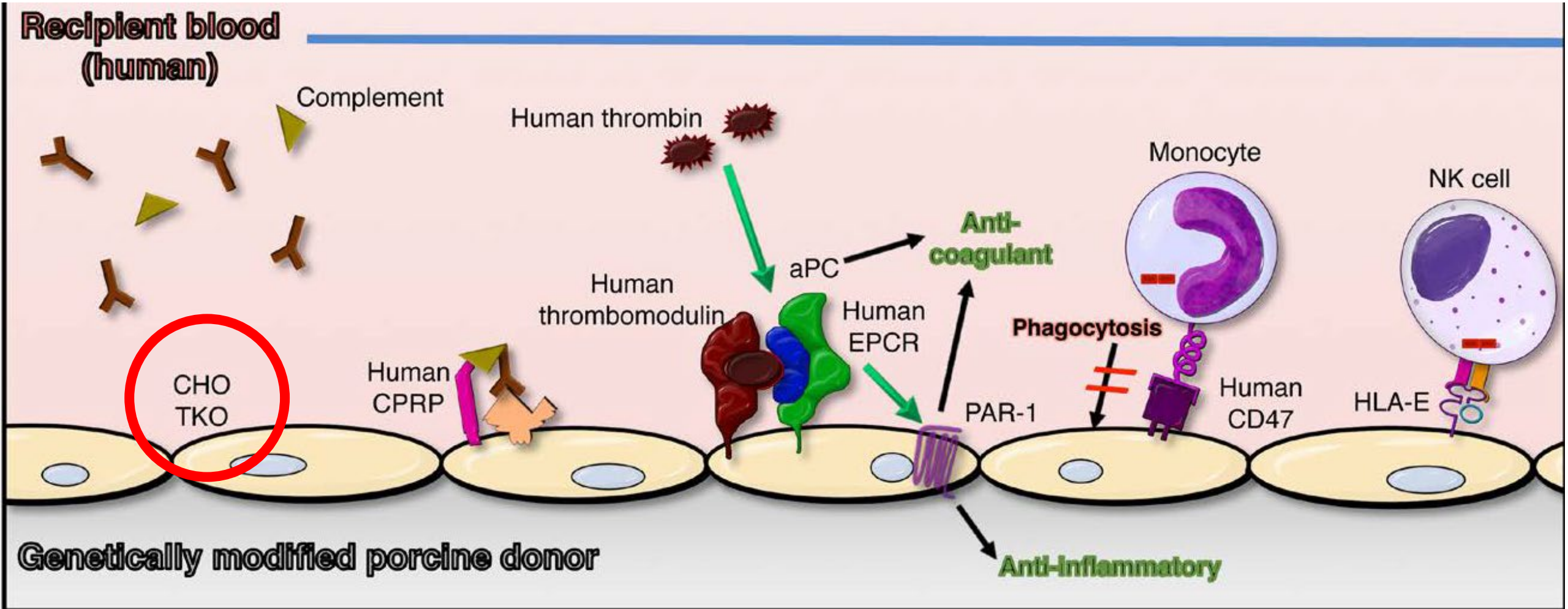
Address residual barriers

-‘Primary Cardiac Xenograft Dysfunction’ (PCXD)

Xenotransplantation

Carbohydrate target removal (CHO TKO: GTKO, CMAHKO, β4GKO)

Added Complement Pathway Regulatory Proteins (hCPRPs: hCD46, hCD55, hCD59)



Xenotransplantation

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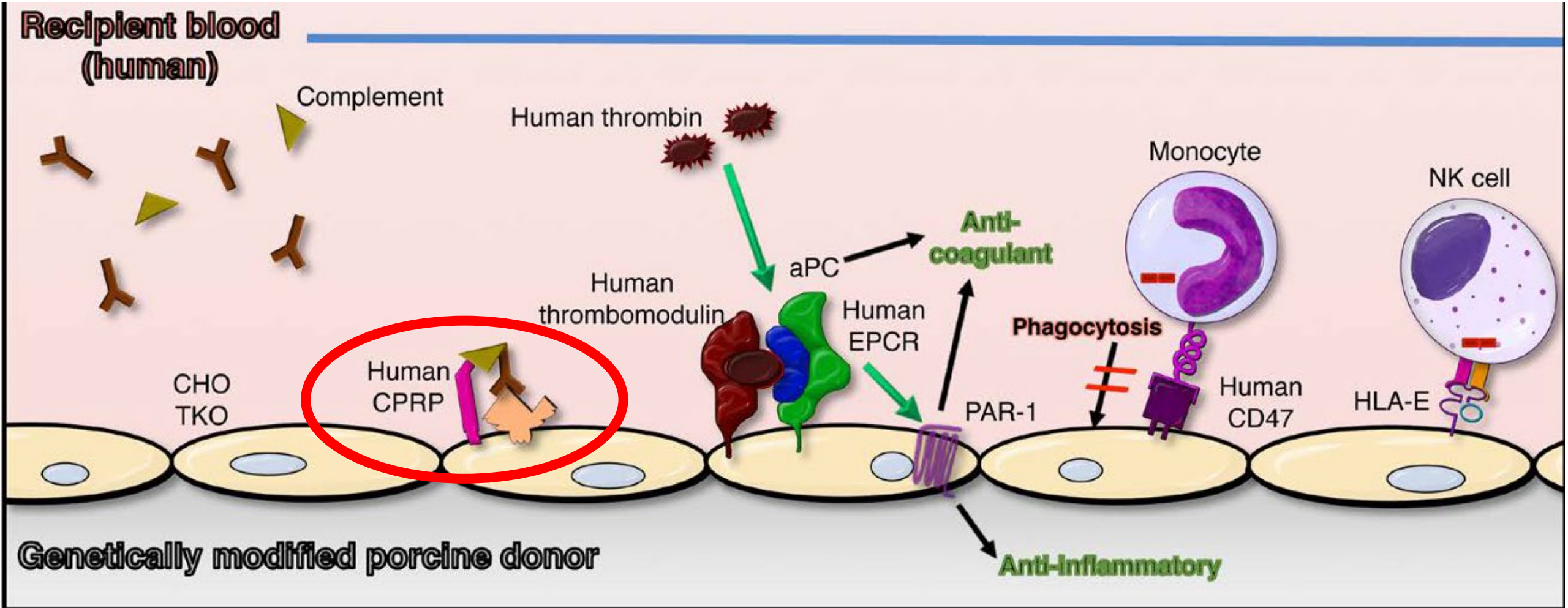
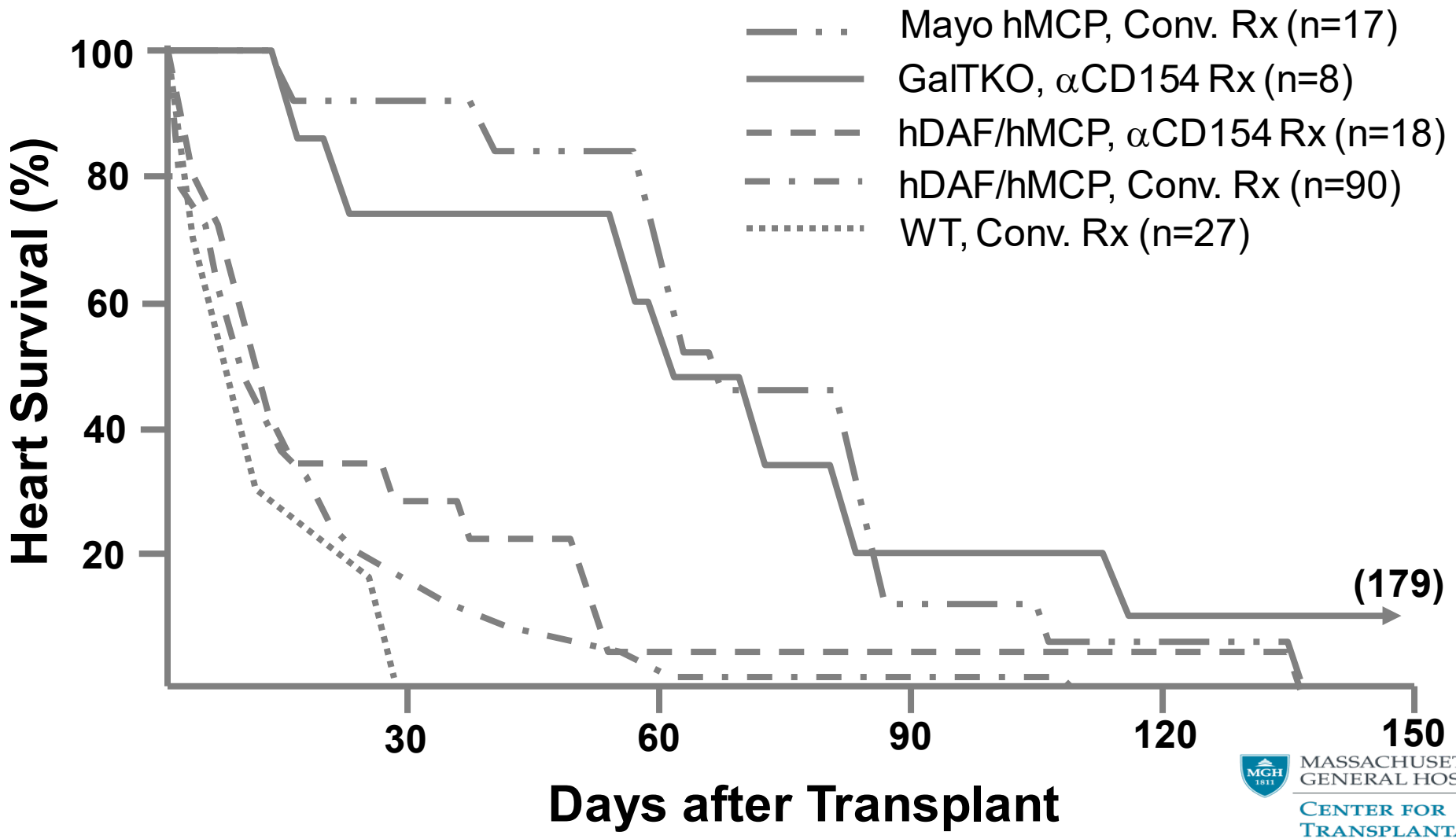


