Manchester International Living Donor Meeting 2018

INDIANA UNIVERSITY

Xenotransplantation: Is the End of Living Donors? What to Expect in the Next 50 Years?

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Acknowledgment – Research Support

* Sponsored Research Agreement with United Therapeutics

* IUPUI Office of the Vice Chancellor for Research

* Indiana Clinical and Translational Sciences Institute

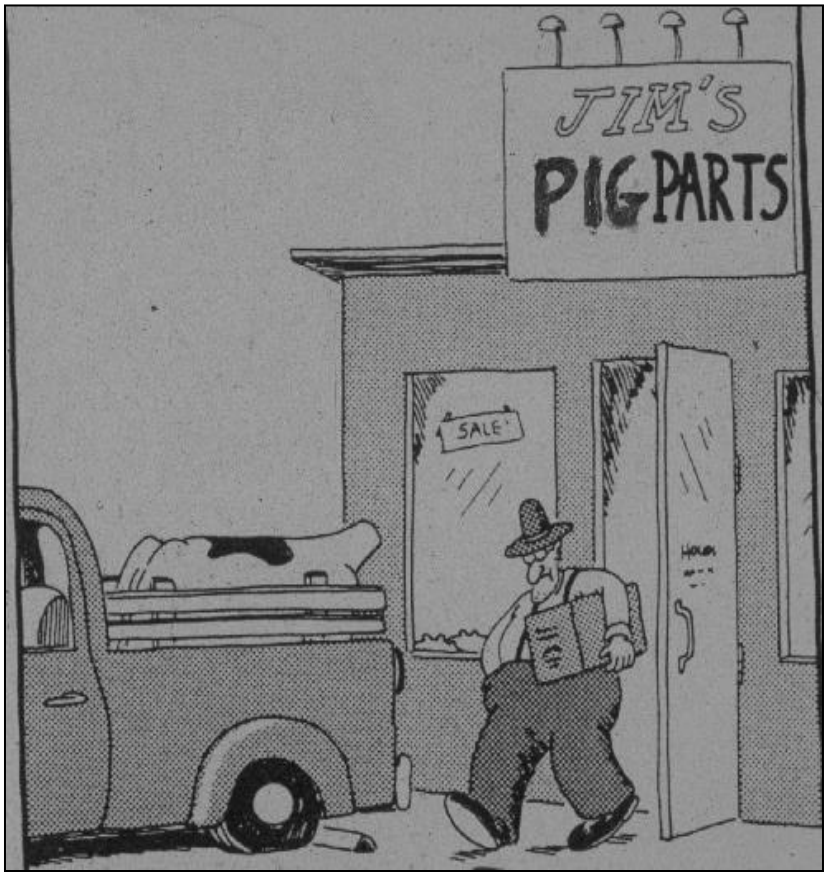
What is Xenotransplantation?

xen·o·trans·plan·ta·tion

/ˌzēnōˌtransplanˈtāSH(ə)n/

noun

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



Why Pig Organs / Kidneys?

The sow, the result of a 10-year breeding programme, takes a nap after giving birth to her huge litter

Non-human primates, such as chimpanzees and baboons are poor organ donors because:

- concerns about spread of infectious disease
- ethical issues
- limited availability
- poor breeding profile

Pigs as donors because:

- they are easy to breed and have large litters
- unlimited supply of donors
- pathogen-free pig breeds are available
- Easy to genetically-engineer
- pig organs are a similar size to human organs
- Similarities to human liver anatomy
- risk of infectious diseases is lower than in non-human primates
- pigs are already killed for food; fewer ethical concerns

Theological Symposium, September 20, 2017, Baltimore, MD

International Xenotransplantation Association Meeting

WELCOME REMARKS
Dr. Wayne Paris
Professor, School of Social Work, Abilene Christian University, Abilene, TX, United States

CURRENT STATUS OF XENOTRANSPLANTATION
Dr. Emanuele Cozzi
Director, Transplant Immunology Unit, Padua University Hospital Padua, Italy

THEOLOGICAL PRESENTATIONS

Rabbi Jerry Seidler
Jewish Religious Views on Theological Issues Associated with Xenotransplantation

Kevin Fitzgerald
Catholic and Christian Views on Theological Issues Associated with Xenotransplantation

Dr. Aasim Padela
Muslim Religious Views on Theological Issues Associated with Xenotransplantation

Barriers to Xenotransplantation

- Pigs as donor source
  - Immunologic barriers preclude clinical application
  - Xenoreactive antibodies bind to antigens on endothelium of pig organs and cause immediate graft loss
  - Coagulation proteins may be somewhat incompatible and lead to thrombotic microangiopathy
  - Cellular immunity

- Genetic engineering can eliminate antigens and physiologic incompatibilities as barriers to clinical application

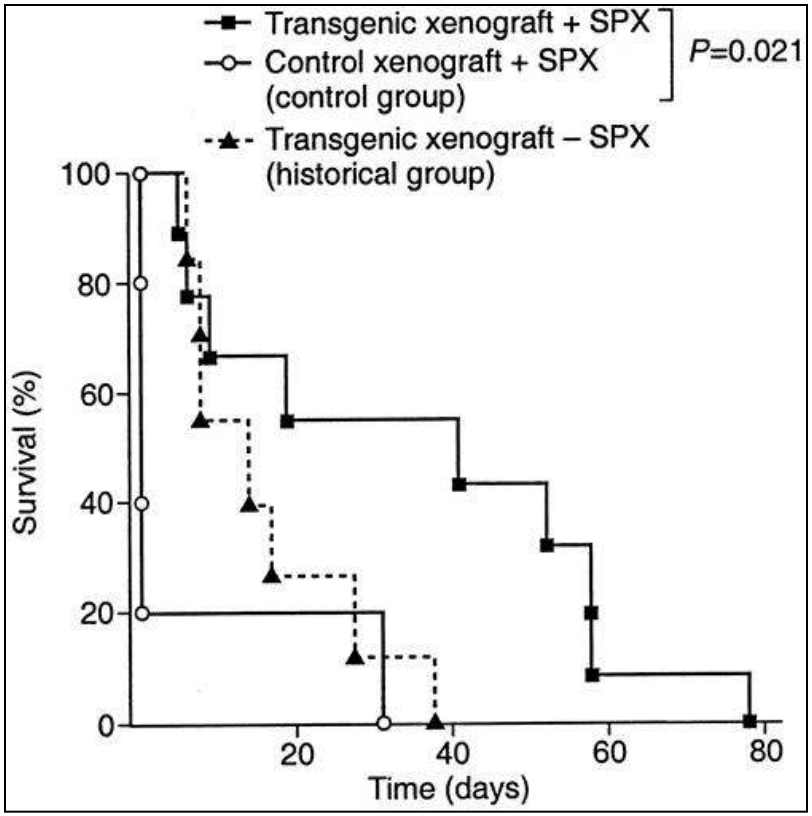
Role of Genetically Engineered Pigs in Xenotransplantation

	Purpose and indications of modification
Human CD59[6]	Complement regulation
Human CD55[7]	Complement regulation
Human H-transferase[8]	Reduction of Gal antigen expression
Human CD46[9]	Complement regulation
GTKO[10]	Deletion of Gal antigen expression
Endo-β-galactosidase C[11]	Reduction (but not deletion) of Gal antigen expression
Human TFPI[12]	Antagonise the function of tissue factor
Human TRAIL[13]	Control mechanisms of rejection mediated by cellular components of immune system
vWF-deficient[14]	Inhibit platelet activation
PERV siRNA[15]	Prevention of PERV activation
Porcine CTLA4-Ig[16]	Local co-stimulation blockade; T-cell suppression
Human thrombomodulin[17]	Anticoagulation (activates protein C)
HLA-E/human beta-2-microglobulin[18]	Protection against cytotoxicity of human natural killer cells
Human A20[19]	Anti-inflammatory; antiapoptosis
CIITA-DN[20]	Suppression of T-cell activation
Human Fas ligand[21]	Protection against cytotoxicity of human CD8+ and natural killer cells
Human GnT-III[22]	Downregulation of antigenicity to human natural antibodies
Human heme oxygenase 1[23]	Antiapoptosis; cytoprotection; anti-inflammatory
Human ENTPD1 (CD39)*	Anticoagulation and anti-inflammatory; conversion of ATP to ADP and AMP

Ekser et al, Lancet 2012 – Cooper, Ekser, et al. J Pathology 2016

Experience with hCD55 Expressing Pigs

Long-term Survival of Nonhuman Primates Receiving Life-Supporting Transgenic Porcine Kidney Xenografts



Cozzi et al. Transplantation 2000;70:15-21

Ureteral Stenosis in HDAF Pig-to-Primate Renal Xenotransplantation: A Phenomenon Related to Immunological Events?

Nicola Baldan[a,b,c,*], Paolo Rigotti[a,b,c], Fiorella Calabrese[d], Roberto Cadrobbi[a,b,c], Arben Dedja[b,c], Ilaria Iacopetti[e], Massimo Boldrin[b], Michela Seveso[b], Luigi Dall'Olmo[b,c], Laura Frison[c], Giulia De Benedictis[e], Daniele Bernardini[e], Gaetano Thiene[d], Emanuele Cozzi[a,b,c] and Ermanno Ancona[a,b,c]

[a]Padua General Hospital, Padua, Italy
[b]C.O.R.I.T. (Consorzio per la Ricerca sul Trapianto d'Organi), Padua, Italy
[c]Department of Medical and Surgical Sciences, [d]Institute of Pathology, and [e]Department of Clinical Veterinary Sciences, University of Padua, Padua, Italy
**Corresponding author: Nicola Baldan, nicola.baldan@unipd.it*

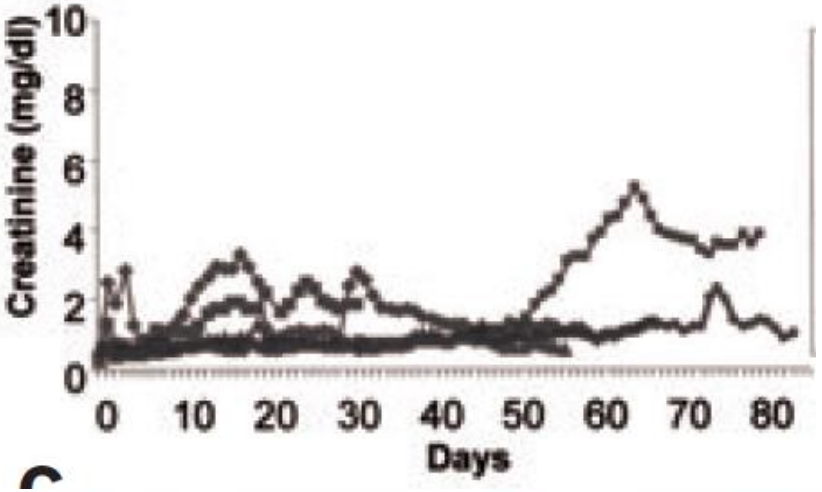
90 days. The longest survival of genetically-modified kidney xenotransplantation in the literature until 2015.

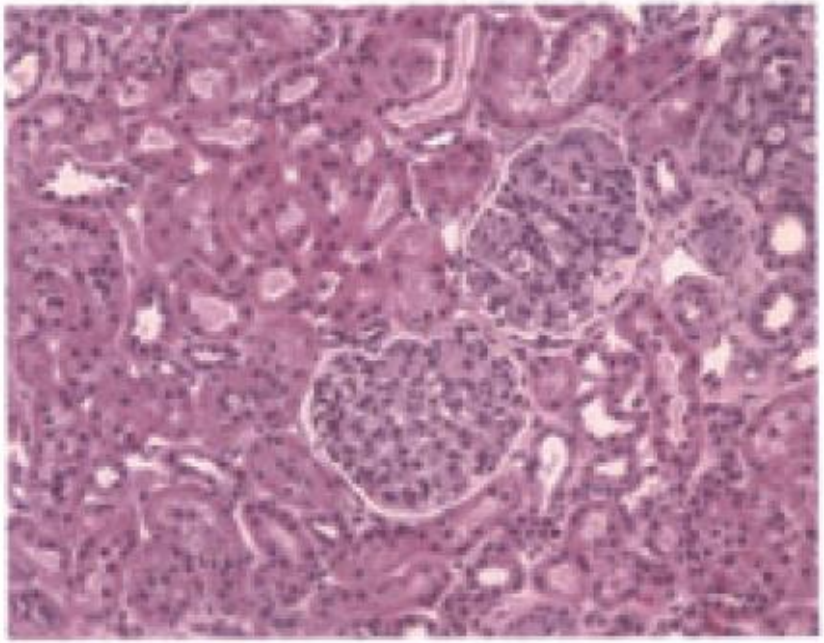
Baldan et al. Am J Transplant 2004;4:475-481

Experience with Gal-Knockout (GTKO) Pigs

Marked prolongation of porcine renal xenograft survival in baboons through the use of α1,3-galactosyltransferase gene-knockout donors and the cotransplantation of vascularized thymic tissue

Kazuhiko Yamada[1], Koji Yazawa[1], Akira Shimizu[1], Takehiro Iwanaga[1], Yosuke Hisashi[1], Matthew Nuhn[1], Patricia O'Malley[1], Shuji Nobori[1], Parsia A Vagefi[1], Clive Patience[2], Jay Fishman[3], David K C Cooper[1], Robert J Hawley[2], Julia Greenstein[2], Henk-Jan Schuurman[2], Michel Awwad[2], Megan Sykes[1] & David H Sachs[1]





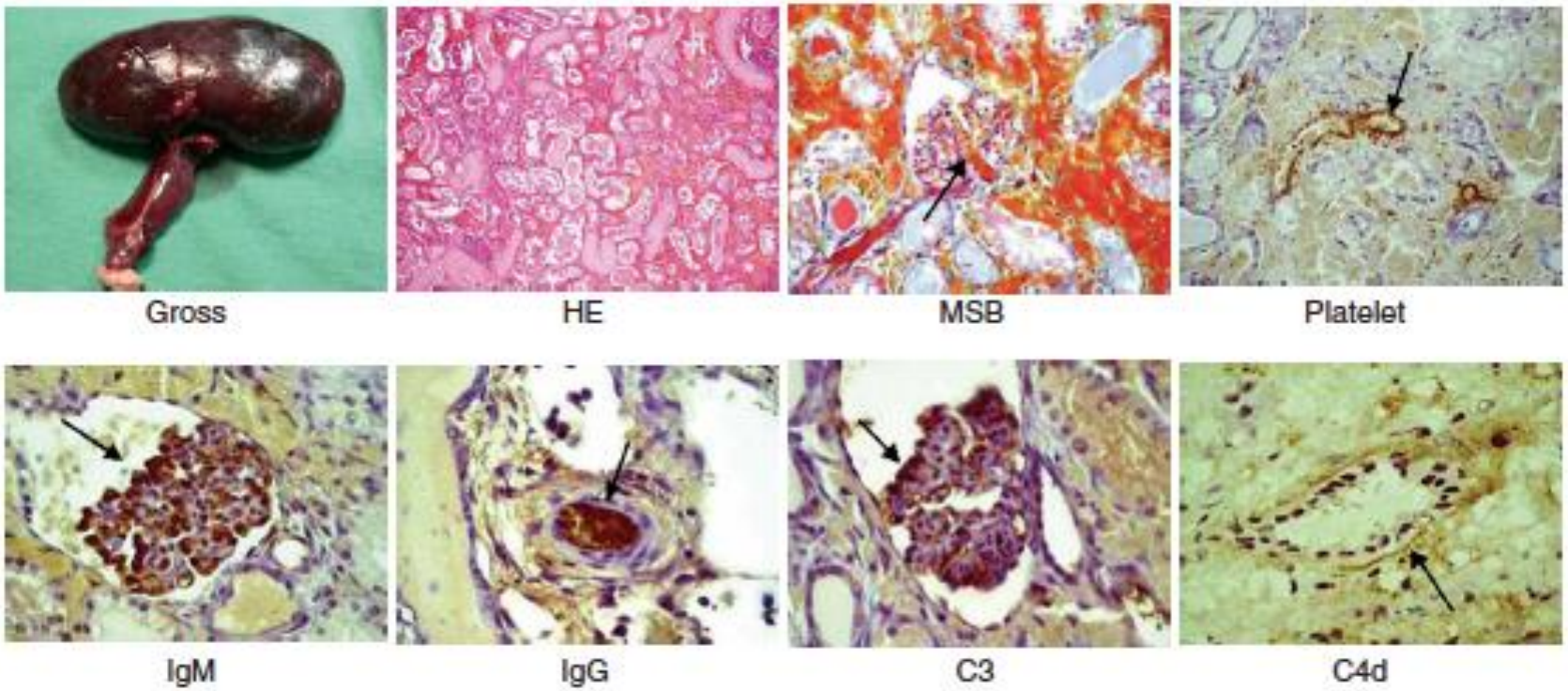
The longest survival was 83 days.

