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Xenotransplantation: Current status and future perspectives

XRC 2단계 바이오이종장기개발사업단
Xenotransplantation Research Center

서울대학교 의과대학 박 정 규

Disclosure of COI

I'm serving as an outside director of Genenbio Inc. and as the CEO of PB Immune Therapeutics Inc.

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History of Xenotransplantation

Human–headed winged lion (Lamassu), 883–859 B.C

History of Xenotransplantation

1682

DOG SKULL WAS USED FOR INJURED SKULL OF RUSSIAN NOBLEMAN

LATE 1800S

FROG SKIN WAS OFTEN GRAFTED ONTO BURN PATIENTS

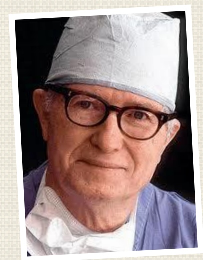
1920

SERGE VORONOFF, TESTICLE OF MONKEYS TO ELDERLY MEN (SEXUAL REJUVENATION)

1963-1965

KEITH REEMTSMA, CHIMPANZEE KIDNEYS INTO 13 PATIENTS, JUST BEFORE HEMODIALYSIS ERA

(9 MONTHS)



1964

JAMES D. HARDY, CHIMPANZEE HEART INTO HUMAN

(2 HOURS) THE HEART WAS TOO SMALL

1977

CHRISTIAAN BARNARD, BABOON AND CHIMPANZEE HEART AS BACKUP PUMPS IN TWO PATIENTS: FAILED

1984

BABY FAE, BABOON HEART INTO BABY BORN WITH MALFORMED HEART

(20 MORE DAYS FIRST USE OF CY)

1992

LIVER TRANSPLANTATION, BABOON LIVER AT U OF PITTSBURGH

(2 MONTHS)

1995

JEFF GETTY, AIDS PATIENT RECEIVED BABOON BM CELLS (SYMPTOM IMPROVED, DIED IN 2006)

Organ Source of Xenotransplantation – Pig

- endangered species
- difficult to breed
- ethical problem
- high risk of zoonosis

- appropriate organ size
- lower risk of zoonosis
- easy to breed
- genetic engineering

Cooper D.K.C. A brief history of cross-species organ transplantation. *Proc (Bayl Univ Med Cent)*. 2012 Jan; 25(1): 49–57.

Successful Cases of Xenotransplantation

Maribeth Cook

34/F

Hemiplegia due to stroke

Transplantation of 3x10[7] porcine neural cells to injured brain (1994)

⇒ She ran a marathon with braces

Amanda Davis

21/F

Left hemiplegia

Transplantation of porcine neural cells to injured brain (1999)

⇒ ambulation without braces

Jim Finn

Parkinson disease

Transplantation of porcine neural cells to injured brain (1997)

⇒ He can stand up and sit down by himself.

Robert Pennington

20/M

Acute hepatic failure

Extracorporeal linkage of porcine liver. (1997)

⇒ Successful bridge for 3 days waiting for allogeneic donor

Barriers in Xenotransplantation

- **Immunological barriers**

  - Hyperacute rejection
  - Acute humoral xenograft rejection
  - Acute cellular xenograft rejection
  - Chronic xenograft rejection