World Experience, 1988-2006
Pig hearts in treated baboons



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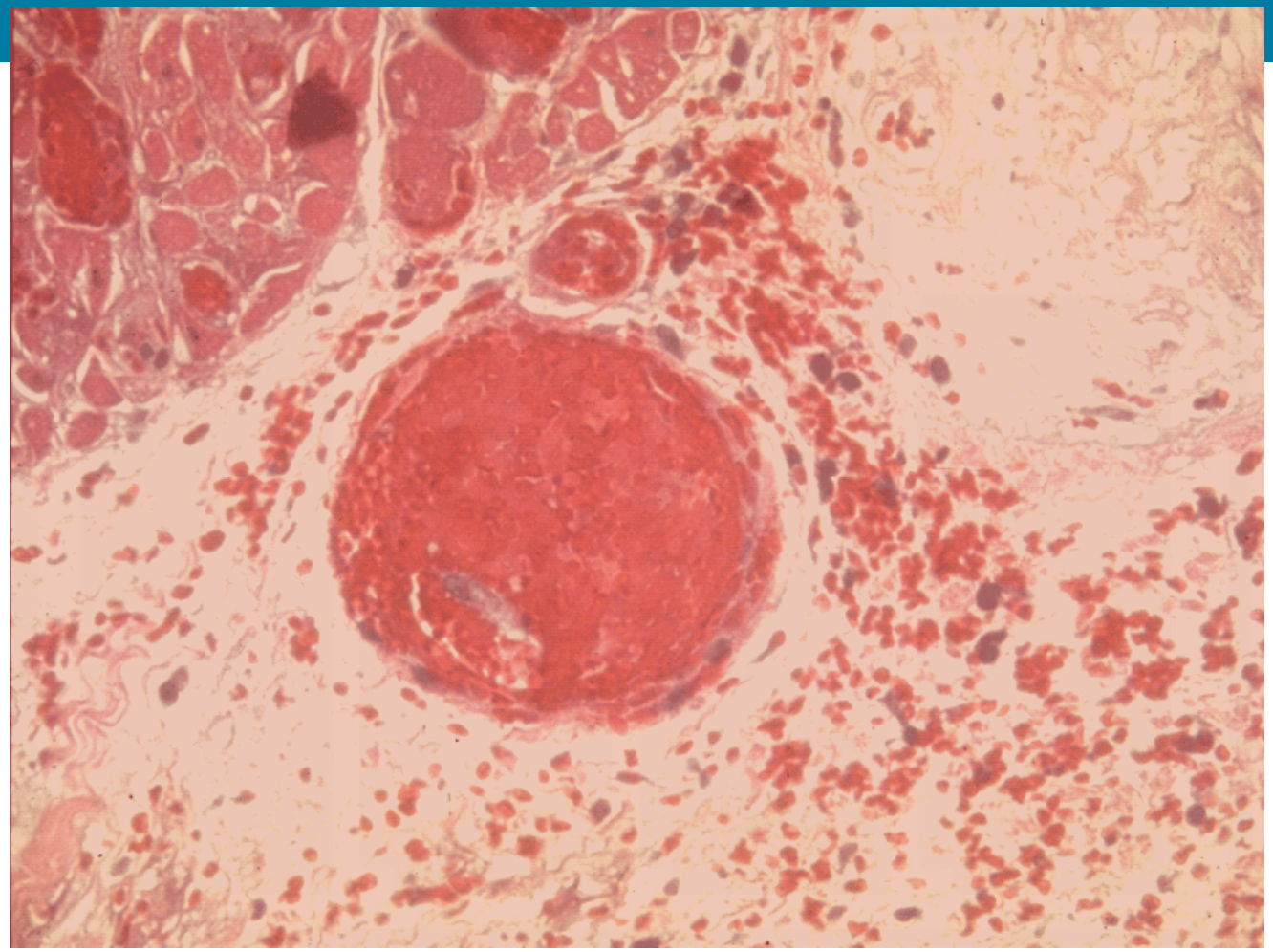
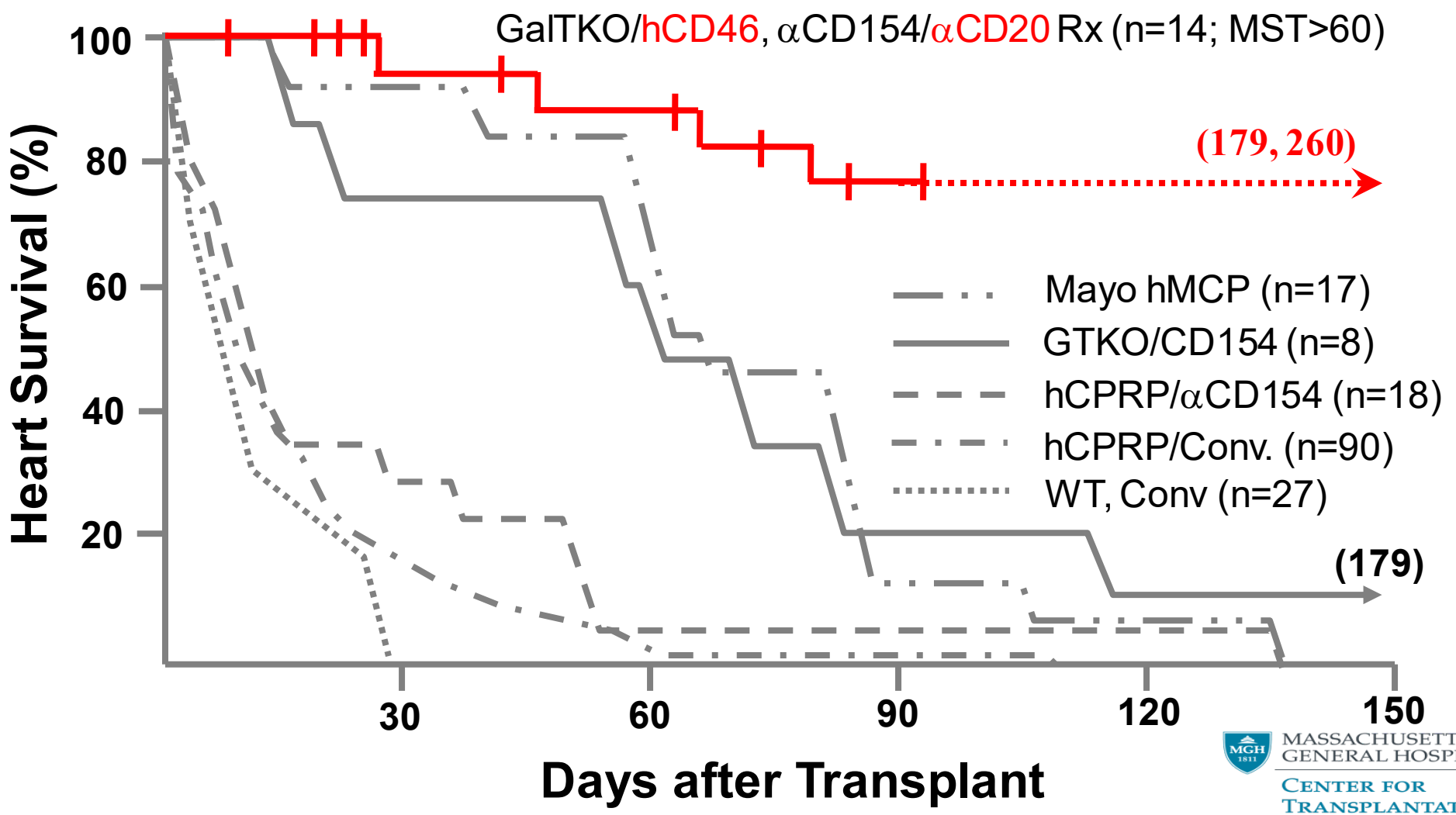
Thrombotic Microangiopathy