Yamada et al. Nat Med January 2005

Experience with Gal-Knockout (GTKO) Pigs

Acute rejection is associated with antibodies to non-Gal antigens in baboons using Gal-knockout pig kidneys

Gang Chen[1,11,12], Hua Qian[1,12], Thomas Starzl[2], Hongtao Sun[3], Bertha Garcia[3], Ximo Wang[1], Yishai Wise[1], Yuanqing Liu[1], Ying Xiang[1], Laura Copeman[4], Weihua Liu[3], Anthony Jevnikar[4,5,6], William Wall[1,7], David K C Cooper[2], Noriko Murase[2], Yifan Dai[2,8], Wanyu Wang[9], Yuliang Xiong[9], David J White[4] and Robert Zhong[1,3,4,6,7,10]

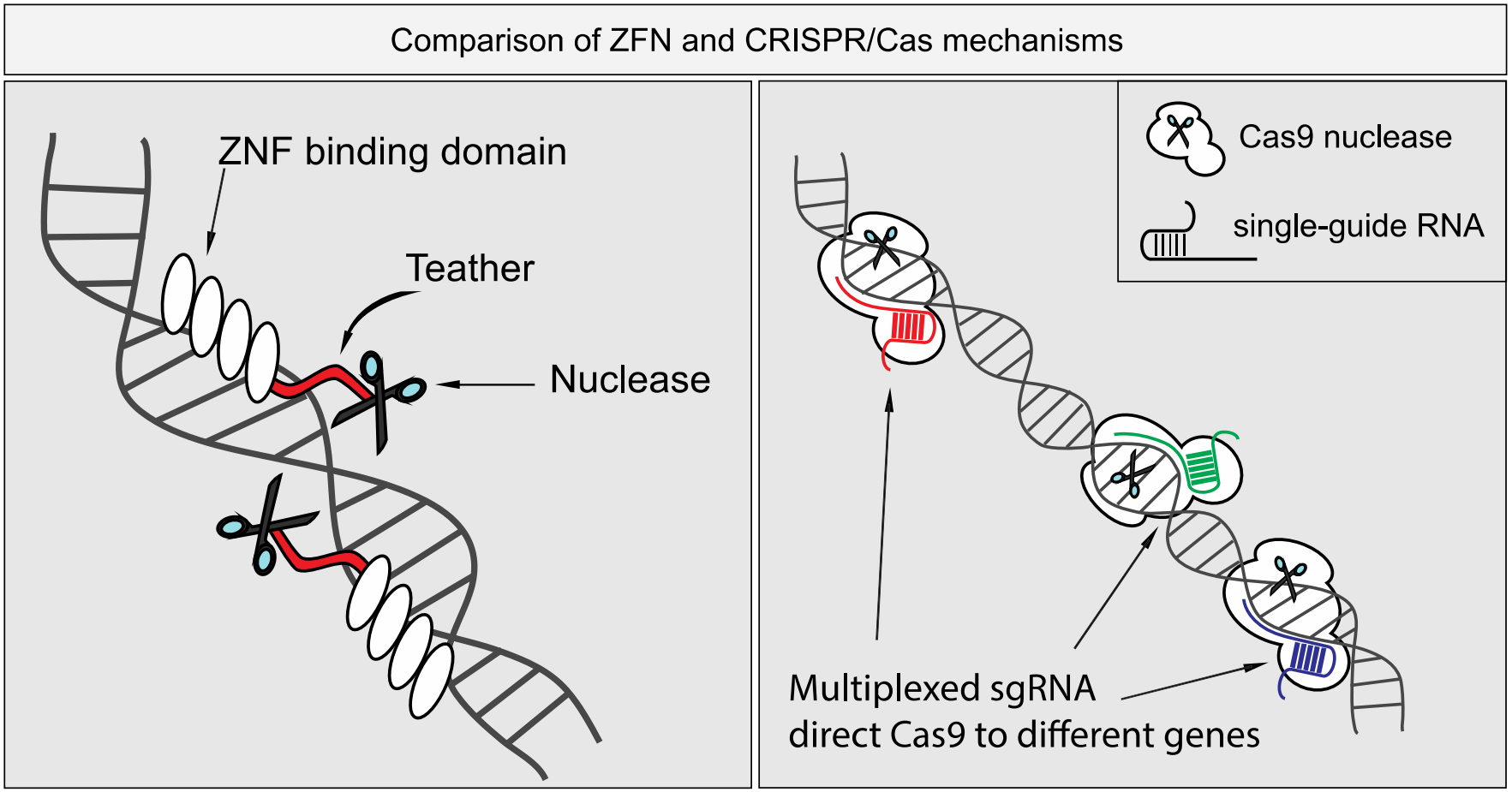


The longest survival was 16 days.

Chen et al. Nat Med December 2005

Genetic Engineering: 2013-present

CRISPR/Cas9 system is better than ZFN or TALENs



- Guide RNA are independent from nuclease allowing multiplexing
- Cas9 plasmid is inexpensive and easy to modify

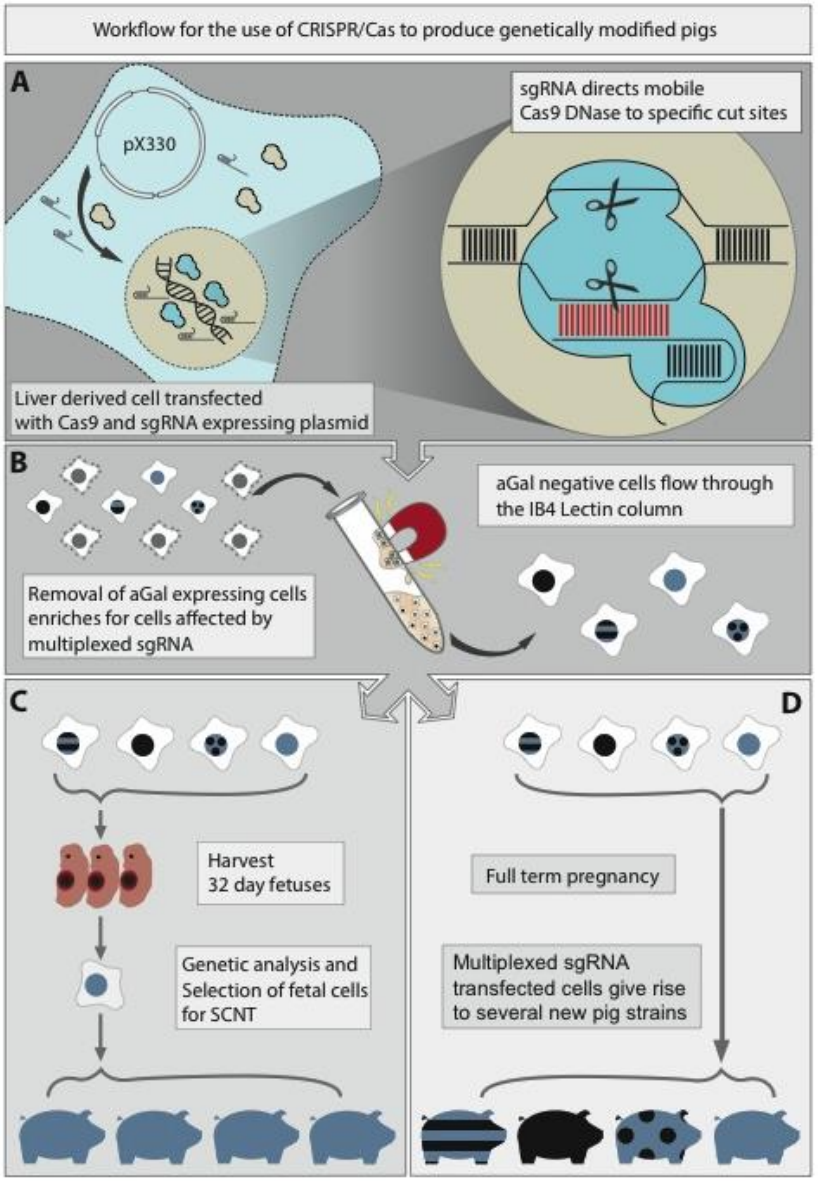
CRISPR: clustered regularly interspaced short palindromic repeats

Genetic Engineering: CRISPR/Cas9

creation of genetically distinct pigs from a single pregnancy

- Selection enriches for KO cells
- Enriched cells used for SCNT
- Harvest 32 day fetuses, collect cells for SCNT or…
- Full term pregnancy produces diverse genetics.
- Pigs in 135 days

1080 days → 210 days → **135 days**



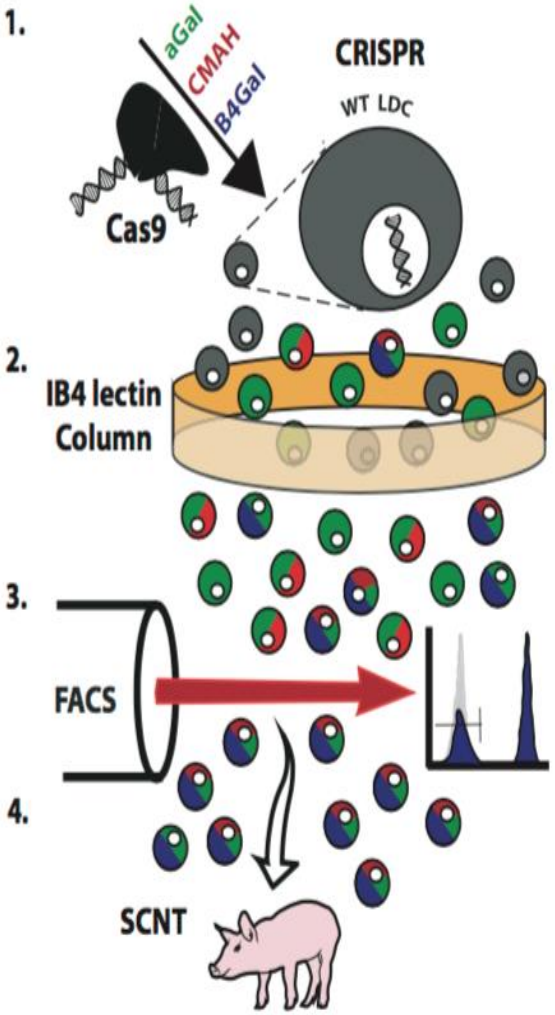
Li et al. Xenotransplantation 2014

Production of Triple Knockout Pigs – GTKO.Neu5Gc-KO.B4GalNT2-KO

Evaluation of human and non-human primate antibody binding to pig cells lacking GGTA1/CMAH/β4GalNT2 genes

Estrada JL, Martens G, Li P, Adams A, Newell KA, Ford ML, Butler JR, Sidner R, Tector M, Tector J. Evaluation of human and non-human primate antibody binding to pig cells lacking GGTA1/CMAH/β4GalNT2 genes. Xenotransplantation 2015: 22: 194–202. © 2015 The Authors. *Xenotransplantation* Published by John Wiley & Sons Ltd

Jose L. Estrada,[1] Greg Martens,[1] Ping Li,[1] Andrew Adams,[2,3] Kenneth A. Newell,[3] Mandy L. Ford,[3] James R. Butler,[1] Richard Sidner,[1] Matt Tector[4] and Joseph Tector[1,5]



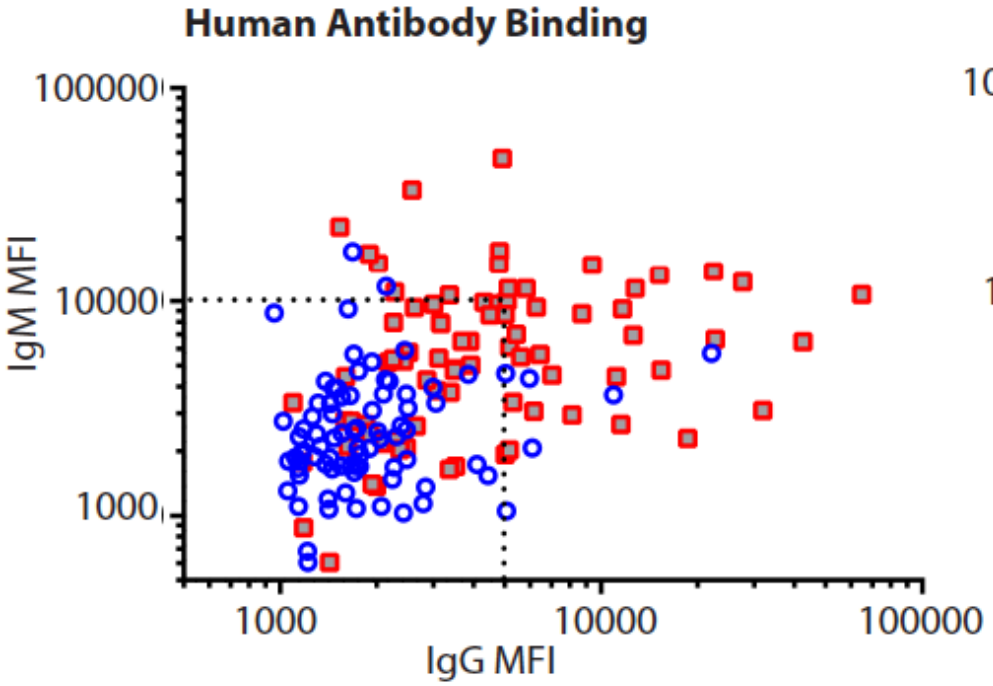
3 known antigens

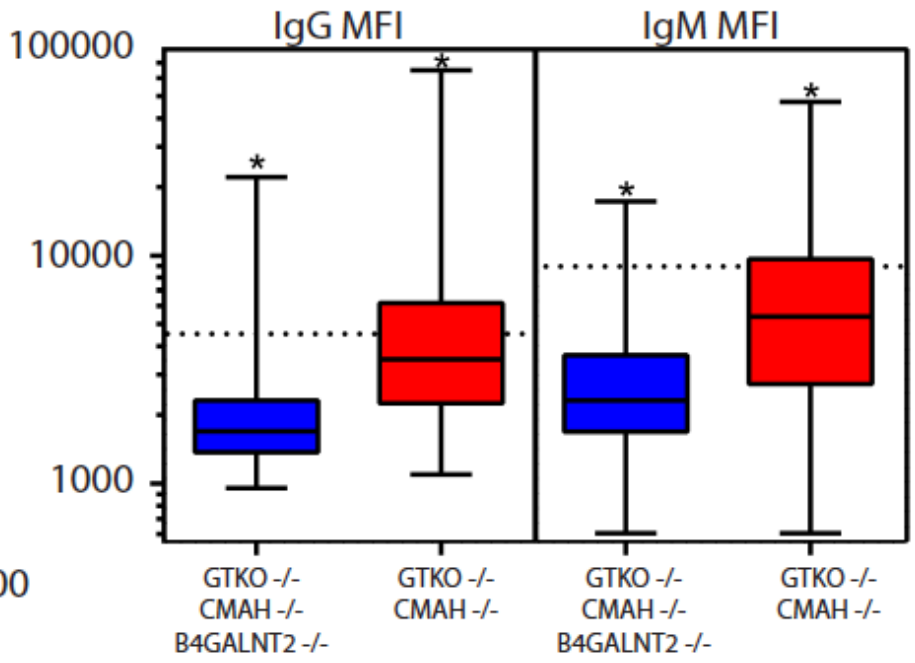
- Gal antigen (a product of the enzyme a1,3-galactocyltransferase)
- Neu5Gc (HD) antigen (a product of the enzyme CMAH)
- Sda antigen (a product of the enzyme B4GalNT2)

- B4GalNT2= Beta-1,4 N-Acetylgalactosaminyltransferase 2
- CMAH= cystidine monophosphate N-acetylneuraminic acid hydroxylase