- **Infectious risks**

  - Zoonosis
  - PERV

- **Ethical Problem**

Hyperacute Rejection

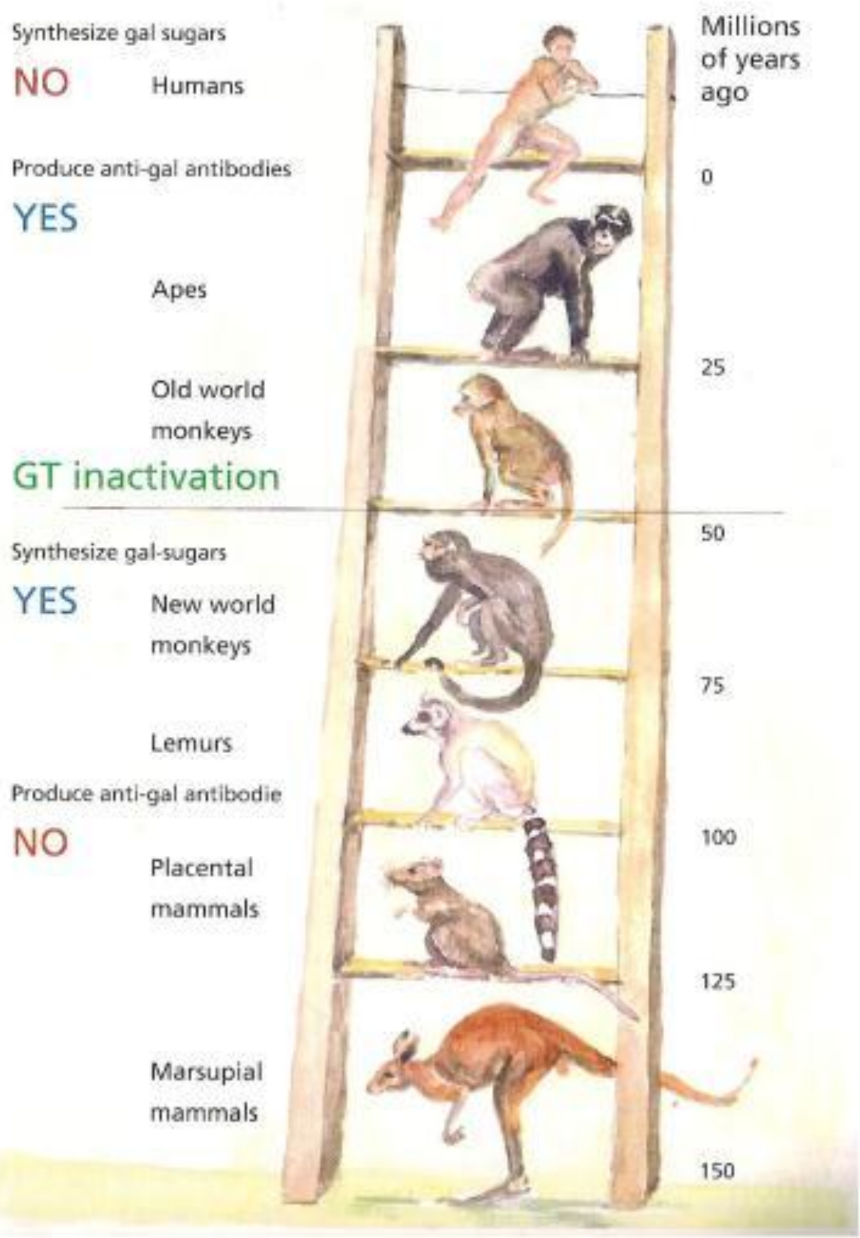
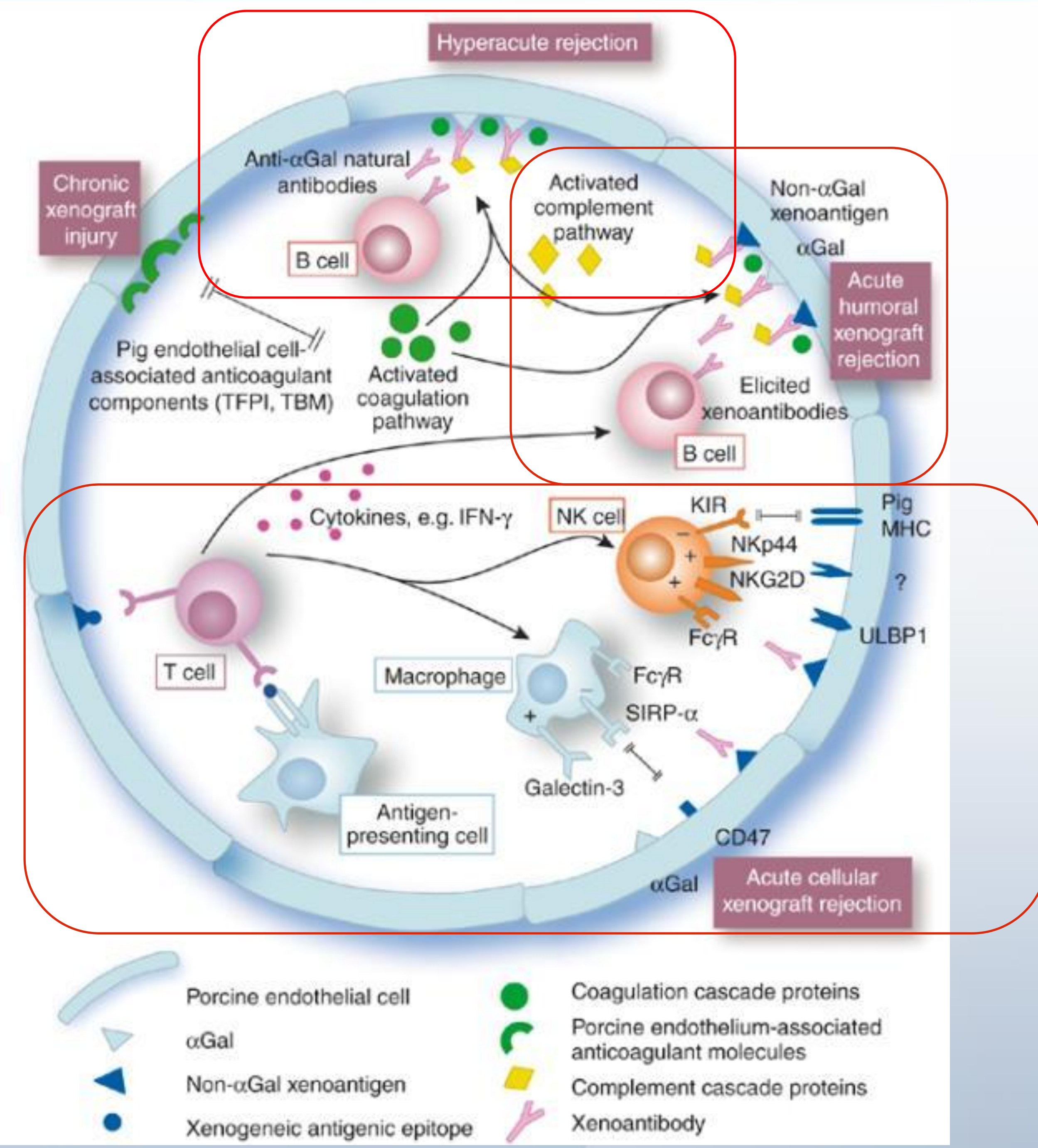