Caused by anti-pig antibody? Complement?
Platelet or Coagulation pathway activation?

World Experience, 1988-2011
Pig hearts in treated baboons



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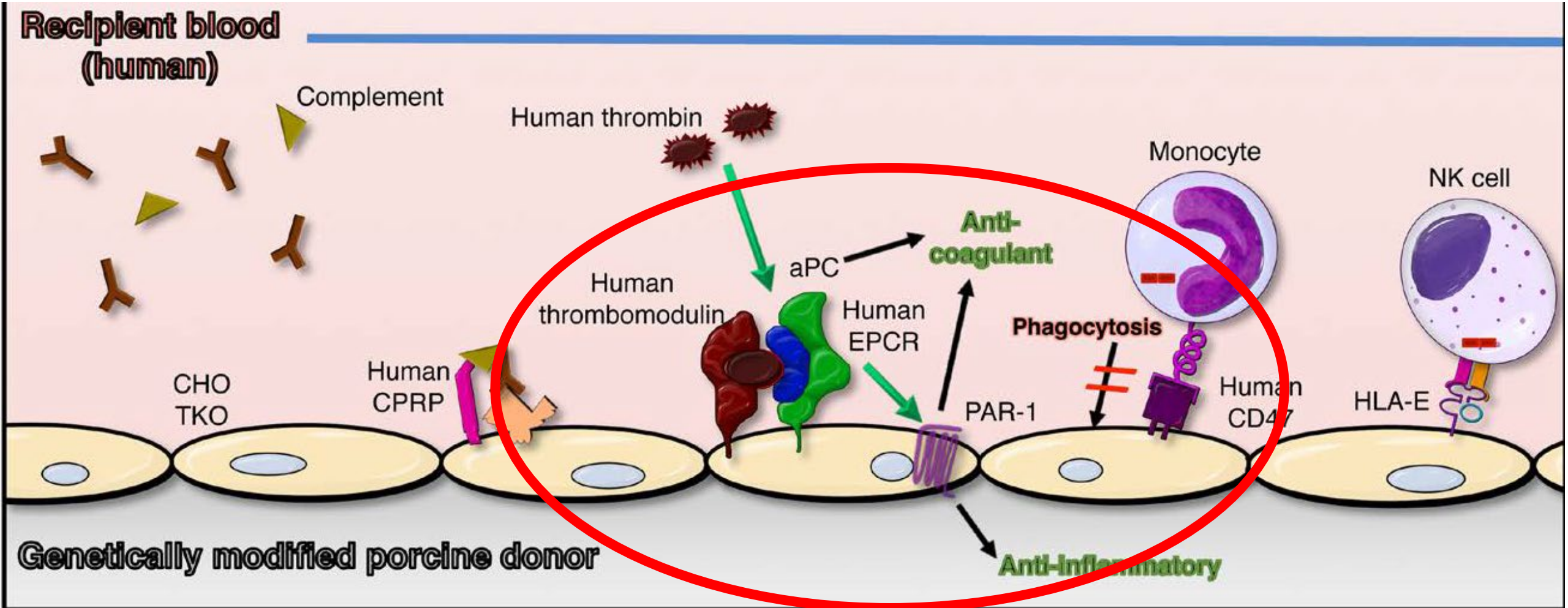
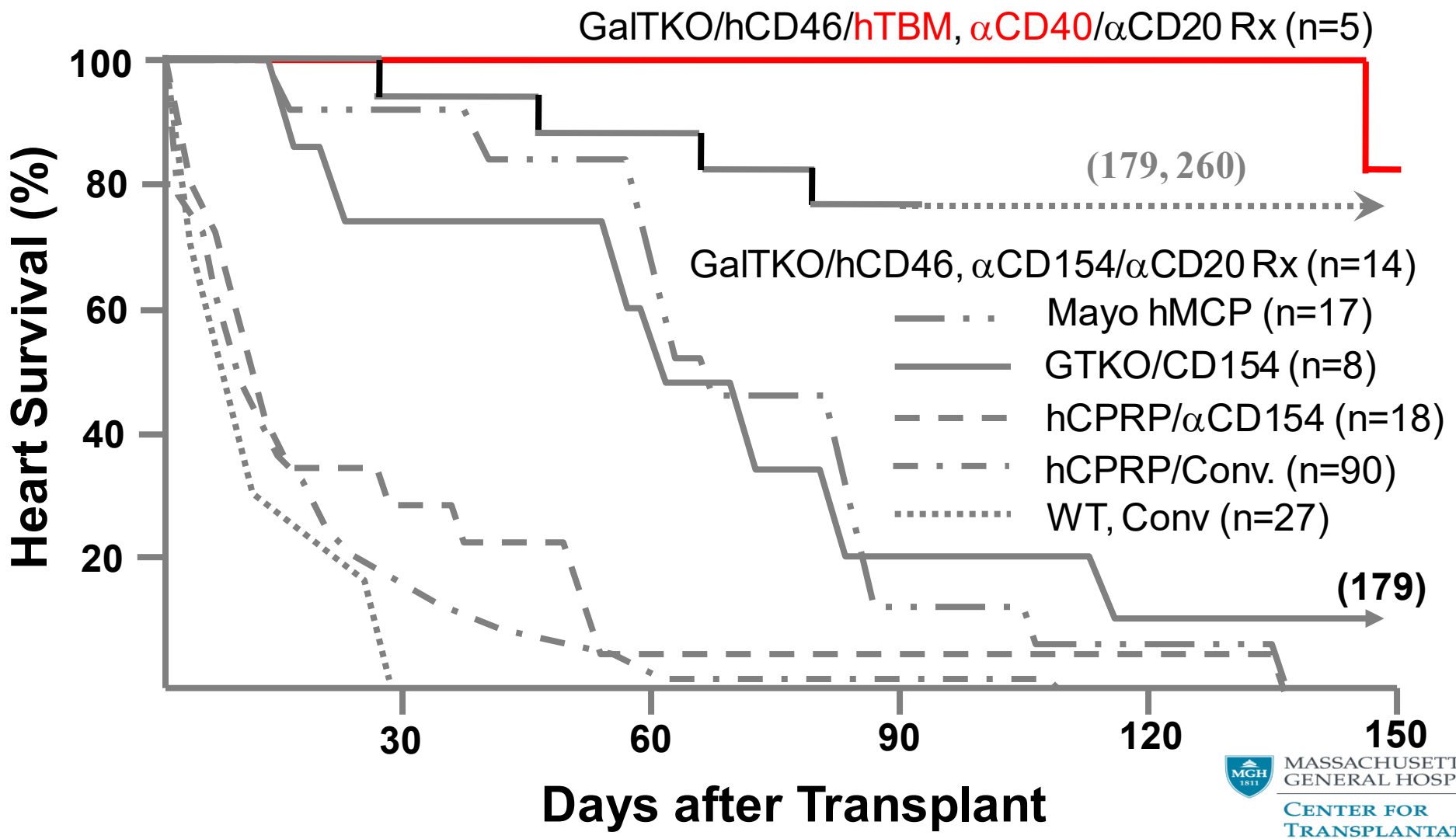
Xenotransplantation