Estrada et al, Xenotransplantation 2015

Genetic Engineering: Impact of B4GALNT2-KO





Indianapolis, IN - Kidney transplant waitlist patients

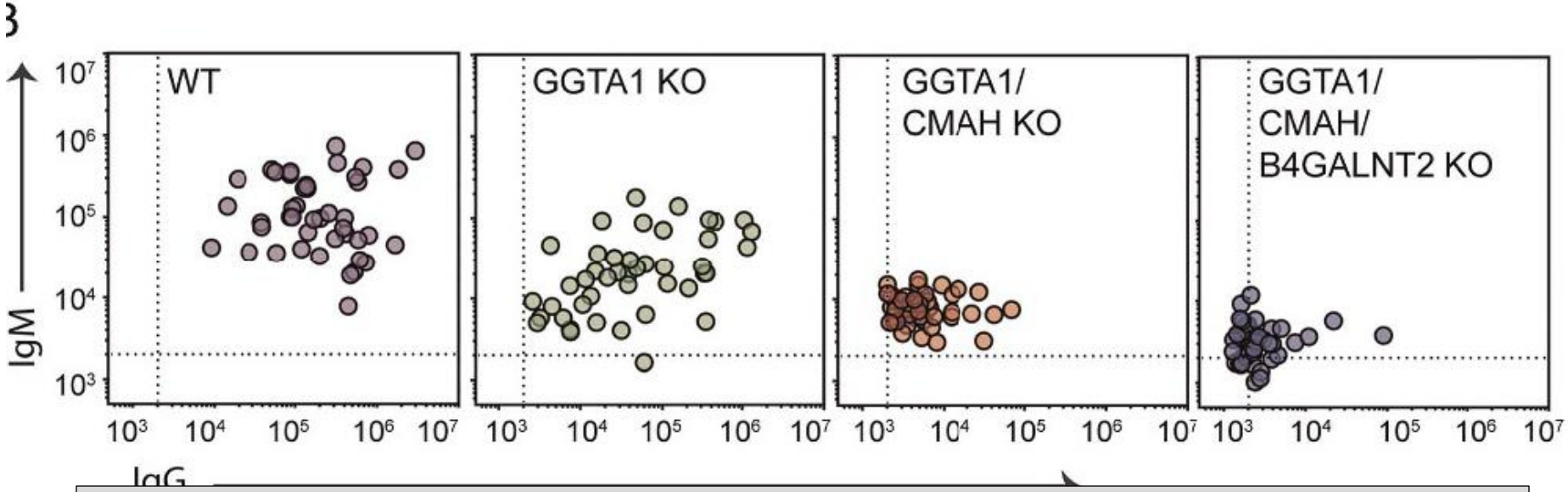
Estrada et al, Xenotransplantation 2015

Ab binding to triple-ko pig cells (GGTA1.CMAH.B4GalNT2)

Original Basic Science—General

Humoral Reactivity of Renal Transplant-Waitlisted Patients to Cells From GGTA1/CMAH/B4GalNT2, and SLA Class I Knockout Pigs

Gregory R. Martens, MD,[1] Luz M. Reyes, PhD,[1] James R. Butler, MD,[2] Joseph M. Ladowski, MS,[1] Jose L. Estrada, DVM, PhD,[1] Richard A. Sidner, PhD,[2] Devin E. Eckhoff, MD,[1,3] Matt Tector, PhD,[1,3] and A. Joseph Tector, MD, PhD[1,3]



Birmingham, AL - Kidney transplant waitlist patients

Martens et al. Transplantation 2017

Xenotransplantation 2015: 22: 221–230
doi: 10.1111/xen.12166

Brief Communication

Pre-transplant antibody screening and anti-CD154 costimulation blockade promote long-term xenograft survival in a pig-to-primate kidney transplant model

Higginbotham L, Mathews D, Breeden CA, Song M, Farris AB III, Larsen CP, Ford ML, Lutz AJ, Tector M, Newell KA, Tector AJ, Adams AB. Pre-transplant antibody screening and anti-CD154 costimulation blockade promote long-term xenograft survival in a pig-to-primate kidney transplant model. Xenotransplantation 2015: 22: 221–230. © 2015 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd.

Abstract: Xenotransplantation has the potential to alleviate the organ shortage that prevents many patients with end-stage renal disease from enjoying the benefits of kidney transplantation. Despite significant advances in other models, pig-to-primate kidney xenotransplantation has met limited success. Preformed anti-pig antibodies are an important component of the xenogeneic immune response. To address this, we screened a cohort of 34 rhesus macaques for anti-pig antibody levels. We then selected animals with both low and high titers of anti-pig antibodies to proceed with kidney transplant from galactose α1,3 galactose

Laura Higginbotham,[1] Dave Mathews,[1] Cynthia A. Breeden,[1] Mingqing Song,[1] Alton Brad Farris III,[2] Christian P. Larsen,[1] Mandy L. Ford,[1] Andrew J. Lutz,[3] Matthew Tector,[4] Kenneth A. Newell,[1] A. Joseph Tector[3] and Andrew B. Adams[1]

[1]Department of Surgery, Emory Transplant Center, Emory University School of Medicine, Atlanta, GA, [2]Anatomic Pathology, Emory University School of Medicine, Atlanta, GA, [3]Department of Surgery, Indiana University Health Transplant Institute, Indiana University School of Medicine, Indianapolis, IN, [4]Indiana University Health Transplant Department, Indianapolis, IN, USA

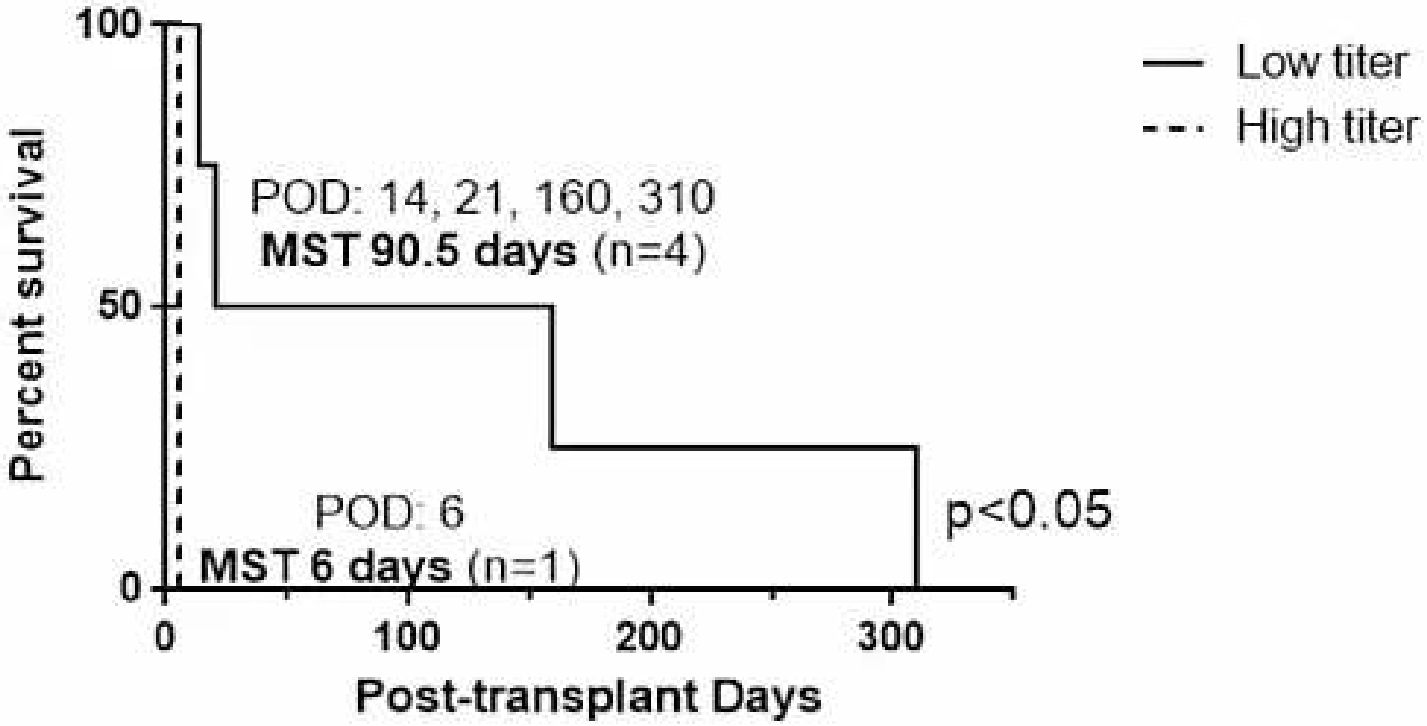
Pig-to-NHP renal xenotransplantation

Treatment regimen: T cell depletion, costimulation blockade, daily MMF/steroids

	Anti-CD154	Belatacept
High titer	n=1	
Low titer	n=2	n=2

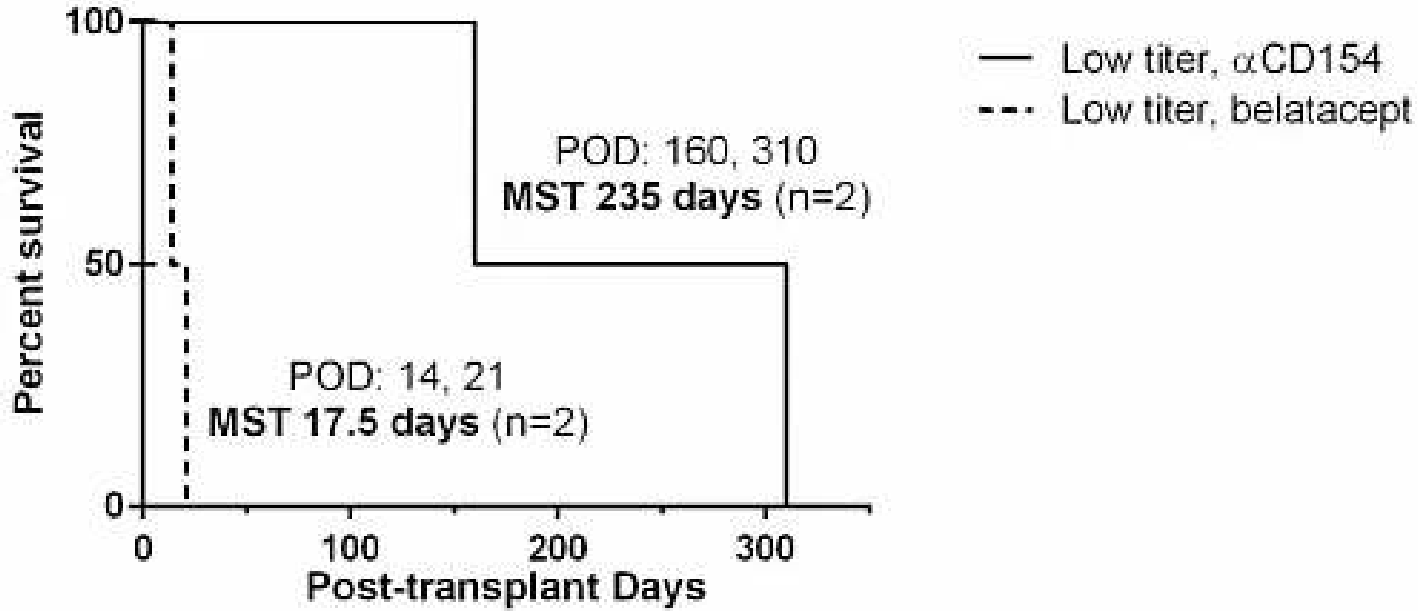
Higginbotham et al. IXA 2015 Meeting

Low anti-pig antibody titers are associated with prolonged survival



Higginbotham et al. IXA 2015 Meeting

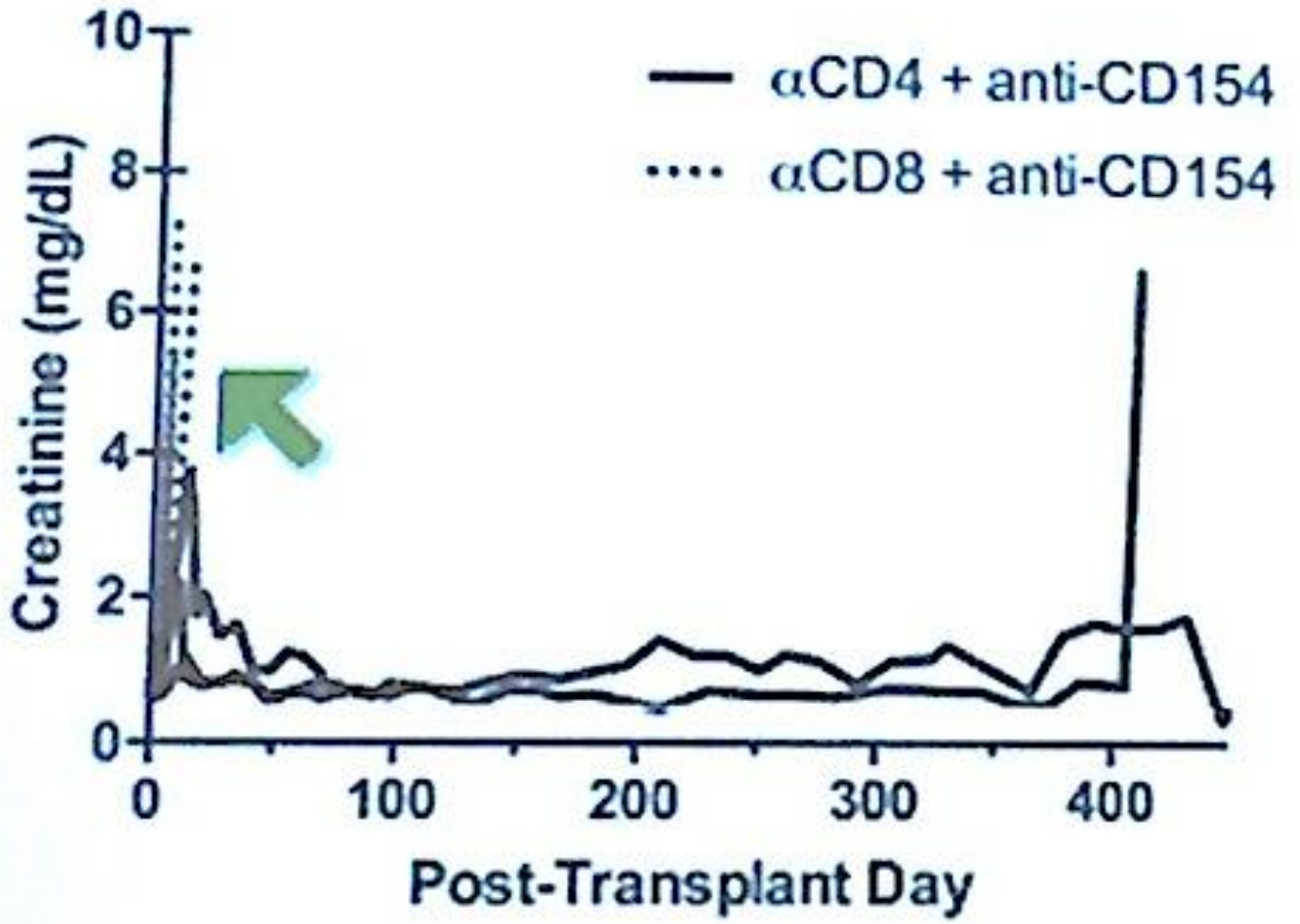
Anti-CD154 more effectively prolongs xenograft survival



Higginbotham et al. IXA 2015 Meeting

Pig-to-Primate Renal Transplantation – IXA 2017

GTKO/hDAF(CD55)
GTKO/B4GalNT2-KO



Tector et al. IXA 2017 Meeting

Xenotransplantation 2015: 22: 302–309
doi: 10.1111/xen.12174

XENOTRANSPLANTATION

Brief Communication

Pig kidney graft survival in a baboon for 136 days: longest life-supporting organ graft survival to date

Iwase H, Liu H, Wijkstrom M, Zhou H, Singh J, Hara H, Ezzelarab M, Long C, Klein E, Wagner R, Phelps C, Ayares D, Shapiro R, Humar A, Cooper DKC. Pig kidney graft survival in a baboon for 136 days: longest life-supporting organ graft survival to date. Xenotransplantation 2015: 22: 302–309. © 2015 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd.