Fig. 14. The mammalian evolution in steps of 25 million years. From the evolution of marsupial mammals, such as the kangaroo, up to New World monkeys in South America, such as the gibbon or the depicted howling monkey, mammals expressed the gene coding for an enzyme, α-1,3-galactosyltransferase (GT), producing one of the most abundant antigens present in their cell membranes. These antigens present themselves on the cell surface as epitopes, the α-Gal sugar antigens or α-Gal for short. They belong to the family of transplantation antigens. During evolution about 50 million years ago, the GT was completely inactivated. Therefore, the ancestral Old World monkeys from Africa and Asia and later evolutionary species such as apes (orangutans, gorillas and chimpanzees) and humans cannot synthesize α-Gal. But they produce large amounts of a natural antibody against α-Gal, named anti-α-Gal, which is absent in New World monkeys and in mammals from previous evolutionary steps.

As a consequence, an organ transplantation from, say, a gibbon to a baboon is impracticable because of the inevitable antigen–antibody reaction which will cause hyperacute graft rejection. Much less risk of acute rejection, with probable survival of the graft, is seen after organ or tissue transplantation between e.g. a baboon and a chimpanzee, or between a chimpanzee and a human. The three species all lack the α-Gal epitope.

This figure is inspired by a version of a similar representations originally by Uri Galili and later by David K.C. Cooper. With kind permission from U.G. and D.K.C.C.


Hyperacute rejection
Chronic xenograft injury
Anti-αGal natural antibodies
B cell
Activated complement pathway
Non-αGal xenoantigen
αGal
Acute humoral xenograft rejection
Pig endothelial cell-associated anticoagulant components (TFPI, TBM)
Activated coagulation pathway
Elicited xenoantibodies
B cell
Cytokines, e.g. IFN-γ
NK cell
KIR
Pig MHC
NKp44
NKG2D
?
ULBP1
FcγR
T cell
Macrophage
FcγR
SIRP-α
Galectin-3
Antigen-presenting cell
CD47
αGal
Acute cellular xenograft rejection
Porcine endothelial cell
αGal
Non-αGal xenoantigen
Xenogeneic antigenic epitope
Coagulation cascade proteins
Porcine endothelium-associated anticoagulant molecules
Complement cascade proteins
Xenoantibody