Coagulation regulation (TBM, EPCR, TFPI, CD39)

‘Self-recognition’ (HLA-E, CD47)



World Experience, 1988-2016
Pig hearts in treated baboons



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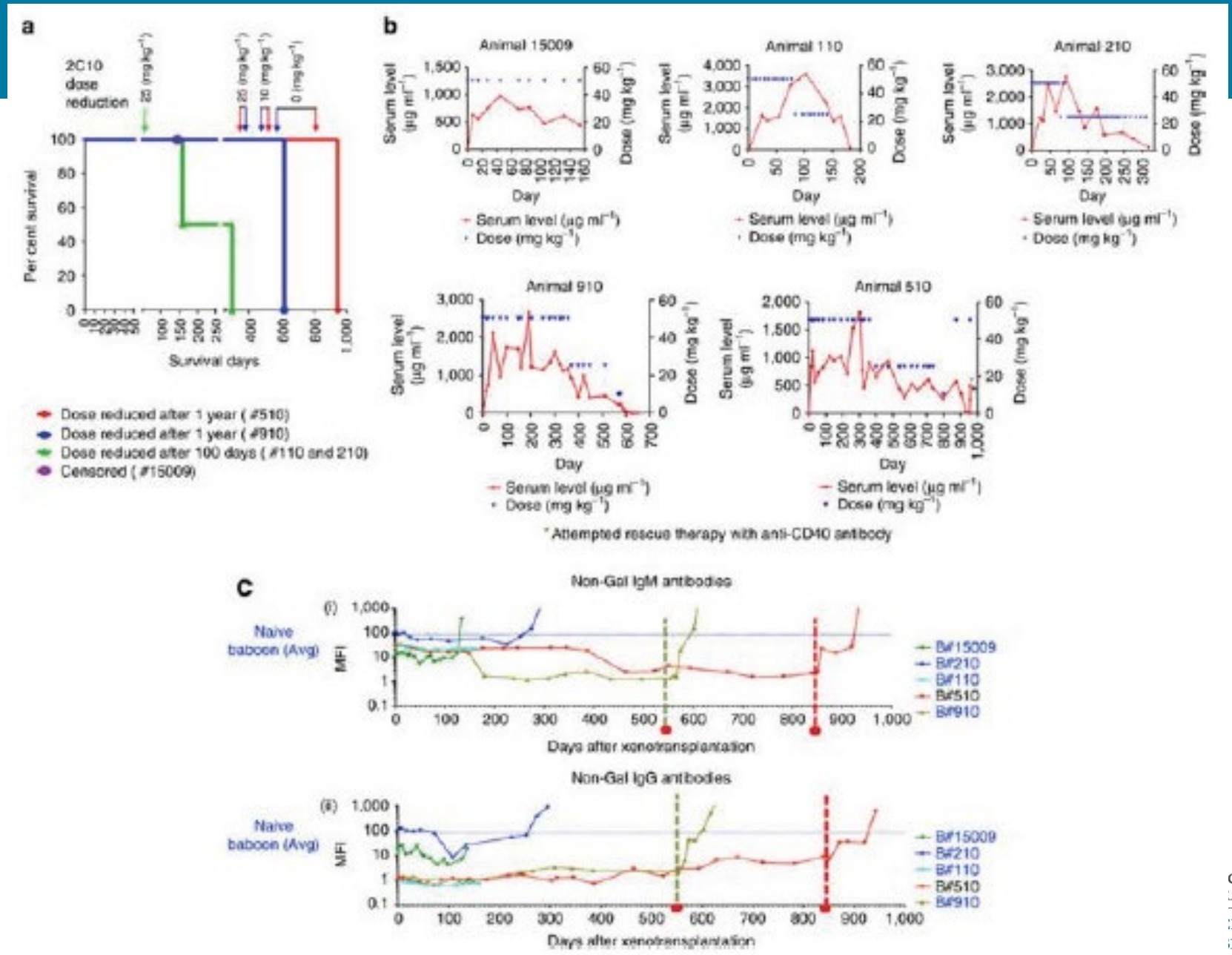
Mohiuddin et al, Nature Communications 2016



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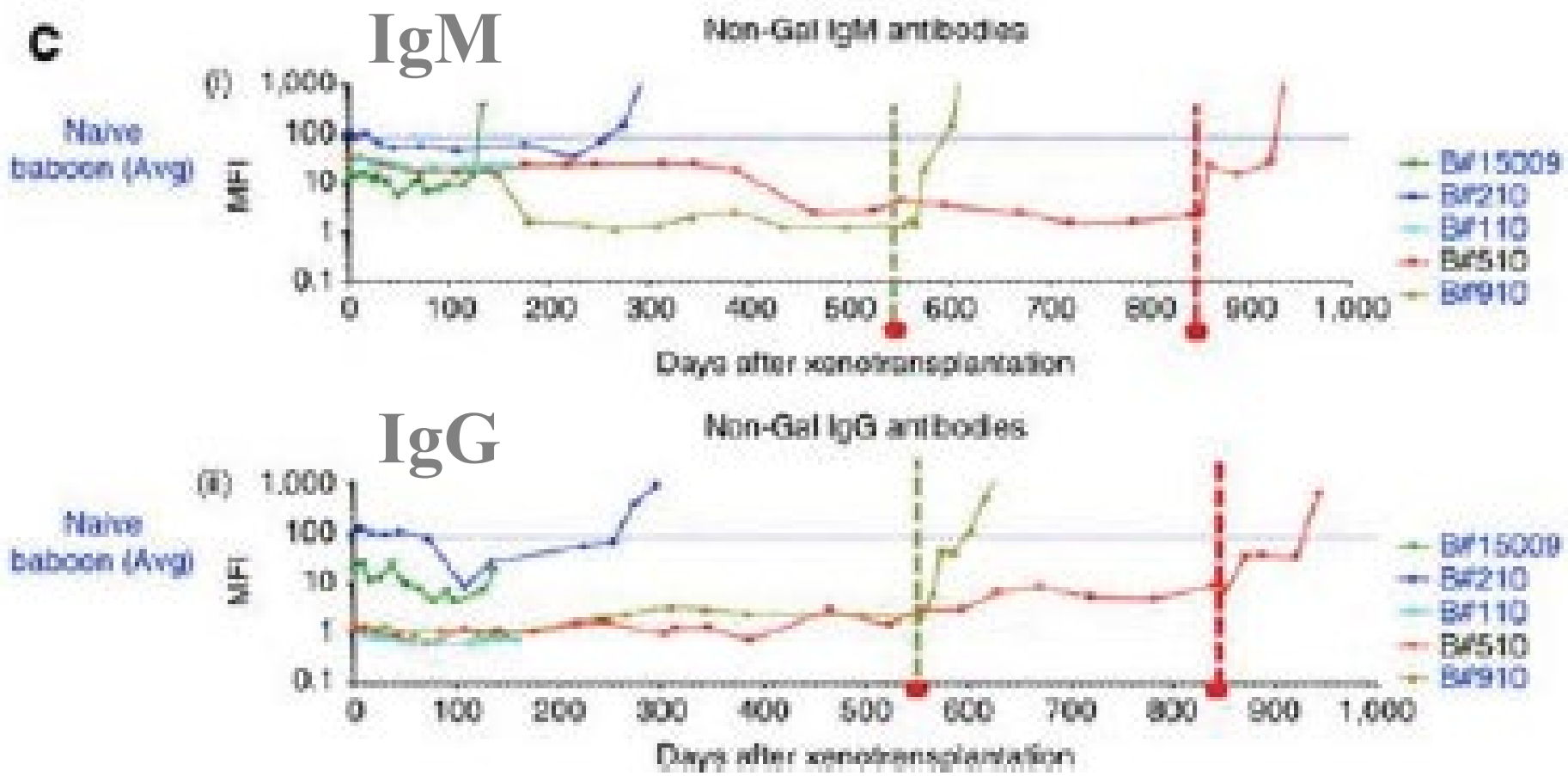