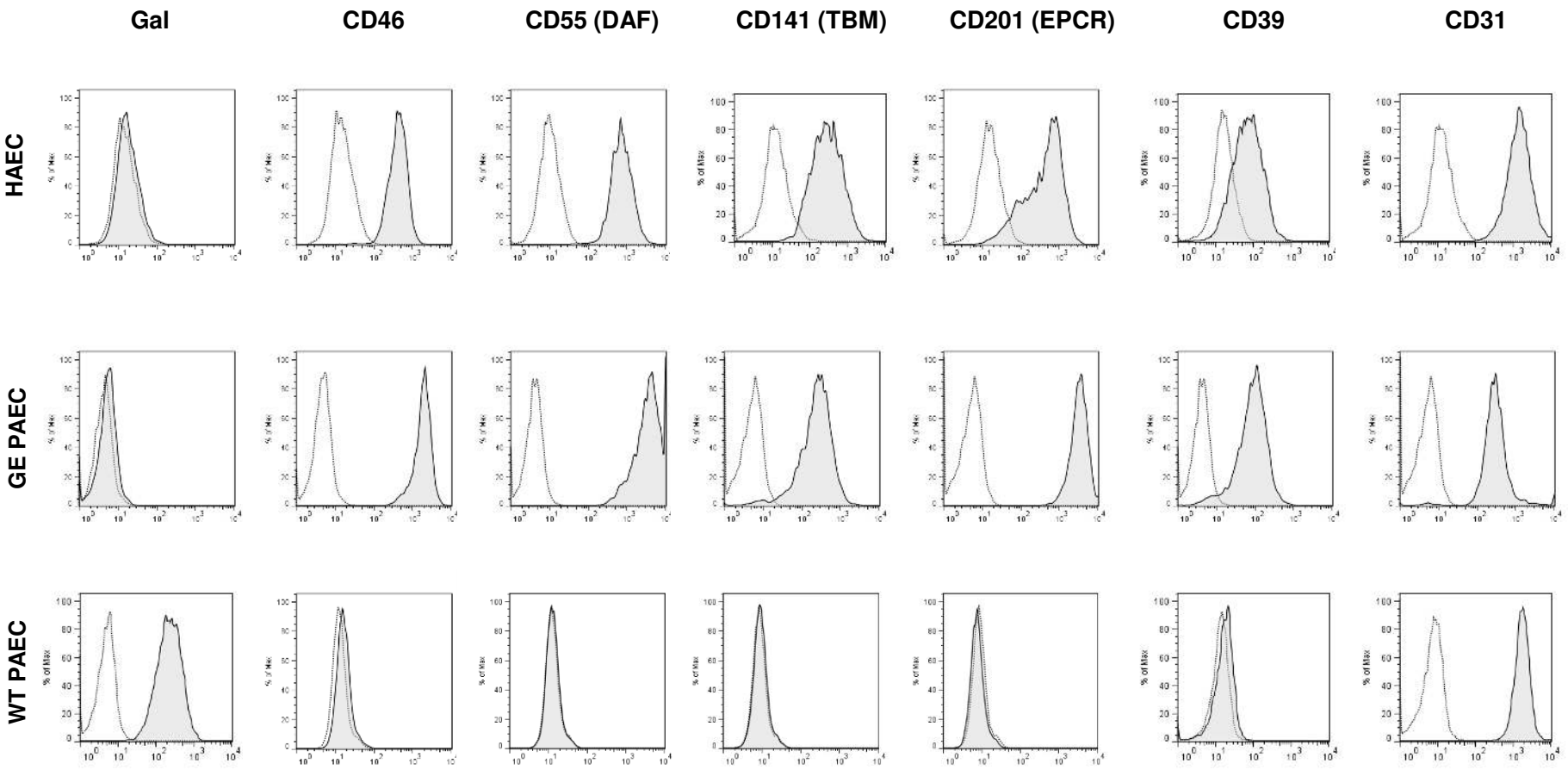
Abstract: The longest survival of a non-human primate with a life-supporting kidney graft to date has been 90 days, although graft survival > 30 days has been unusual. A baboon received a kidney graft from an α-1,3-galactosyltransferase gene-knockout pig transgenic for two human complement-regulatory proteins and three human coagulation-regulatory proteins (although only one was expressed in the kidney). Immunosuppressive therapy was with ATG+anti-CD20mAb (induction) and anti-CD40mAb+rapamycin+corticosteroids (maintenance). Anti-TNF-α and anti-IL-6R were administered. The baboon survived 136 days with a generally stable serum creatinine (0.6 to 1.6 mg/dl) until

Hayato Iwase,[1,*] Hong Liu,[1,2,*] Martin Wijkstrom,[1] Huidong Zhou,[1,3] Jagjit Singh,[1] Hidetaka Hara,[1] Mohamed Ezzelarab,[1] Cassandra Long,[1] Edwin Klein,[4] Robert Wagner,[4] Carol Phelps,[5] David Ayares,[5] Ron Shapiro,[1] Abhinav Humar[1] and David K. C. Cooper[1]

[1]Thomas E. Starzl Transplantation Institute, University of Pittsburgh, Pittsburgh, PA, USA, [2]Department of General Surgery, First Hospital of Shanxi Medical University, Taiyuan, Shanxi, [3]Center for Kidney Transplantation, Second Affiliated Hospital of the University of South China, Hengyang, Hunan, China, [4]Division of Laboratory Animal Resources, University of Pittsburgh, Pittsburgh, PA, [5]Revivicor, Blacksburg, VA, USA

Iwase et al. Xenotransplantation 2015

Comparison of surface expression on between HAECs and PAECs



GE: GTKO/CD46/CD55/TBM/EPCR/CD39

Iwase et al. Xenotransplantation 2015

GTKO/CD46/CD55/TBM/EPCR/CD39

Pig-to-Baboon Kidney XenoTx

Induction:

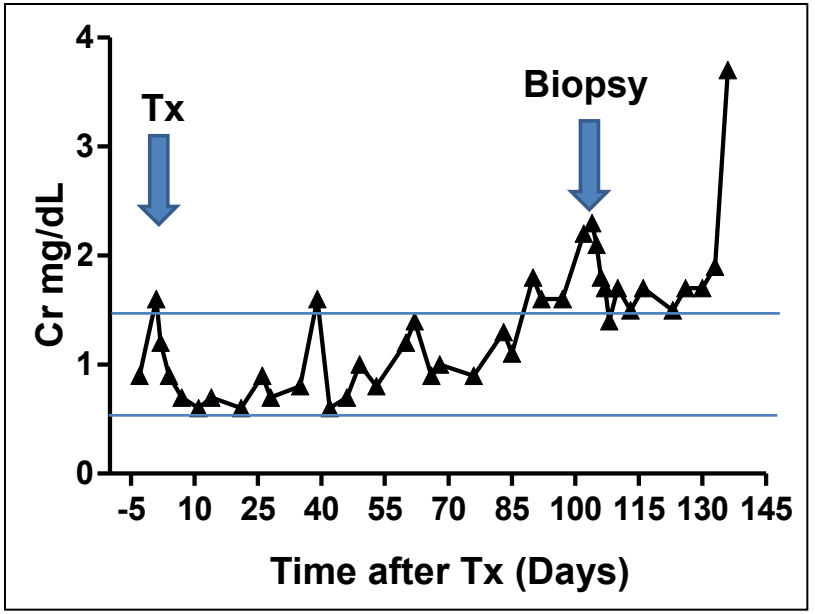
- ATG + RTX + CVF

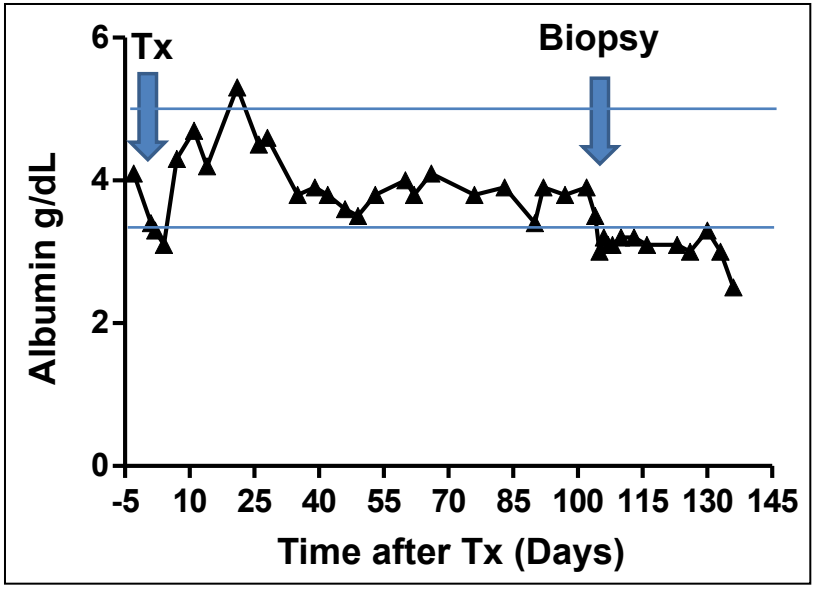
Maintenance:

- Anti-CD40mAb
- Rapamycin
- Methylprednisolone

Anti-inflammation:

- Tocilizumab
- Etanercept

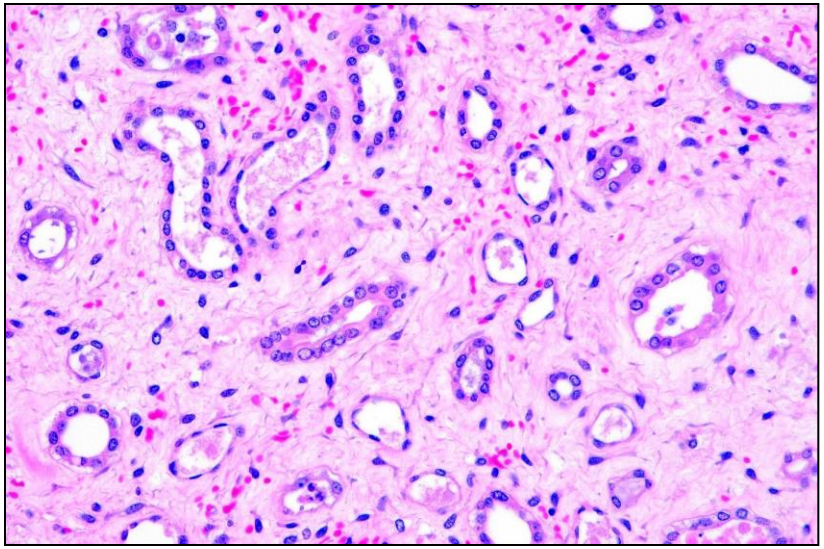




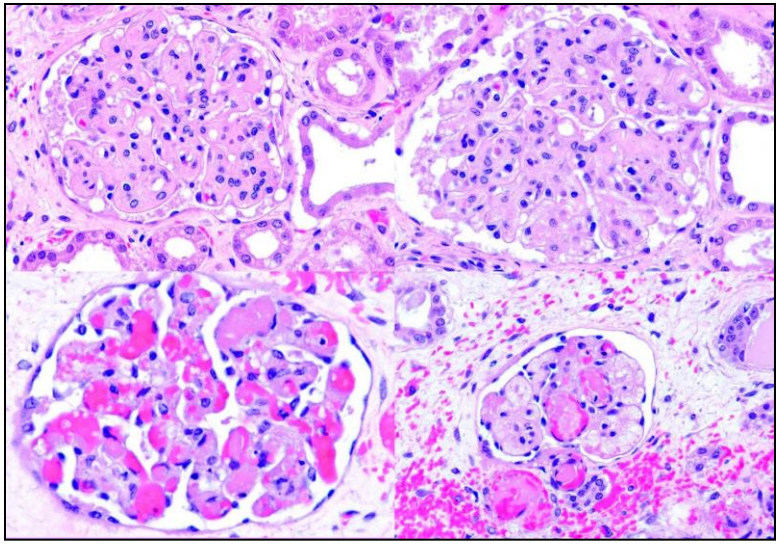
Iwase et al. Xenotransplantation 2015

Next to The Future: GTKO/CD46/CD55/TBM/EPCR/CD39

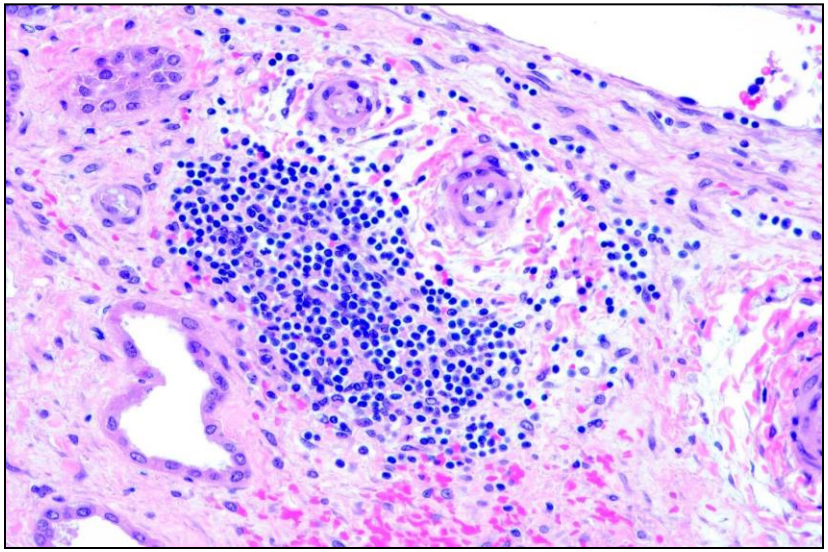
Life-supporting pig-to-baboon kidney xenotransplantation ~ 5 months survival



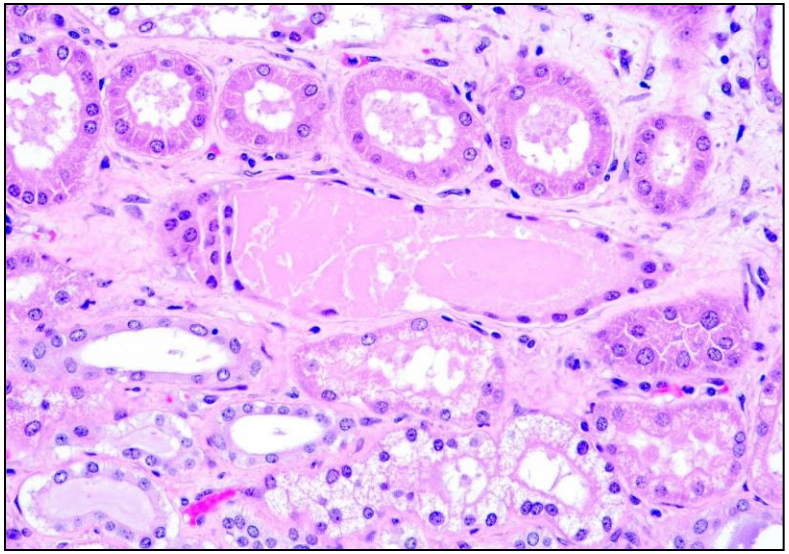
Interstital expansion



Glomerular enlargement, thrombi, mesangial expansion



Foci of lymphoid aggregates



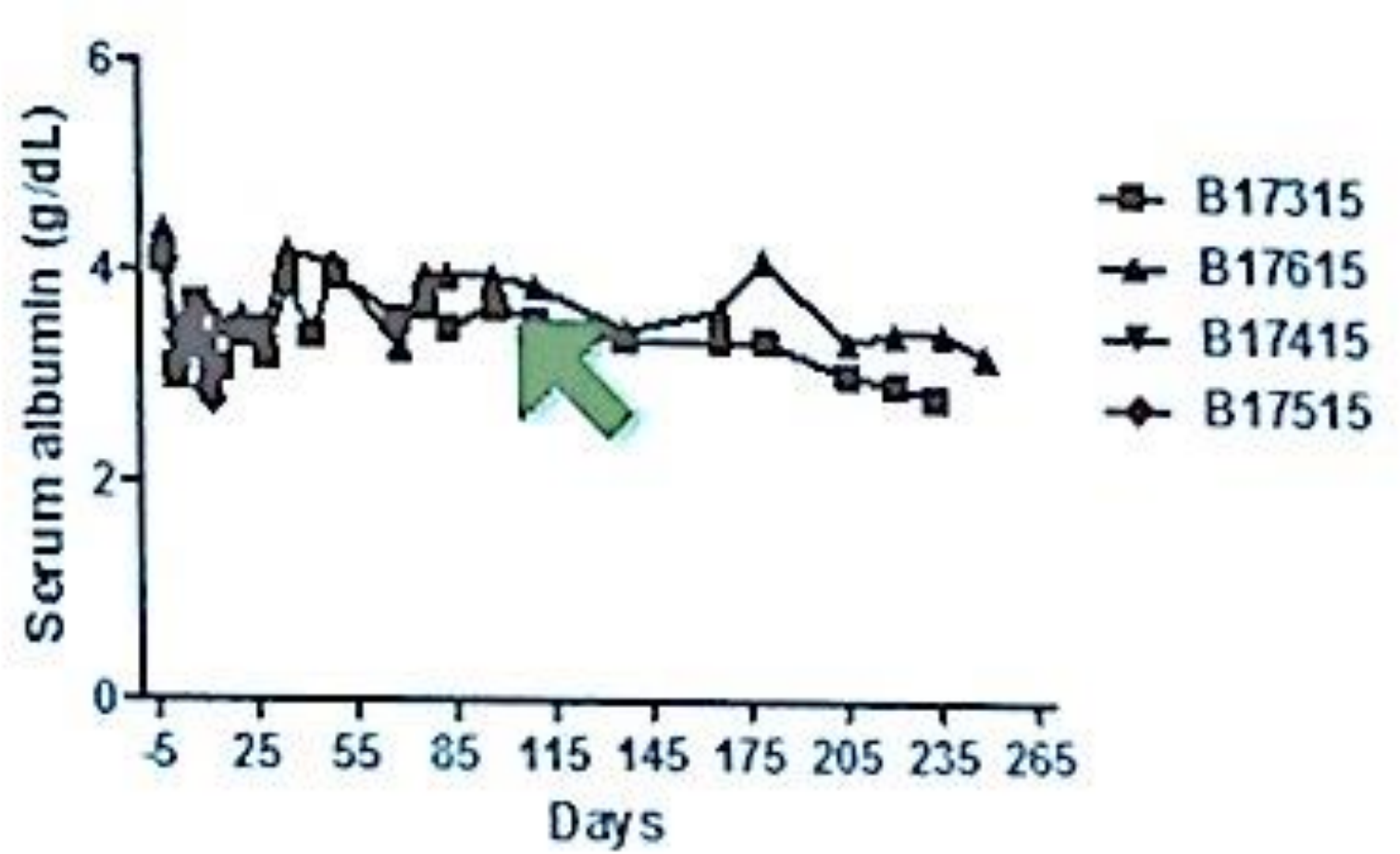
Mild, patchy, tubular proteinosis

Iwase et al. Xenotransplantation 2015

Pig-to-Primate Renal Transplantation – IXA 2017

GTKO/CD46/CD55/
TBM/EPCR/CD39

Baboon



Iwase et al. IXA 2017 Meeting

THE LANCET

Clinical xenotransplantation: the next medical revolution?

Burcin Ekser, Mohamed Ezzelarab, Hidetaka Hara, Dirk J van der Windt, Martin Wijkstrom, Rita Bottino, Massimo Trucco, David K C Cooper

Lancet 2012; 379: 672–83

Published Online October 21, 2011 DOI:10.1016/S0140-6736(11)61091-X

Thomas E Starzl Transplantation Institute, University of Pittsburgh Medical Center, Pittsburgh, PA, USA (B Ekser MD, M Ezzelarab MD, H Hara MD, D J van der Windt MD, M Wijkstrom MD, Prof D K C Cooper MD);

The shortage of organs and cells from deceased individuals continues to restrict allotransplantation. Pigs could provide an alternative source of tissue and cells but the immunological challenges and other barriers associated with xenotransplantation need to be overcome. Transplantation of organs from genetically modified pigs into non-human primates is now not substantially limited by hyperacute, acute antibody-mediated, or cellular rejection, but other issues have become more prominent, such as development of thrombotic microangiopathy in the graft or systemic consumptive coagulopathy in the recipient. To address these problems, pigs that express one or more human thromboregulatory or anti-inflammatory genes are being developed. The results of preclinical transplantation of pig cells—eg, islets, neuronal cells, hepatocytes, or corneas—are much more encouraging than they are for organ transplantation, with survival times greater than 1 year in all cases. Risk of transfer of an infectious microorganism to the recipient is small.

Ekser B et al, Lancet, 2012

THE LANCET

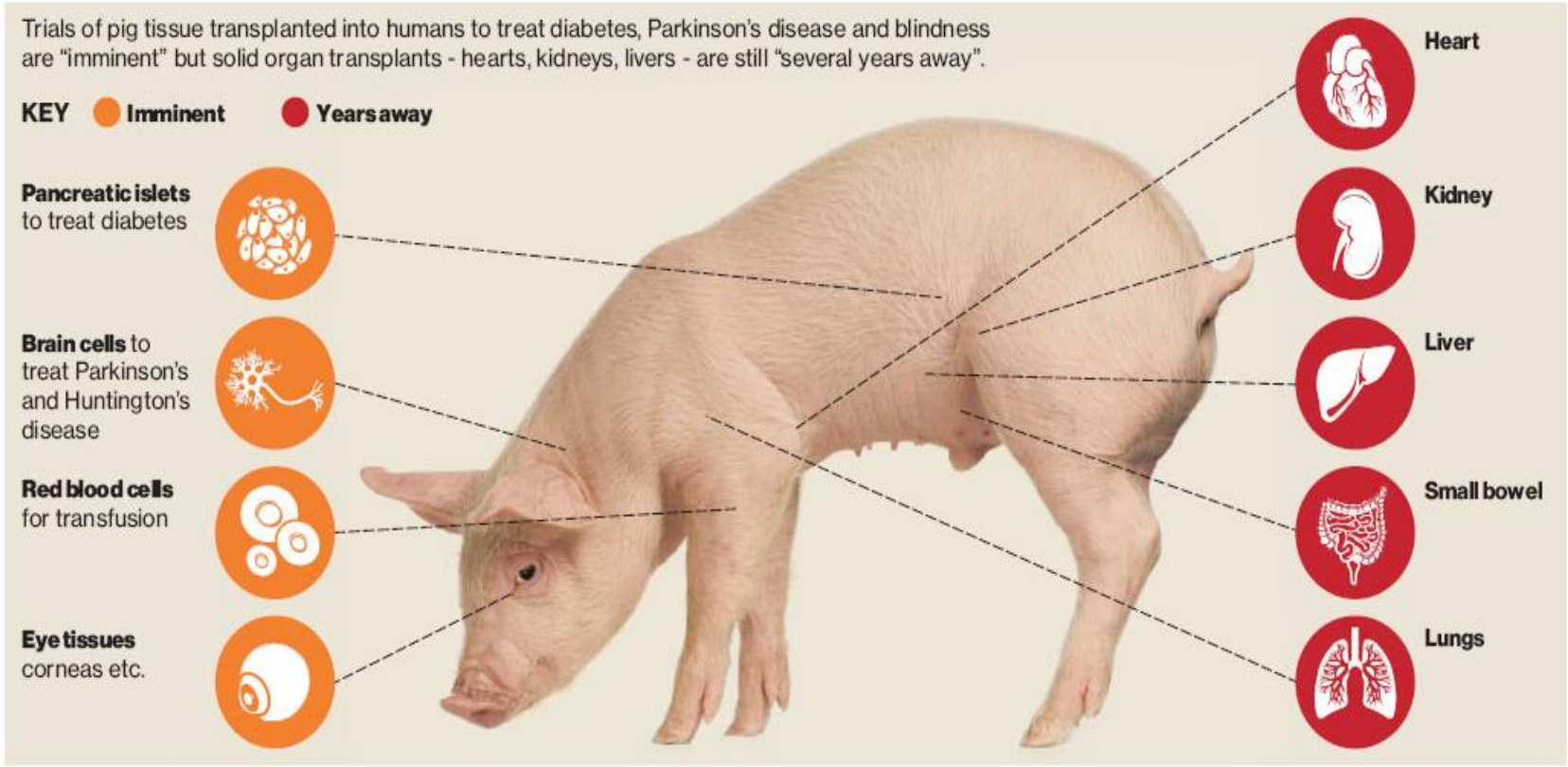
Clinical xenotransplantation: the next medical revolution?

Burcin Ekser, Mohamed Ezzelarab, Hidetaka Hara, Dirk J van der Windt, Martin Wijkstrom, Rita Bottino, Massimo Trucco, David K C Cooper

Lancet 2012; 379: 672–83

The shortage of organs and cells from deceased individuals continues to restrict allotransplantation. Pigs could

THE INDEPENDENT



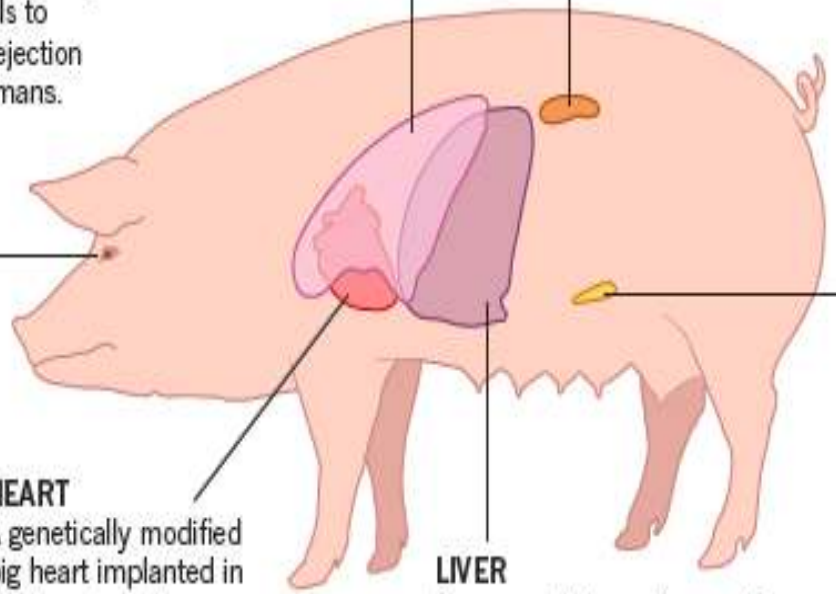
Ekser B et al, Lancet, 2012

NEW LIFE FOR PIG ORGANS

Gene-editing technologies have breathed life into the languishing field of xenotransplantation.

CHOICE CUTS

Researchers are looking to source an increasing variety of living tissues, including solid organs, from pigs. Many are attempting to genetically engineer the animals to reduce the risk of rejection and infection in humans.



LUNG
A factory farm is being designed to produce 1,000 pig lungs per year.

KIDNEY
A kidney with six genetic modifications supported a baboon's life for 4 months.

CORNEA
Pig corneas were approved for marketing in China in April.

PANCREAS
Phase III clinical trials of insulin-producing islet cells are under way.

HEART
A genetically modified pig heart implanted in a baboon's abdomen survived for 2.5 years.

LIVER
Livers could be engineered to produce their own antibodies against primate immune cells.

The Economist

CRISPR/Cas9 gene editing

No pig in a poke

Genome engineering may help make porcine organs suitable for use in people

Oct 17th 2015 | From the print edition

GENOME EDITING

Genome-wide inactivation of porcine endogenous retroviruses (PERVs)

Luhan Yang,[1,2,3*†] Marc Güell,[1,2,3†] Dong Niu,[1,4†] Haydy George,[1†] Emal Lesha,[1] Dennis Grishin,[1] John Aach,[1] Ellen Shrock,[1] Weihong Xu,[6] Jürgen Poci,[1] Rebeca Cortazio,[1] Robert A. Wilkinson,[5] Jay A. Fishman,[5] George Church[1,2,3*]

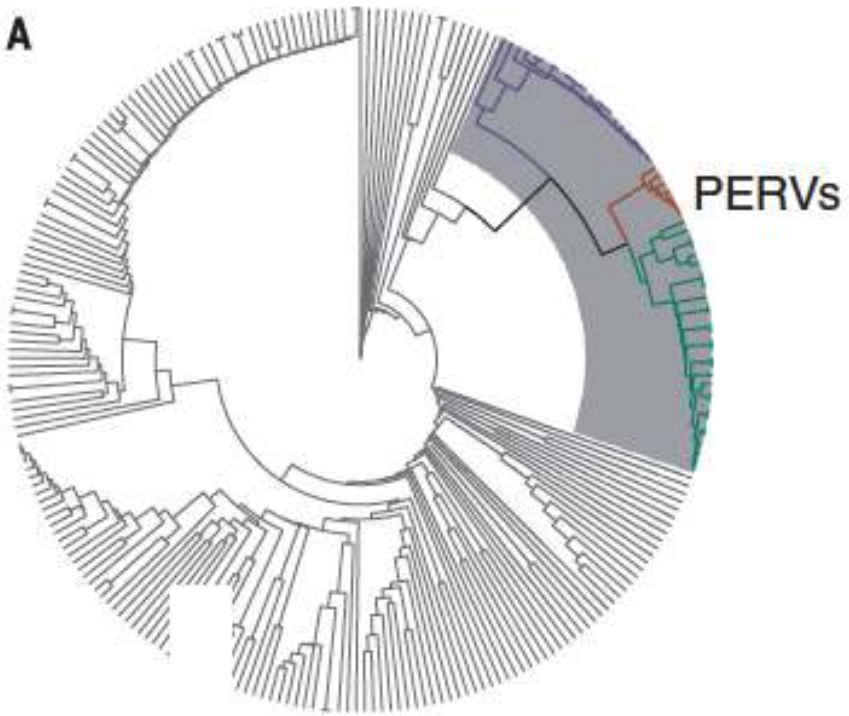


Fig. 1. CRISPR-Cas9 gRNAs were designed to specifically target the *pol* gene in 62 copies of PERVs in PK15 cells. (**A**) Phylogenetic tree representing endogenous retroviruses present in the pig genome. PERVs are highlighted in blue. (**B**) Copy number determination of PERVs in PK15 cells via digital droplet PCR. The copy number of *pol* elements was estimated to be 62, using three independent reference genes: *ACTB*, *GAPDH*, and *EB2*. $n = 3$ independent reference genes, mean ± SEM. (**C**) We designed

Science 2015 November

Xenotransplantation makes a comeback

A newly announced genome-editing experiment—the largest documented to date—is the latest in a series of advances reinvigorating the field of xenotransplantation. In October, Harvard University geneticist George Church and colleagues at Boston-based startup eGenesis described (*Science* **350**, 1101–1104, 2015) the use of CRISPR-Cas9 to disrupt all 62 genomic copies of porcine endogenous retrovirus (PERV) in cultured pig kidney epithelial cells. That feat, which dwarfs the greatest number of simultaneous DNA changes ever recorded using genome editing, removes one potential hurdle facing use of pig xenotransplants in human patients—zoonosis. But eGenesis is not the only company actively exploring the commercial prospects of xenotransplantation; Silver Spring, Maryland–based United Therapeutics has been quietly building infrastructure and partnerships—most notably with La Jolla–based Synthetic Genomics—to bring xenotransplantation closer to reality.

The recent *Science* paper addresses a widely perceived risk that has never been documented. "Nobody's ever shown that PERVs could actually cause a disease in a human," points out David Sachs, director of the Transplantation

Today, interest is once again waxing thanks both to a better understanding of organ rejection and to the use of genome editing tools, such as CRISPR-Cas9, to manipulate pig genes encoding proteins that trigger immune recognition. Though regulators have yet to set definitive rules for how long xenotransplants must survive before clinical trials can begin, Muhammad Mohiuddin, chief of the transplantation section at the National Heart, Lung, and Blood Institute (NHLBI), notes that a 1999 document (http://www.fda.gov/ohrms/dockets/ac/99/other/6_3-4mtg.doc) from the US Food and Drug Administration suggested a threshold of 90% survival at 60 days and 50% survival at 90

Perkel. Nat Biotechnol January 2016

EDITORIAL

nature biotechnology

Xenotransplantation 2.0

Will targeted immunosuppressants and new tools in genome engineering be enough to finally give xenopigs wings?

Nat Biotechnol January 2016

2-week-old piglet whose genome has been engineered to inactivate porcine endogenous retroviruses (PERVs).