Conclusions

Known mechanisms of GalTKO.hCPRP Heart Injury

-Preformed or elicited non-gal antibody, complement
Addressed with TKO pigs, αCD20+ATG induction, αCD40/154-based IS

-Consumptive coagulopathy (recip), thrombotic microangiopathy (graft)
Addressed with hTBM and hCPRP, effective IS (αCD40/154-based)

Additional barrier in orthotopic translation

-Perioperative Cardiac Xenograft Dysfunction (PCXD)

Orthotopic Heart Results

State of the art in 2017

-Vial et al, JHLT 1999

up to 37 days with hDAF heart in monkeys, conventional IS

-Mohiuddin/McGregor/Reichart, 2015, 2017

up to 53 days with GTKO, GTKO.hCPRP hearts in baboons

intensified conventional IS toxic, ineffective

The barriers:

-PCXD: "Perioperative Cardiac Xenograft Dysfunction"

>50% mortality within 1 day, survival >14 days rare

-Graft hypertrophy

Nature, December 2018

LETTER

https://doi.org/10.1038/s41586-018-0765-z

Consistent success in life-supporting porcine cardiac xenotransplantation

Matthias Längin[1,2,18], Tanja Mayr[1,2,18], Bruno Reichart[2]*, Sebastian Michel[3], Stefan Buchholz[3], Sonja Guethoff[2,3], Alexey Dashkevich[3], Andrea Baehr[4], Stefanie Egerer[4], Andreas Bauer[1], Maks Mihalj[3], Alessandro Panelli[2], Lara Issl[2], Jiawei Ying[2], Ann Kathrin Fresch[2], Ines Buttgereit[2], Maren Mokelke[2], Julia Radan[2], Fabian Werner[1], Isabelle Lutzmann[2], Stig Steen[5], Trygve Sjöberg[5], Audrius Paskevicius[5], Liao Qiuming[5], Riccardo Sfriso[6], Robert Rieben[6], Maik Dahlhoff[4], Barbara Kessler[4], Elisabeth Kemter[4], Katharina Klett[7,8,9], Rabea Hinkel[7,8,9], Christian Kupatt[7,9], Almuth Falkenau[10], Simone Reu[11], Reinhard Ellgass[3], Rudolf Herzog[3], Uli Binder[12], Günter Wich[13], Arne Skerra[14], David Ayares[15], Alexander Kind[16], Uwe Schönmann[17], Franz-Josef Kaup[17], Christian Hagl[3], Eckhard Wolf[4], Nikolai Klymiuk[4], Paolo Brenner[2,3,19] & Jan-Michael Abicht[1,2,19]

Nature, December 2018

LE

1586-018-0765-z

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Matthias Lä
Alexey Dash
Ann Kathri
Trygve Sjöb
Elisabeth K
Reinhard El
Franz-Josef

, Jiawei Ying[2],
en[5],
Kessler[4],
l
,
Schönmann[17],
9

Agent	Dose	Timing
Induction		
anti-CD20 Ab	19 mg kg^{-1}, i.v. short infusion	days -7, 0, 7 and 14
ATG	5 mg kg^{-1}, continuously i.v.	days -2 and -1
anti-CD40 mAb or anti-CD40L PASylated Fab*	50 mg kg^{-1} or 20 mg kg^{-1}; i.v. short infusion	days -1 and 0
Maintenance		
MMF	40 mg kg^{-1}, continuously i.v.	daily, started on day -2
anti-CD40 mAb or anti-CD40L PASylated Fab*	50 mg kg^{-1} or 20 mg kg^{-1} i.v. short infusion	days 3, 7, 10, 14, 19, then weekly
methylprednisolone	10 mg kg^{-1}, bolus i.v.	daily, tapered down
Anti-inflammatory therapy		
IL6-receptor antagonist	8 mg kg^{-1}, short infusion i.v.	monthly
TNFα inhibitor	0.7 mg kg^{-1}, bolus s.c.	weekly
IL1-receptor antagonist	1.3 mg kg^{-1}, bolus s.c. or i.v.	daily
Additive therapy		
acetylsalicylic acid	2 mg kg^{-1}, bolus i.v.	daily
unfractionated heparin	20-40 U $kg^{-1}h^{-1}$, continuously i.v.	daily, started on day 5
C1 esterase inhibitor	17.5 U kg^{-1}, i.v. short infusion	days 0, 1, 7 and 14
ganciclovir	5 mg kg^{-1}, continuously i.v.	daily
cefuroxim	50 mg kg^{-1}, continuously i.v.	daily, prophylaxis from day 0 to 5
epoetin beta	2,000 U, bolus s.c. or i.v.	days -7, 0 and if necessary

Nature, December 2018

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cardi

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Orthotopic Heart Results

State of the art: 2022

-Vial et al, 1997:	***≤ 37 days***
-Mohiuddin/McGregor/Reichart, 2017:	***≤ 53 days***
-Langin, Reichart, Brenner et al, 2018-21:	**> 180 days**
-Mohiuddin et al, 2022:	**up to 270 days**

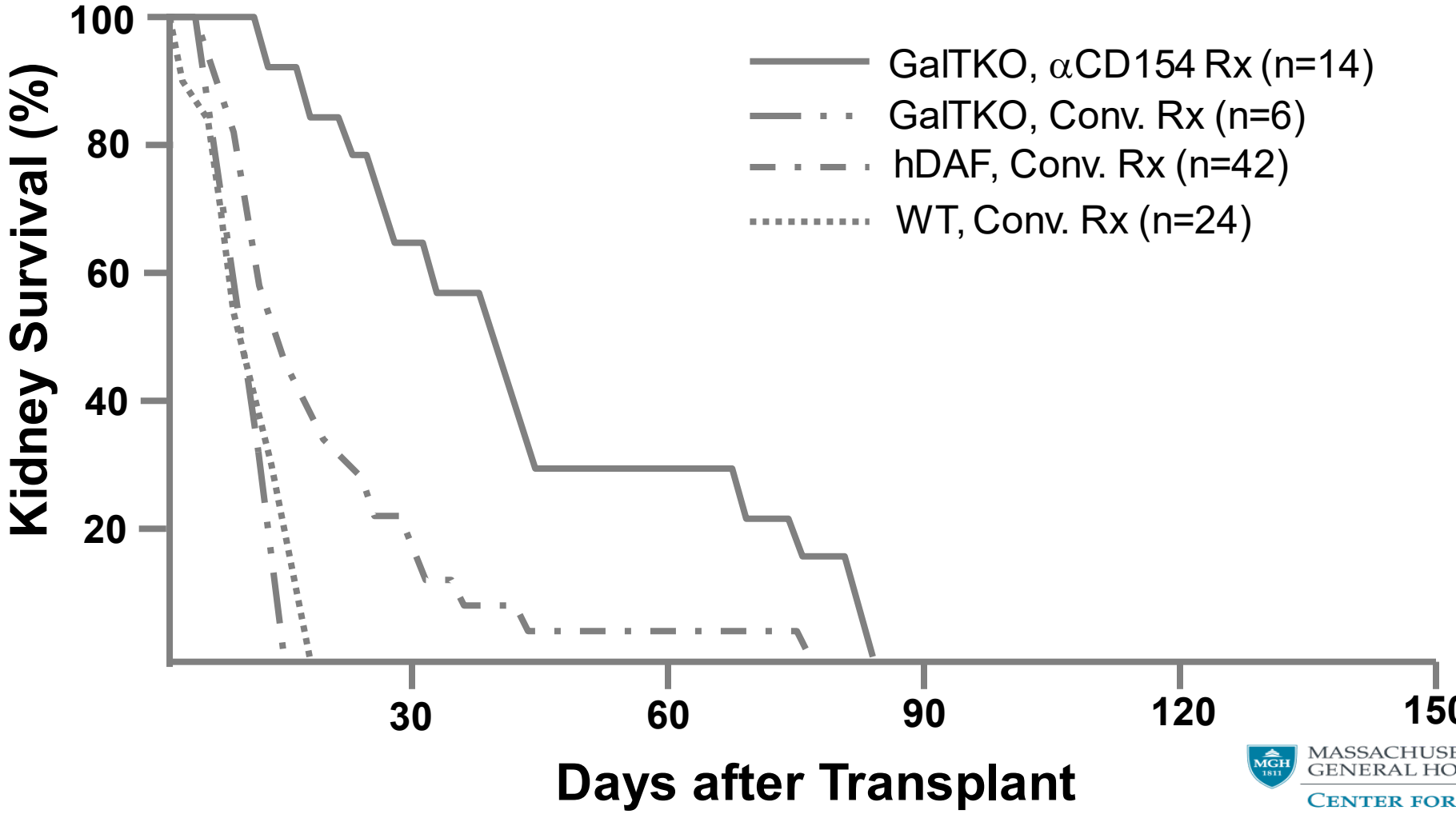
IS: ATG+αCD20 induction, αCD40/154 + MMF, mTOR inhibitor