22 September 2017

Exciting Developments in Xenotransplantation

Genetically engineered pigs produced in Munich, Germany, were recently used in a record-breaking baboon heart transplant. JAN-MICHAEL ABICHT

Scientists grow bullish on pig-to-human transplants

By Kelly Servick | Sep. 22, 2017 , 1:47 PM

Exciting Developments in Xenotransplantation

TIME | Health

RESEARCH

Why People May Have Pig Organs Inside Them One Day

Alexandra Sifferlin
Aug 25, 2017

TIME Health *For more, visit TIME Health.*

International Business Times

Home Society

Chinese scientists to soon perform world's first pig organ transplant in humans

2017-08-27 17:00:13 IST
By K Vidhya

Exciting Developments in Xenotransplantation

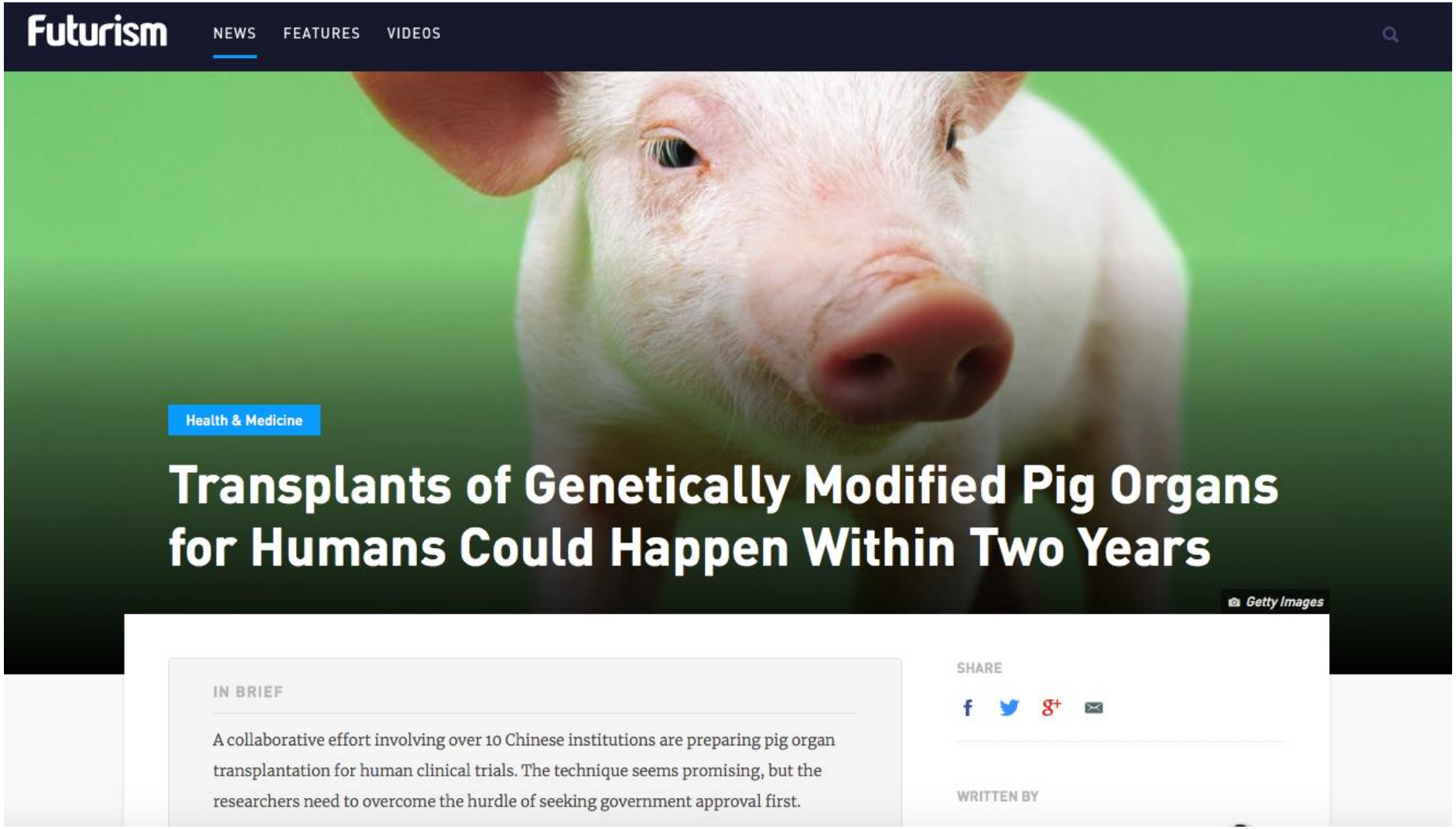
INDEPENDENT

Pig organs could soon be transplanted into humans after major 'xenotransplantation' breakthrough

The shortage of organs for transplants is one of the biggest challenges to modern medicine

Andrew Griffin Science reporter | @_andrew_griffin | Thursday 10 August 2017 17:58 BST | 99 comments

Exciting Developments in Xenotransplantation



Exciting Developments in Xenotransplantation

04.17.18 | WORLD CHANGING IDEAS

We Are Getting Closer To Transplanting Pig Organs Directly Into People

Scientists using CRISPR to edit pig organs so they'll be accepted by human bodies think a breakthrough is coming that will end the organ-donation waiting list.

Exciting Developments in Xenotransplantation

Saturday, April 21, 2018

The Tribune

VOICE OF THE PEOPLE

Chandigarh

Posted at: Apr 15, 2018, 2:43 AM; last updated: Apr 15, 2018, 2:43 AM (IST)

Pig may come to rescue of renal failure patient: Experts

Also in this section

Hit by Fortuner, woman dies

Mohali MLA Sidhu makes Cabinet cut

Ex-guard planned robbery, 3 held

Mother's body hanging, child in

TOP STORIES


Oppn submits impeachment motion against CJI on 5 counts


9 new Cabinet ministers for Punjab, swearing-in today

Told PM ministers had to go:

Exciting Developments in Xenotransplantation

** First FDA-approved clinical trials in xenotransplantation are expected to start in the United States in 2019-2020.*

Future of Xenotransplantation Behind the Scenes

Identification of new genes for the use in clinical transplantation

- Expression of human CD47 in order to achieve tolerance
- Expression of human HLA-G/E in order to achieve tolerance
- Swap of HLA with SLA
- Knocking out porcine tetraspanins (CD37/CD81)

Future of Xenotransplantation Behind the Scenes

Xenotransplantation. 2010 ; 17(4): 267–273. doi:10.1111/j.1399-3089.2010.00601.x.

CD47 in Xenograft Rejection and Tolerance Induction

Yong-Guang Yang*
Transplantation Biology Research Center, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts, United States

Abstract

Robust immune responses to xenografts remain a major obstacle to clinical translation of xenotransplantation, which could otherwise be a potential solution to the worldwide shortage of organ donors. The more vigorous xenograft rejection relative to allograft rejection is largely accounted for by the extensive genetic disparities between the donor and recipient. Xenografts activate host immunity not only by expressing immunogenic xenoantigens that provide the targets for immune recognition and rejection, but also by lacking ligands for the host immune inhibitory receptors. This review is focused on recent findings regarding the role of CD47, a ligand of an immune inhibitory receptor SIRPα, in xenograft rejection and induction of xenotolerance.

Future of Xenotransplantation Behind the Scenes

Cellular & Molecular Immunology (2011) 8, 285–288

www.nature.com/cmi

REVIEW

CD47: a new player in phagocytosis and xenograft rejection

Nalu Navarro-Alvarez and Yong-Guang Yang

Organ transplantation is limited by the availability of human donor organs. The transplantation of organs and tissues from other species (xenotransplantation) would supply an unlimited number of organs and offer many other advantages for which the pig has been identified as the most suitable source. However, the robust immune responses to xenografts remain a major obstacle to clinical application of xenotransplantation. The more vigorous xenograft rejection relative to allograft rejection is largely accounted for by the extensive genetic disparities between the donor and recipient. Xenografts activate host immunity not only by expressing immunogenic xenoantigens that provide the targets for immune recognition and rejection, but also by lacking ligands for the host immune inhibitory receptors. This review is focused on recent findings regarding the role of CD47, a ligand of an immune inhibitory receptor, signal regulatory protein alpha (SIRPα), in phagocytosis and xenograft rejection.
Cellular & Molecular Immunology (2011) **8,** 285–288; doi:10.1038/cmi.2010.83; published online 24 January 2011

Future of Xenotransplantation Behind the Scenes

Original Basic Science—General

Swine Leukocyte Antigen Class II Is a Xenoantigen

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

Background. Over 130 000 patients in the United States alone need a lifesaving organ transplant. Genetically modified porcine organs could resolve the donor organ shortage, but human xenoreactive antibodies destroy pig cells and are the major barrier to clinical application of xenotransplantation. The objective of this study was to determine whether waitlisted patients possess preformed antibodies to swine leukocyte antigen (SLA) class II, homologs of the class II HLA. **Methods.** Sera from people currently awaiting solid organ transplant were tested for IgG binding to class II SLA proteins when expressed on mammalian cells. Pig fibroblasts were made positive by transfection with the class II transactivator. As a second expression system, transgenes encoding the alpha and beta chains of class II SLA were transfected into human embryonic kidney cells. **Results.** Human sera containing IgG specific for class II HLA molecules exhibited greater binding to class II SLA positive cells than to SLA negative cells. Sera lacking antibodies against class II HLA showed no change in binding regardless of the presence of class II SLA. These antibodies could recognize either SLA-DR or SLA-DQ complexes. **Conclusions.** Class II SLA proteins may behave as xenoantigens for people with humoral immunity toward class II HLA molecules.

(*Transplantation* 2018;102: 249–254)

Ladawski et al. J Immunol 2018

Future of Xenotransplantation Behind the Scenes

The Journal of Immunology

Examining the Biosynthesis and Xenoantigenicity of Class II Swine Leukocyte Antigen Proteins

Joseph M. Ladowski,* Gregory R. Martens,* Luz M. Reyes,* Zheng-Yu Wang,* Devin E. Eckhoff,* Vera Hauptfeld-Dolejsek,† Matt Tector,* and A. Joseph Tector*

Genetically engineered pig organs could provide transplants to all patients with end-stage organ failure, but Ab-mediated rejection remains an issue. This study examines the class II swine leukocyte Ag (SLA) as a target of epitope-restricted Ab binding. Transfection of individual α- and β-chains into human embryonic kidney cells resulted in both traditional and hybrid class II SLA molecules. Sera from individuals on the solid organ transplant waiting list were tested for Ab binding and cytotoxicity to this panel of class II SLA single-Ag cells. A series of elution studies from an SLA-DQ cell line were performed. Our results indicate that human sera contain Abs specific for and cytotoxic against class II SLA. Our elution studies revealed that sera bind the SLA-DQ molecule in an epitope-restricted pattern. Site-specific mutation of one of these epitopes resulted in statistically decreased Ab binding. Humans possess preformed, specific, and cytotoxic Abs to class II SLA that bind in an epitope-restricted fashion. Site-specific epitope mutagenesis may decrease the Ab binding of highly sensitized individuals to pig cells. *The Journal of Immunology*, **2018, 200: 2957–2964.**

Ladawski et al. J Immunol 2018

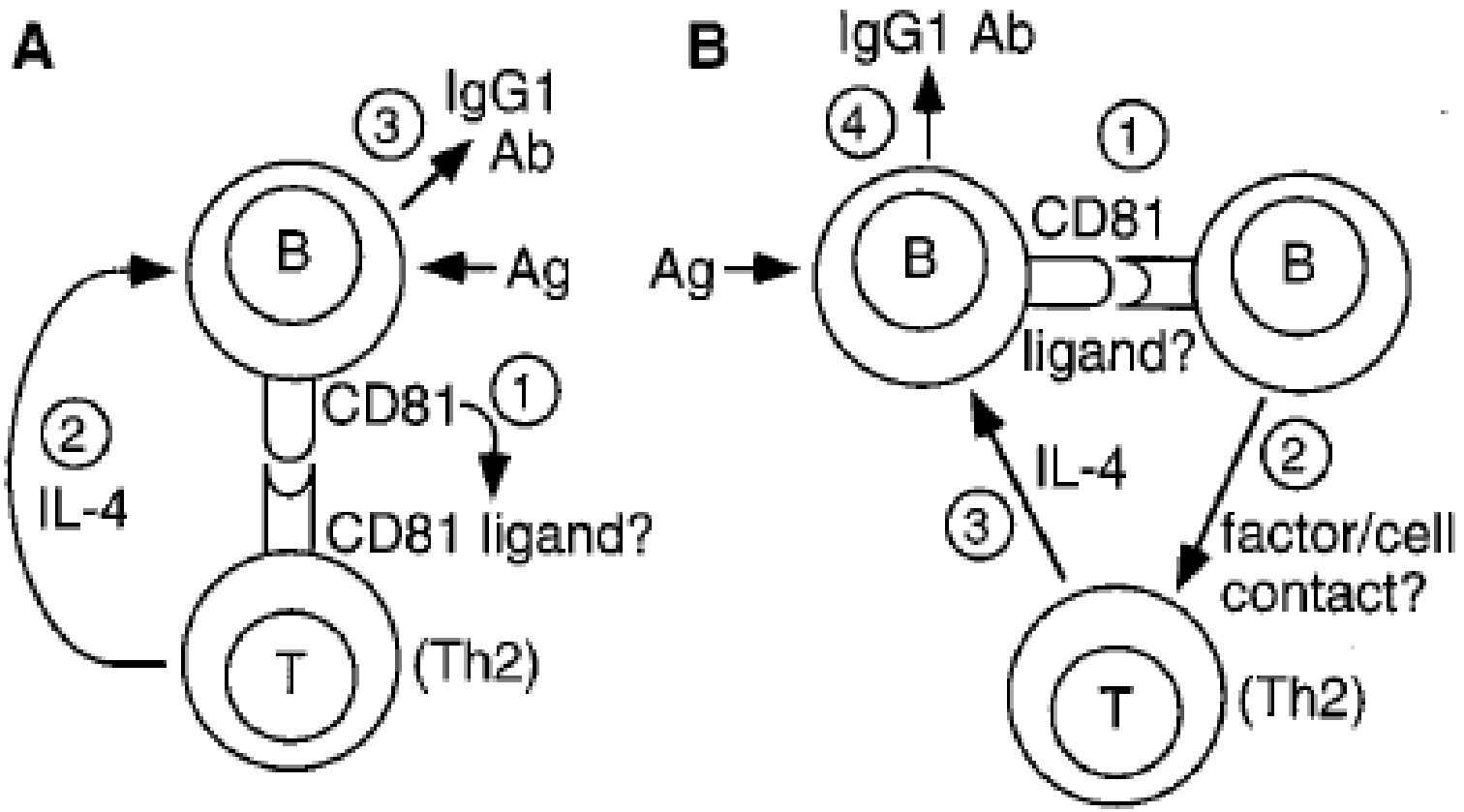
Identification of novel xenoreactive non-Gal antigens: Tetraspanins CD37 and CD81

Ping Li, PhD, **Burcin Ekser, MD, PhD**

Division of Transplant Surgery, Indiana University School of Medicine

Presented at IXA 2017

Tetraspanins (CD37/CD81/CD9/CD151)

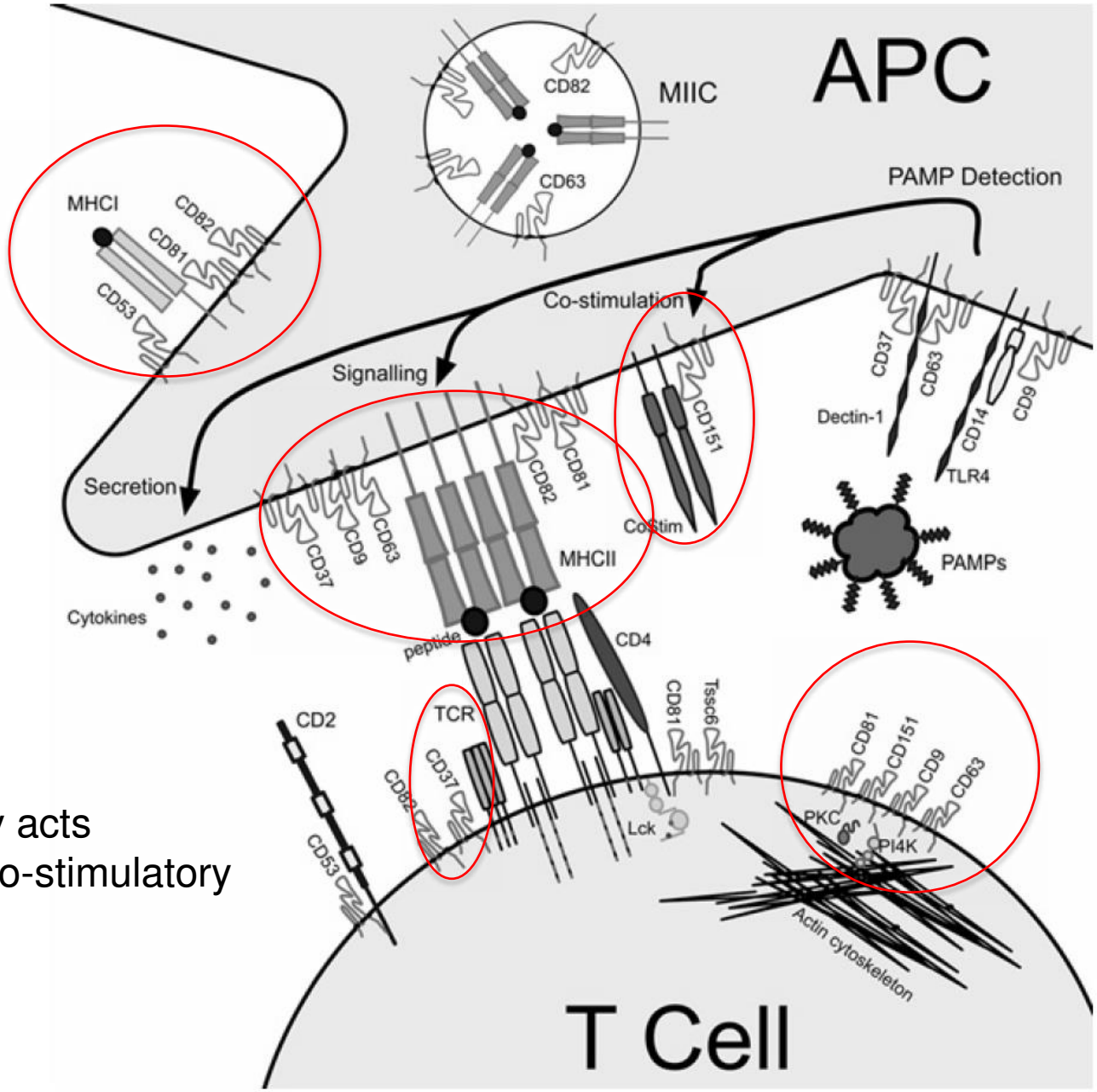


T-cell dependent B-cell activation
CD81 on B cells interacts directly with a ligand on T cells, leading to IgG1 production

Direct B-cell activation
B cells interact with a ligand on B cells, leading to IgG1 production

Maecker et al. PNAS 1998

Tetraspanins (CD37/CD81/CD9/CD151)



Tetraspanin family acts as non-classical co-stimulatory molecules.

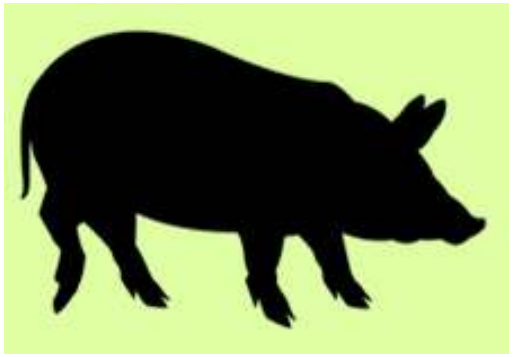
Next 50 Years …

HLA
& any tissue typing

Heart, Lung,
Liver, Kidney, Pancreas

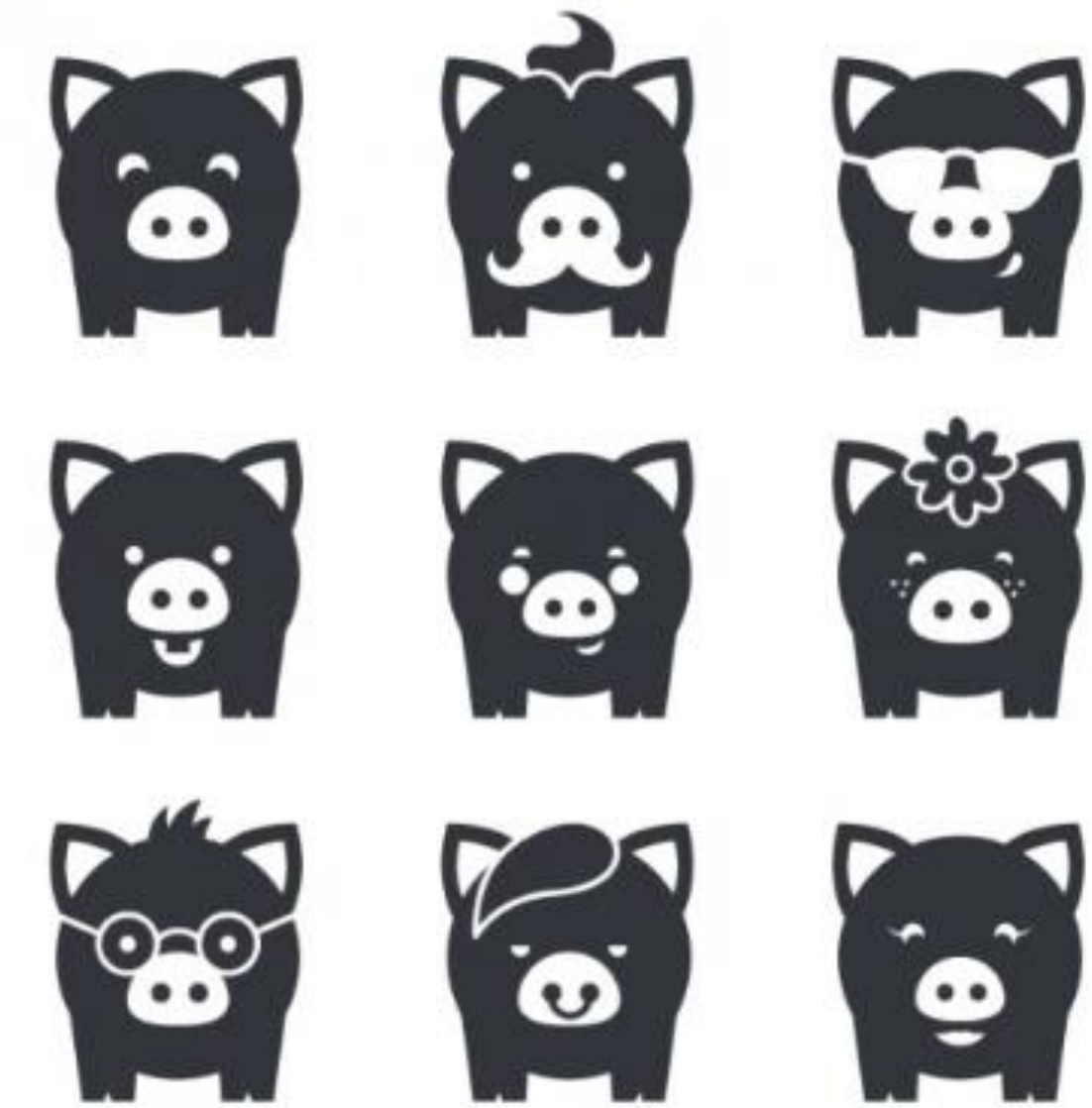
SLA to HLA Swap

SLA-knockout
Personalized HLA
Expressing Pig

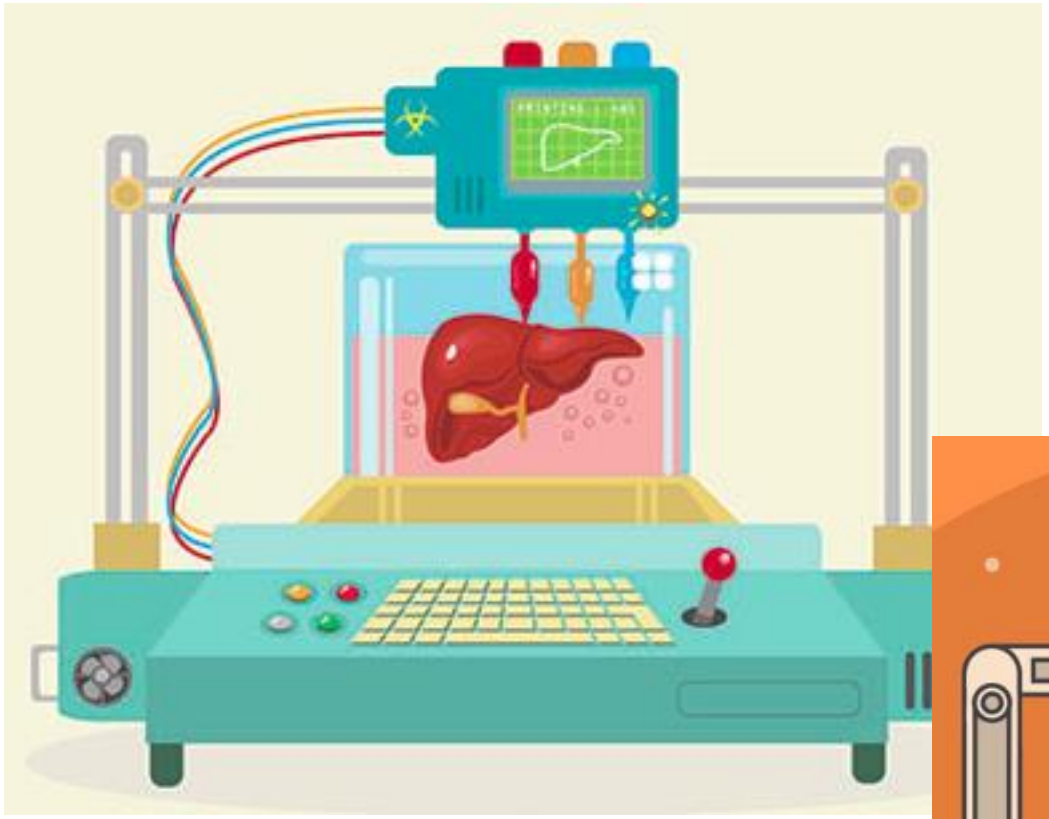


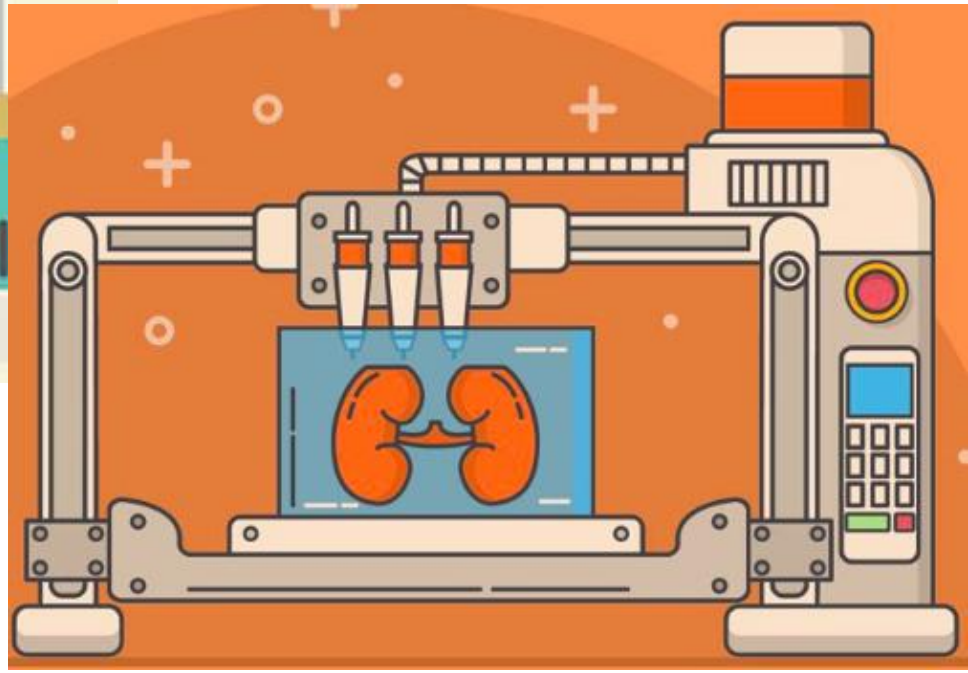
Next 50 Years …

Personalized clinical grade pigs for any organ demand and any immunological type



3D-Bioprinting of Organs





Why is 3D Bioprinted Organ Model Needed?

- **2D** in vitro models are **limited**
- Small animal studies are often **not** considered translational
- Large animal (nonhuman primate/pig) studies are very **expensive, difficult, time consuming** & limiting due to animal rights
- Human studies are **often impossible for drug safety**
- Human studies are also limited due to the **difficulty of reaching the exact number** of patients for the studied pathology
- **Positive ethical consequences** (creating alternative of animal testing, saving lives of thousands, hundred thousands of animals
- **Easy disease model bioprinting**
- Using own iPSCs, could create **personalized medicine**

Scaffold-Free 3D-Bioprinting of Organs in Indianapolis

The "Kenzan" method is a novel platform technology for high-density cell architecture

Step 3
Maturation
Culture 3D-printed tissue in a bioreactor to promote self-organization of cells until the tissue presents the desired function and strength.

Step 2
3D Printing with the "Kenzan" method
Assemble cellular spheroids into a three-dimensional shape on Kenzan according to the pre-designed 3D data.

Step 1
Spheroid preparation
Form cellular aggregates with single or mixed cell types.

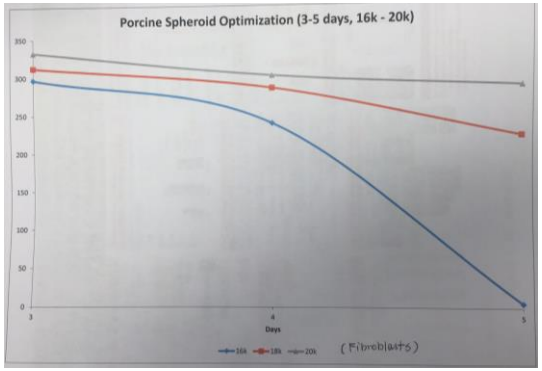
Scaffold-Free 3D-Bioprinting at Indiana University

Smith, Li, Ekser, Nat Sci Reports 2018, in press

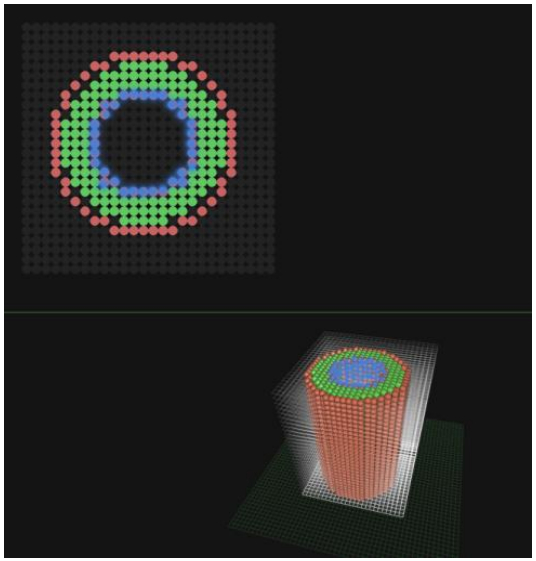
Scaffold-free 3D Bioprinting

(i) Optimal size of spheroids

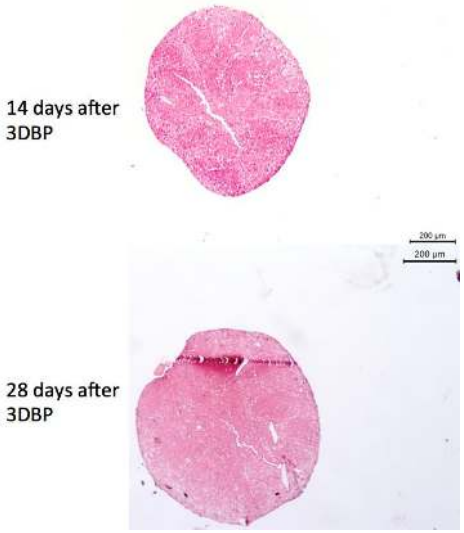
(ii) Optimal time for spheroid formation