PCXD prevented with ischemia minimization

CC/TM, DXR not observed with GTKO.hCD46.hTBM heart

Graft hypertrophy not seen on mTOR inhibitor

World Experience, 1998-2014
Pig kidneys in treated baboons



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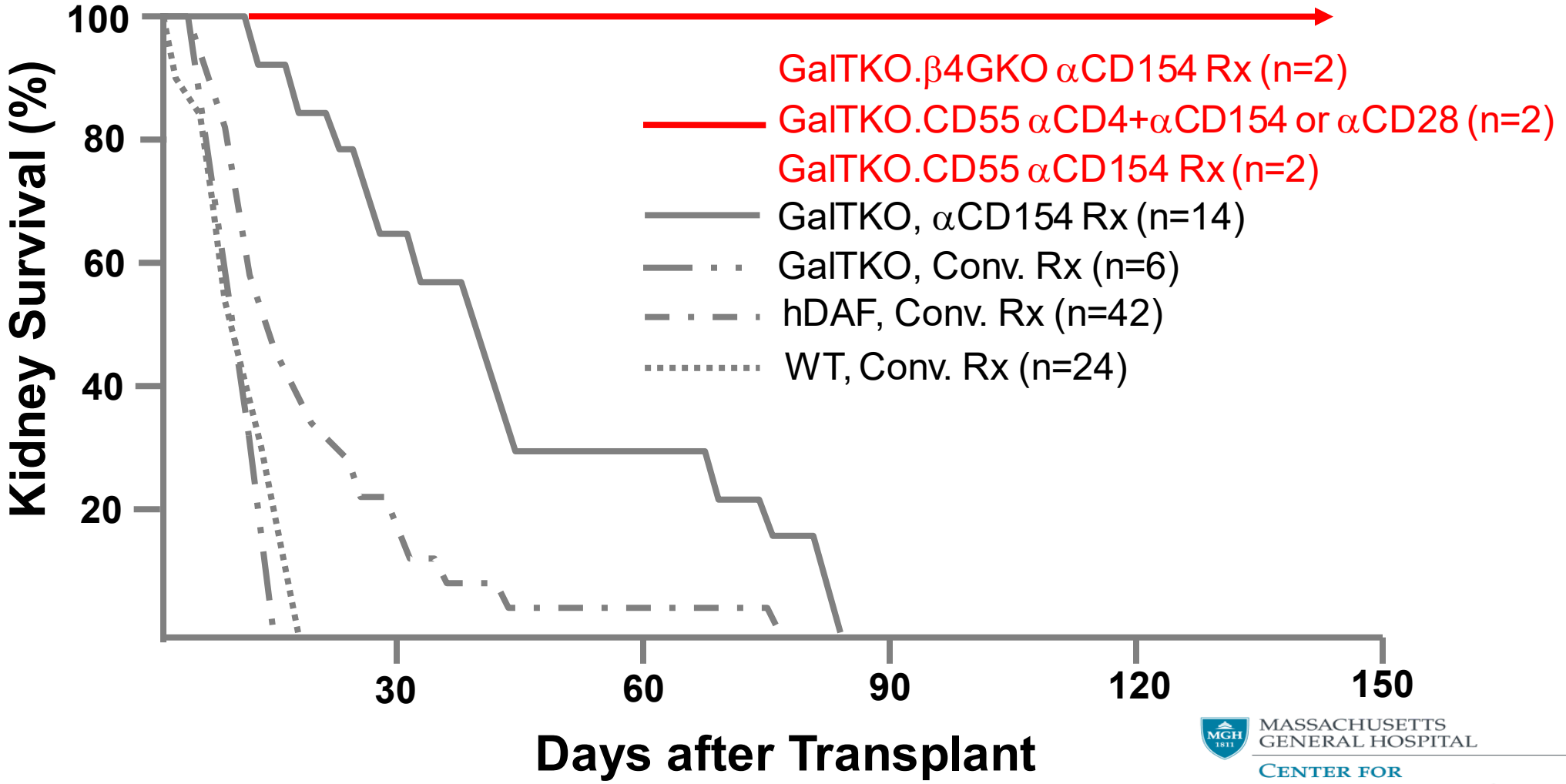
World Experience, 2014-2018
Pig kidneys in treated baboons



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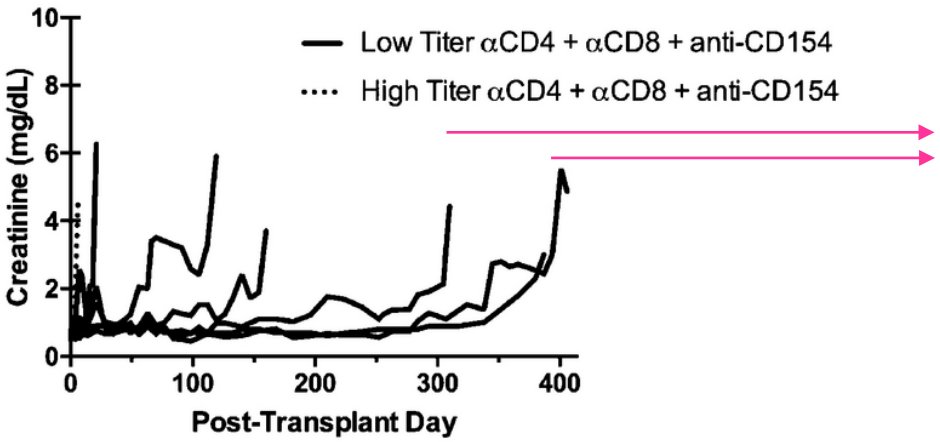
Emory Kidney Xeno Study

Kim SC et al. Am J Transplant Jan 2019
Rhesus recipients, screened for negative XM

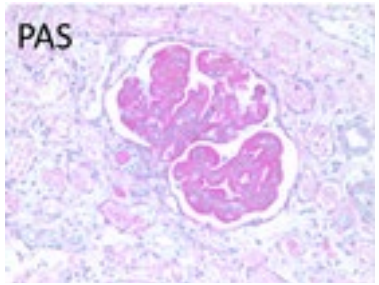
Lessons:

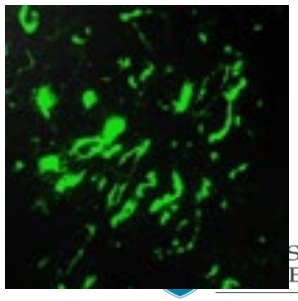
1) Xeno response is particularly dependent on CD4 T cells
2) Sd antigen (encoded by β4Gal) is probably important
3) Rapid rejection with positive crossmatch

Pig	Rx	Graft survival (days)
αGTKO/CD55	αCD4+αCD8+αCD154	310, 160, 406, 18, 115, >400
	αCD4+αCD154	499, 414, >70
	αCD8+αCD154	15, 6, 6