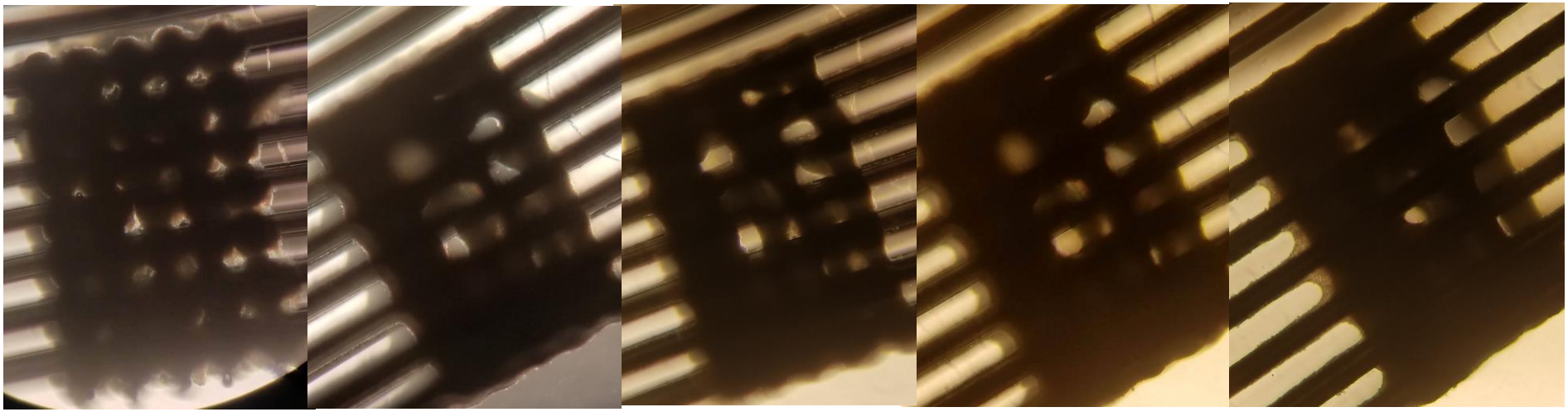
(iii) Design and print the 3D structure



(iv) Viability of the 3D-bioprinted structure



Optimized First Scaffold-Free 3D-Bioprinted Pig Liver Model



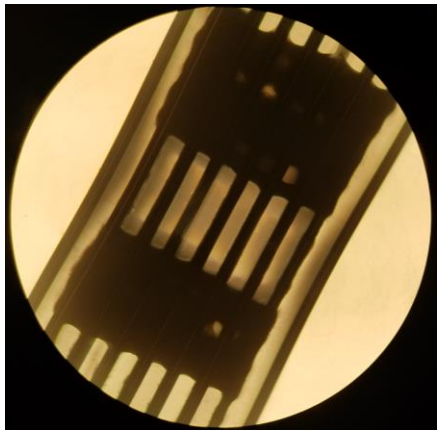
Day 1 Day 2 Day 3 Day 4 Day 5

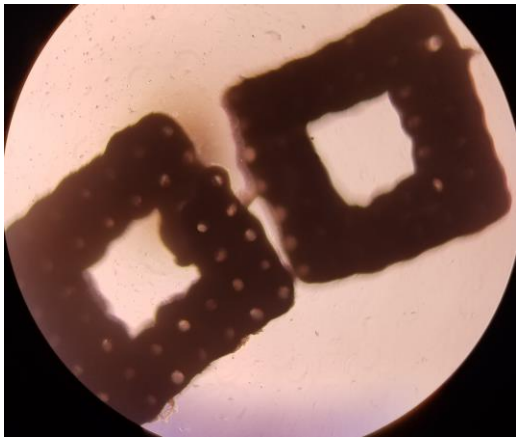
Day 5

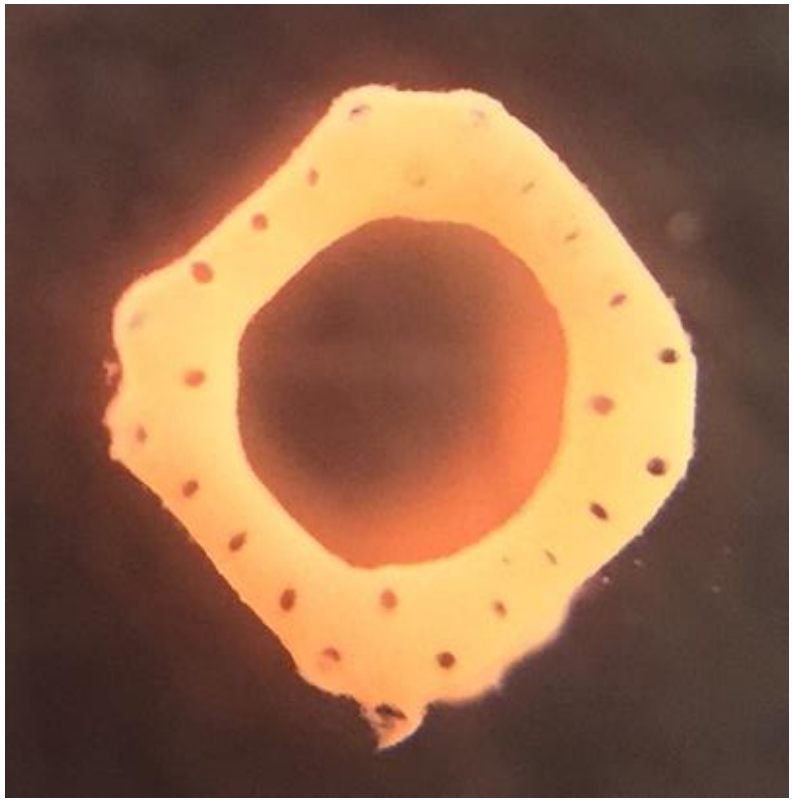
Li, Ekser, et al unpublished

Exciting Developments in Parallel with Xenotransplantation

Scaffold-free 3D-Bioprinting of Tissues/Organs



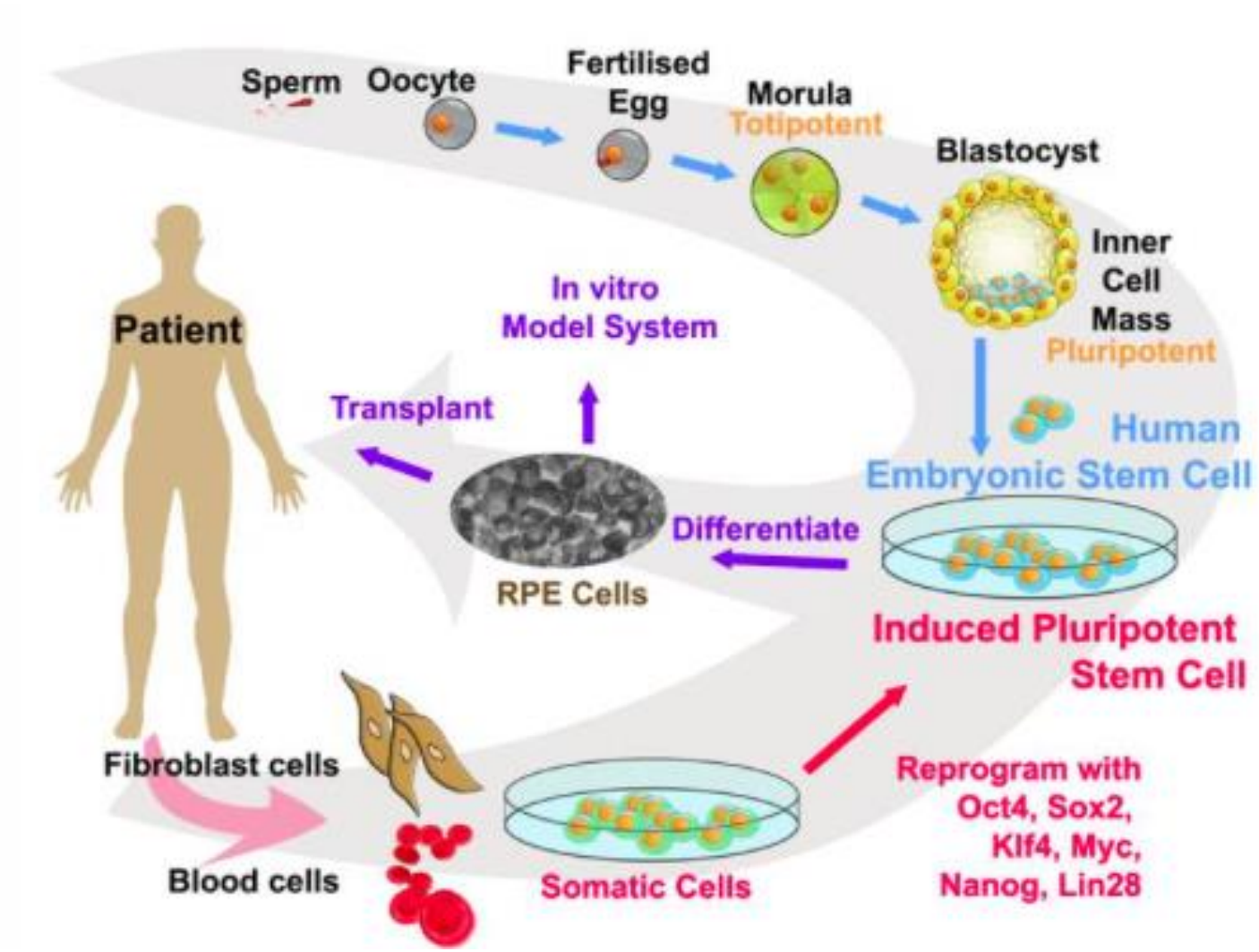




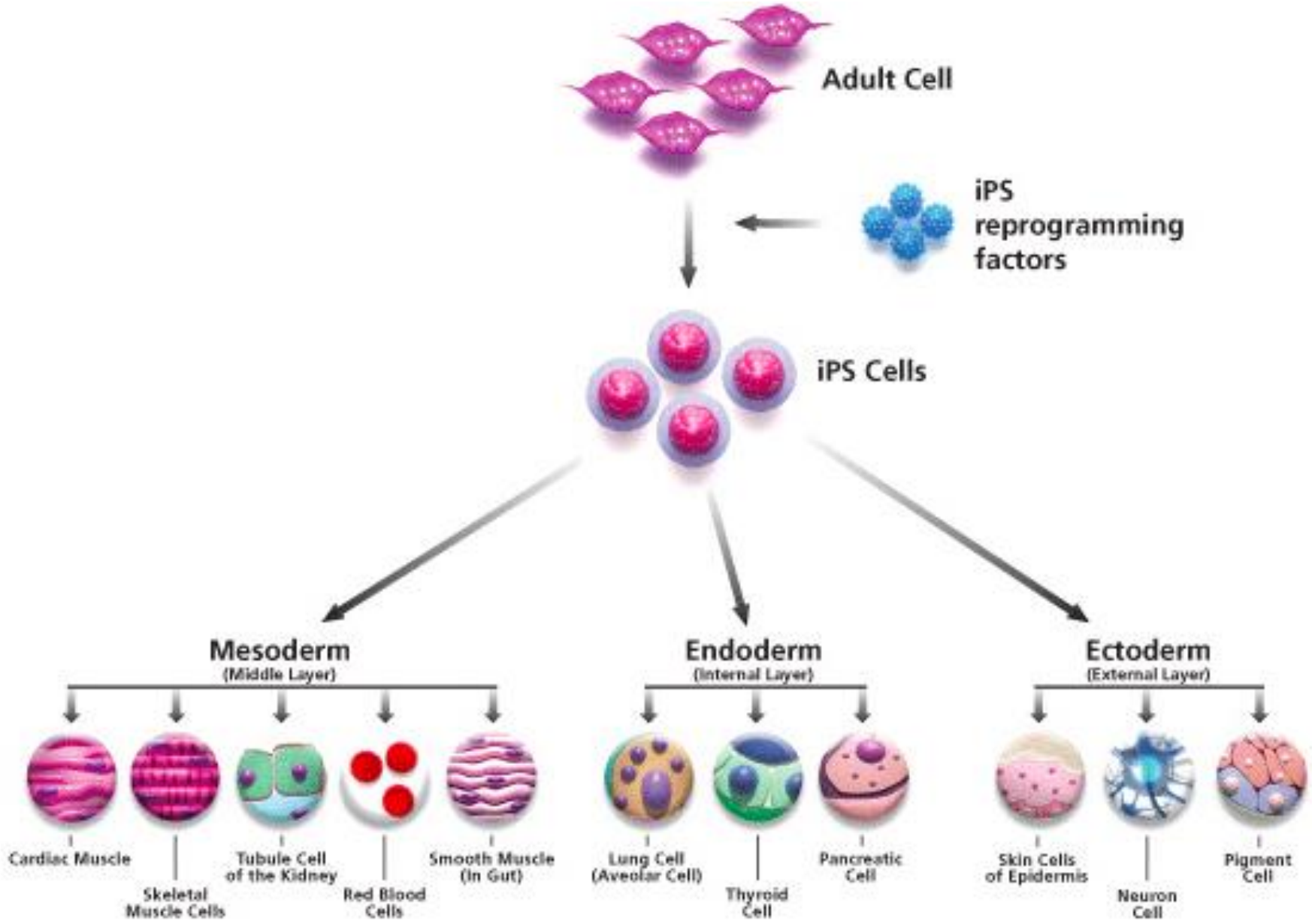
Scaffold-free 3D-Bioprinted Pig Liver Using Genetically-Engineered Cells & Perfused via Bioreactor continuously for 1 week.

Ekser Lab, unpublished

Generation of iPSC

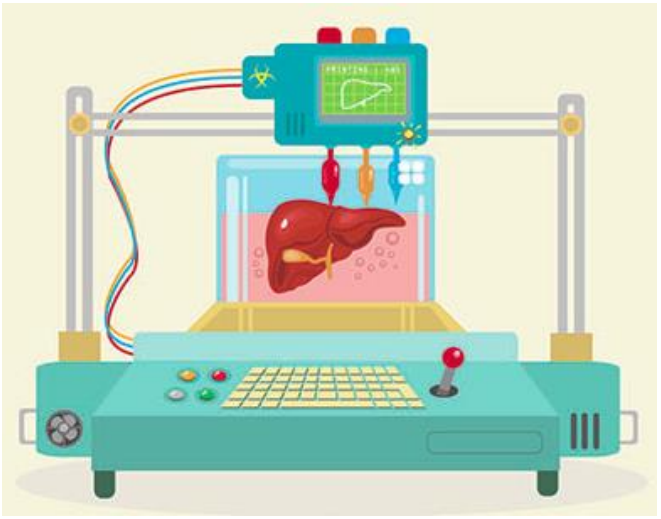


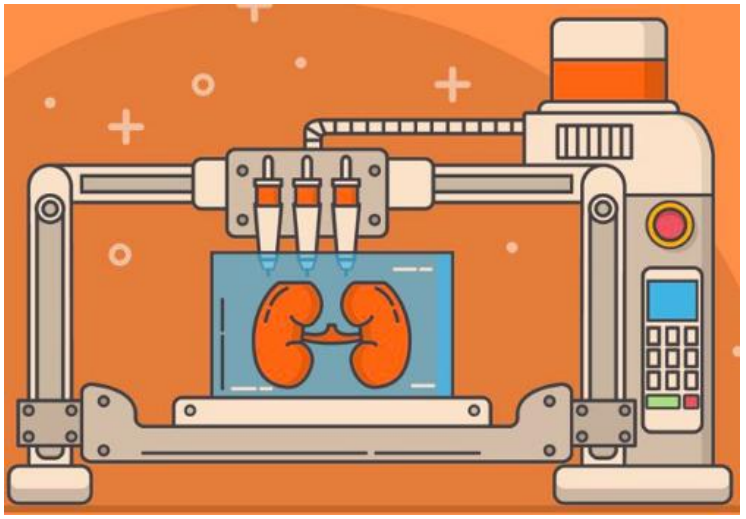
Next 50 Years …



Personalized 3D-Bioprinted Organs

- No need for immunosuppression
- No rejection
- No need to induce tolerance





Ethical Concerns in Living Donors – Friend vs. Selena Gomez

selenagomez • Follow

selenagomez I'm very aware some of my fans had noticed I was laying low for part of the summer and questioning why I wasn't promoting my new music, which I was extremely proud of. So I found out I needed to get a kidney transplant due to my Lupus and was recovering. It was what I needed to do for my overall health. I honestly look forward to sharing with you, soon my journey through these past several months as I have always wanted to do with you. Until then I want to publicly thank my family and incredible team of doctors for everything they have done for me prior to and post-surgery. And finally, there aren't words to describe how I can possibly thank my beautiful friend Francia Raisa. She gave me the ultimate gift and sacrifice by donating her kidney to me. I am incredibly blessed. I love you so much sis. Lupus

10,580,489 likes

SEPTEMBER 14, 2017

Living Donors / Organ Trafficking – Declaration of Istanbul

No need to discuss organ trafficking due to unlimited supply of pig organs and eventually ‘self’ 3D-bioprinted organ supply.

David K.C. Cooper *Thomas E. Starzl* *Burcin Ekser*

"…history of medicine tells us that procedures that were inconceivable yesterday, and barely achievable today often become the ***routine of tomorrow****…"*

Starzl 1982