Chronic antibody mediated rejection





Sd antigen+

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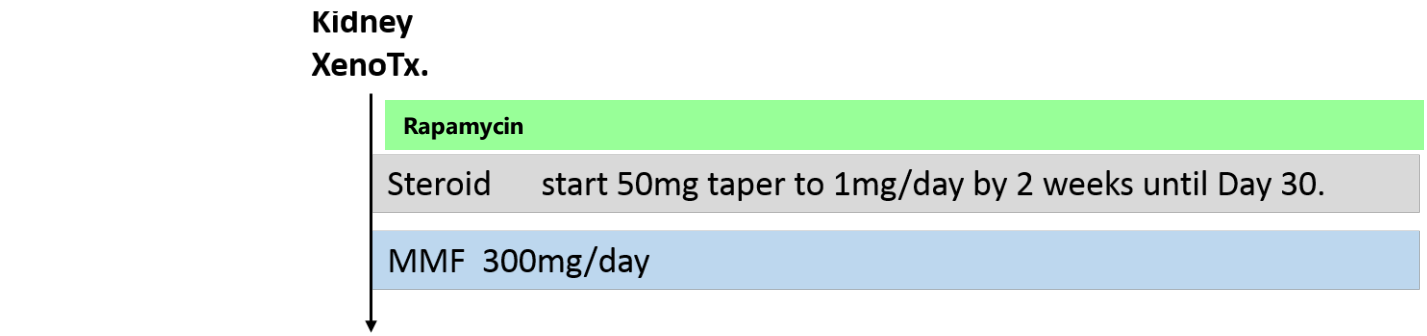
MGH Protocol for Renal xenotransplantation



Rabbit ATG (5mg/kg)	X	X	X												
Rituximab			X												x (when B cells come back)
Anti-CD154 20mg/kg			X		X			X			X				X weekly
Gancyclovir 5mg/kg			X	daily for 50 days											
Enrofloxacin			X	until T cells come back											
Ketorolac (1mg/kg)			X	before anti-CD154 mAb administration											
Epogen (200U/kg)	X	X	X	daily once HCT is stable the administration will be adjusted as necessary to keep HCT>30%											
Lovenox			X	daily											

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Xenotransplantation

Yamada, Sachs, Sykes (MGH/Columbia)

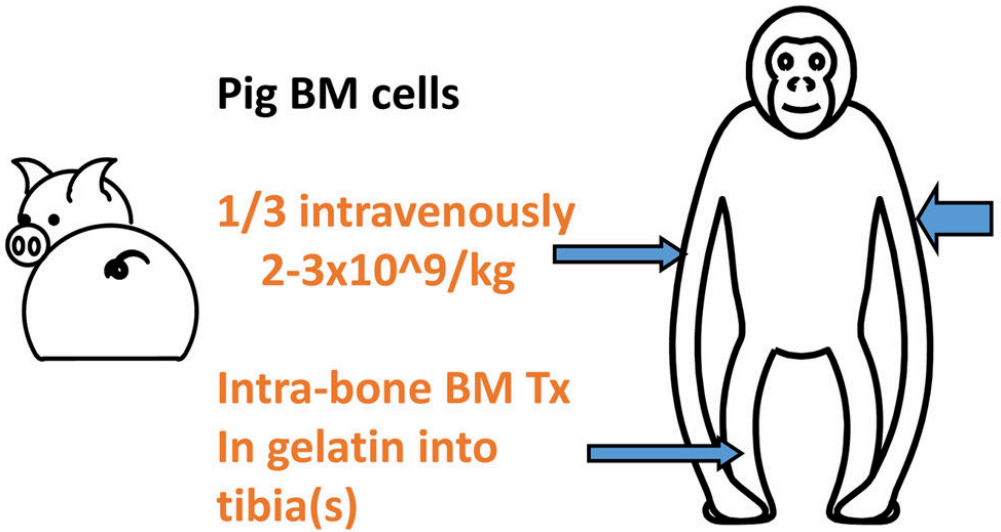
- **Tolerance induction regimen**
  - **Kidney or Thymokidney**
    - **Inbred GTKO.hCD55 miniswine, +/-hCD47**
  - **Mixed hematopoetic chimerism**
    - **Conditioning: ATG, αCD20, TBI, Thymic Irradiation**
    - **BMT around transplant (IV, intra-bone marrow)**
    - **IS: αCD154, Tac tapered, *MMF/Rapa***

Xenotransplantation

Yamada, Sachs, Sykes (MGH/Columbia)

Tolerance by Intra-bone BM Transplantation

Pig GalT-KO BM to Baboons (Intra-bone)



Pre-op
WBI and TI*
T cell depletion
Rituximab

Pre-op (tapering)
Costimulatory blockade
MMF
Tacrolimus

Lung: <14 days **Thymokidney: <100 days**

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Immunosuppression and Tolerance for Xenotransplantation

Conclusions

Costimulation-based IS is effective

In context of GKTO.hCPRP.hTBM heart,

GTKO.β4GKO or GTKO.hCPRP kidney

Prevents ACR, Consumptive Coagulopathy, Thrombotic Microangiopathy

Requirement for induction remains unproven

Efficacy of CNI, MMF, mTOR remains unproven

Tolerance may be achievable with GTKO.hCPRP.hCD47

In context of mixed hematopoietic chimerism

Xenotransplantation

Thank you

for your attention!

Xenotransplantation

Pierson

Discussion Slides

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Xenotransplantation : What is "Success"?

Outcomes

Benchmark outcomes relative to:

Preclinical results: *As good as predicted?*

Alternative therapies: *Satisfy ethical equipoise?*

Infection control: *Diagnostic, therapeutic strategies effective?*

Learning curve must be anticipated

Process

Education: *Patient, professional peers, public*

Transparency: *Acknowledge uncertainties*

Deliberation: *Non-emergent until efficacy established*

Informed consent: adherence to protocol by subject, close contacts

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Xenotransplantation: Heart

U Maryland Pig-to Human Heart Transplant 1/7/22

FDA exemption: 'compassionate use' approval for single case

Reported in scientific literature 6/23/22

10-gene pig heart into critically-ill patient

Preop VA ECMO for 42 days

Intraop complications well-tolerated

Organ function 'excellent' by report

Off VA ECMO POD 3 or 4; acute renal failure on dialysis

Multiple infectious complications; Bed-bound for rehab

Graft failure associated with Porcine CMV in heart xenograft, anti-TKO Ab

Died ~3/7/22, after 10 days back on VA ECMO

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Xenotransplantation: Kidney

NYU Kidney experience *No FDA approval: 'Not a clinical trial'*

NEJM 5/2022

GTKO thymokidney in two brain-dead human patients

Organ function (life-supporting?) for ~2 days

Thymokidney effect not measurable

UAB Kidney experience *No FDA approval: 'Not a clinical trial'*

AJT 1/2022

10-gene pig in a brain-dead human patient with DIC

Native nephrectomy performed

Organ function not demonstrably life-supporting

Concerning histology on biopsies, low U/O after 4 hr

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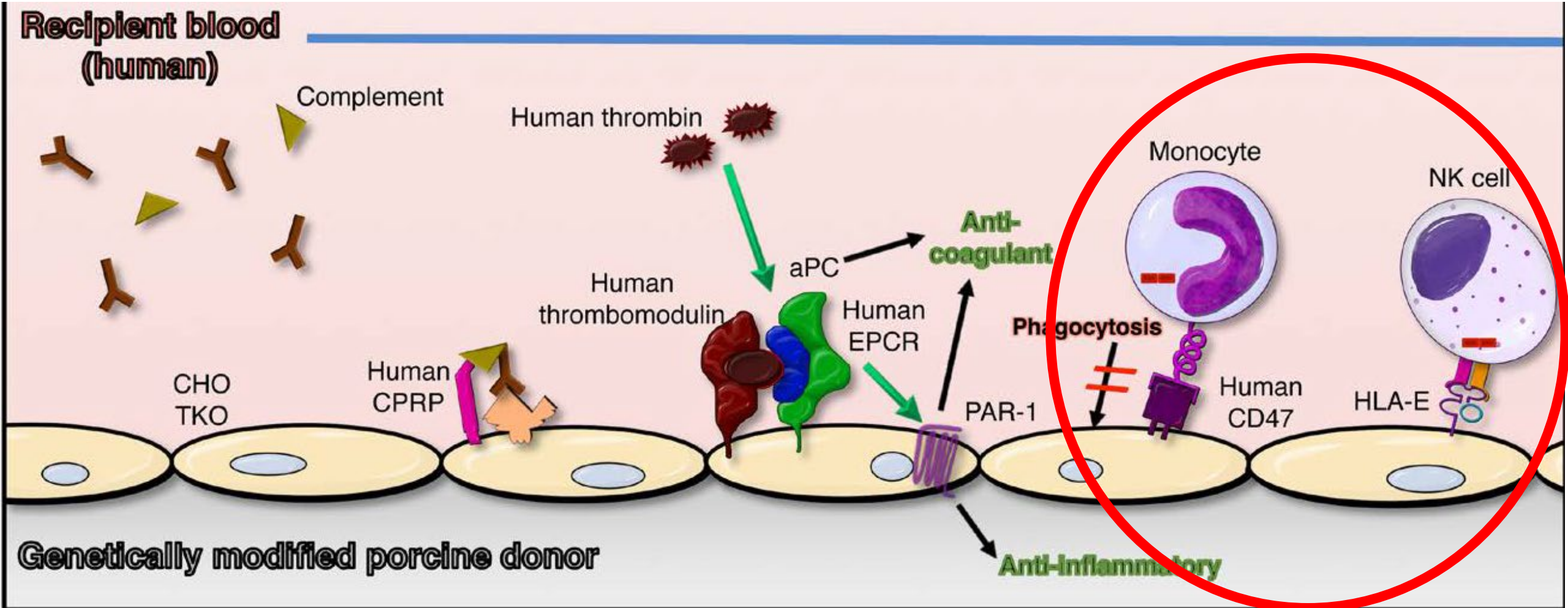
Xenotransplantation

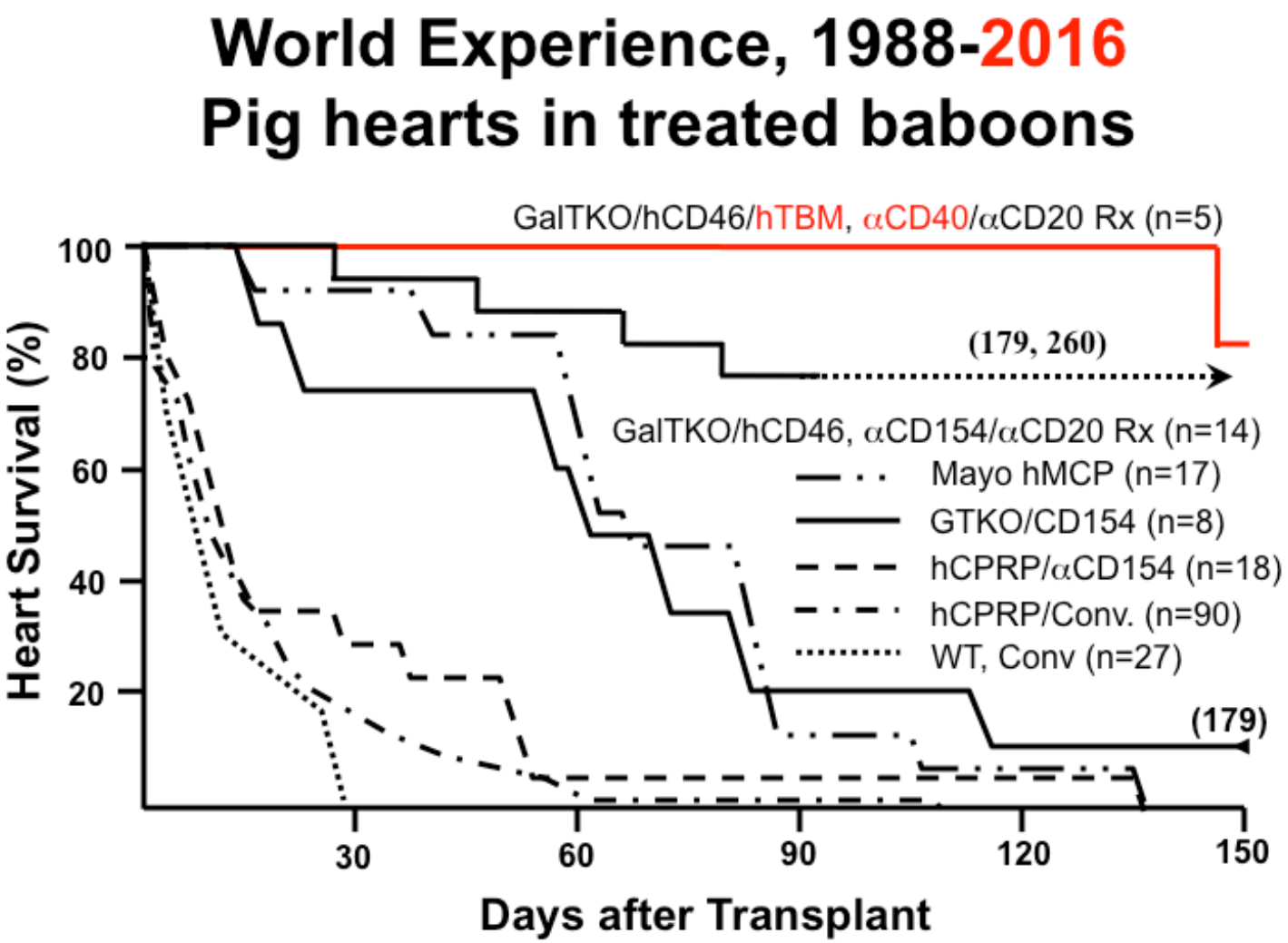
Coagulation regulation (TBM, EPCR, TFPI, CD39)

‘Self-recognition’ (HLA-E, CD47)



Xenotransplantation

Preclinical Results: GalTKO, hCD46, hTBM



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Xenotransplantation

Preclinical efficacy endpoint met for GalTKO.hCD46.hTBM heart

Consistent life-supporting organ function >3 mo; occasional to >6 mo

Failures predictable, and manageable, or model-related

Tolerable regimen

Langin et al,
Nature, 12/2018

Reichart et al
JHLT, 8/2020

LETTER

Ischemia minimization; Tensirolimus

https://doi.org/10.1038/s41586-018-0765-z

4/5 beyond 90 days; no LVH during rapa Rx

Consistent success in life-supporting porcine cardiac xenotransplantation

Table 1 | Serum levels of liver and heart enzymes, platelet counts and prothrombin ratio at the end of experiments that lasted longer than two weeks

	Group I	Group II			Group III					
Experiment	**3**	**6**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**Reference**
Bilirubin (mg dl^{-1})	1.2	0.9	2.7	4.5	0.3	0.2	0.2	0.2	0.2	≤1.2
AST (U l^{-1})	646	896	792	354	101	27	23	63	28	≤49
PR (%)	30	6	6	6	101	96	117	26	99	70–130
CHE (kU l^{-1})	1.6	1.6	1.4	1.1	2.1	9.4	14.4	7.3	7.2	4.6–11.5
Troponin T (ng ml^{-1})	0.233	0.660	1.460	1.470	0.218	0.037	0.018	0.556	0.140	≤0.014
CK total (U l^{-1})	654	636	1017	953	3053	143	66	461	96	≤189
LDH (U l^{-1})	3252	6853	2842	1627	436	311	511	962	497	≤249
Platelets (billion particles per litre)	99	101	65	29	216	202	128	271	303	150–300
Survival (days)	30	18	27	40	51	90	90	195	182	
Causes of death	Heart and liver failure	Heart and liver failure	Heart and liver failure	Heart and liver failure	SVC thrombosis, thoracic duct occlusion	Euthanasia	Euthanasia	Euthanasia	Euthanasia	

Normal reference values are given in the right-most column. Animals from groups I and II exhibited pathological biochemical alterations that correspond to heart and liver failure; platelet counts were low and LDH was elevated. By contrast, most parameters remained close to, or within, normal ranges in animals of group III. The baboon in experiment 10 had to be euthanized because of severe

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