First GalT–KO miniature swine

www.sciencemag.org SCIENCE VOL 295 8 FEBRUARY 2002

Production of α-1,3-Galactosyltransferase Knockout Pigs by Nuclear Transfer Cloning

Liangxue Lai,[1] Donna Kolber-Simonds,[3] Kwang-Wook Park,[1] Hee-Tae Cheong,[1,4] Julia L. Greenstein,[3] Gi-Sun Im,[1,5] Melissa Samuel,[1] Aaron Bonk,[1] August Rieke,[1] Billy N. Day,[1] Clifton N. Murphy,[1] David B. Carter,[1,2] Robert J. Hawley,[3] Randall S. Prather[1*]

www.sciencemag.org SCIENCE VOL 299 17 JANUARY 2003

Production of α1,3-Galactosyltransferase–Deficient Pigs

Carol J. Phelps,[1] Chihiro Koike,[3,4] Todd D. Vaught,[1] Jeremy Boone,[1] Kevin D. Wells,[1] Shu-Hung Chen,[1] Suyapa Ball,[1] Susan M. Specht,[3,4] Irina A. Polejaeva,[1] Jeff A. Monahan,[1] Pete M. Jobst,[1] Sugandha B. Sharma,[3,4] Ashley E. Lamborn,[1] Amy S. Garst,[1] Marilyn Moore,[2] Anthony J. Demetris,[3,5] William A. Rudert,[3,6] Rita Bottino,[3,6] Suzanne Bertera,[3,6] Massimo Trucco,[3,6] Thomas E. Starzl,[3,4] Yifan Dai,[1*] David L. Ayares[1*]

Transplantation of GalT-KO porcine organ to non-human primate

NATURE MEDICINE VOLUME 11 | NUMBER 1 | JANUARY 2005

Heart transplantation in baboons using α1,3-galactosyltransferase gene-knockout pigs as donors: initial experience

graft survival > 180 days

NATURE MEDICINE VOLUME 11 | NUMBER 1 | JANUARY 2005

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

graft survival > 120 days

Lessons from GalT-KO swine

- Hyperacute rejection can be overcome

- Acute humoral rejection:
  additional genetic engineerings are required

- Cell-mediated rejection:
  clinical immuno-suppression regimen can be applied

Infectious Risks

Designated pathogen free (DPF)

- Prevention of zoonosis

Potentially problematic microbes are:

- Porcine endogenous retrovirus (PERV)
  - Difficult to eliminate

- **As yet unidentified**

PERV Infection

No PERV infection report in the primates which had received porcine organs

- porcine organ transplantation to non-human primates
  - Nat Med. 2006 Mar;12(3):304-6.
  - Nat Med. 2006 Mar;12(3):301-3
  - J Virology. 2008 Dec;82(24):12441-8
- porcine islet transplantation to humans
  - 1994 Groth et al.; 2000, 2005 Elliott et al.; 2005 Wang et al.

PERV Infection

Sciencexpress Reports

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*]

[1]Department of Genetics, Harvard Medical School, Boston, MA, USA. [2]Wyss Institute for Biologically Inspired Engineering, Harvard University, Cambridge, MA, USA. [3]eGenesis Biosciences, Boston, MA 02115, USA. [4]College of Animal Sciences, Zhejiang University, Hangzhou 310058, China. [5]Transplant Infectious Disease and Compromised Host Program, Massachusetts General Hospital, Boston, MA 02115, USA. [6]Department of Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA.

*Corresponding author. E-mail: gchurch@genetics.med.harvard.edu (G.C.); luhan.yang@egenesisbio.com

targeted all PERVs but no other endogenous retrovirus or other sequences in the pig genome (methods).

Initial experiments showed inefficient PERV editing when Cas9 and the gRNAs were transiently transfected (fig. S2). Thus we used a PiggyBac transposon (*12*) system to deliver a doxycycline-inducible Cas9 and the two gRNAs into the genome of PK15 cells (figs. S2 and S3). Continuous induction of Cas9 led to increased targeting frequency of the PERVs (fig. S5), with a max-

Sciencexpress / sciencemag.org/content/early/recent / 11 October 2015 / Page 1 / 10.1126/science.aad1191

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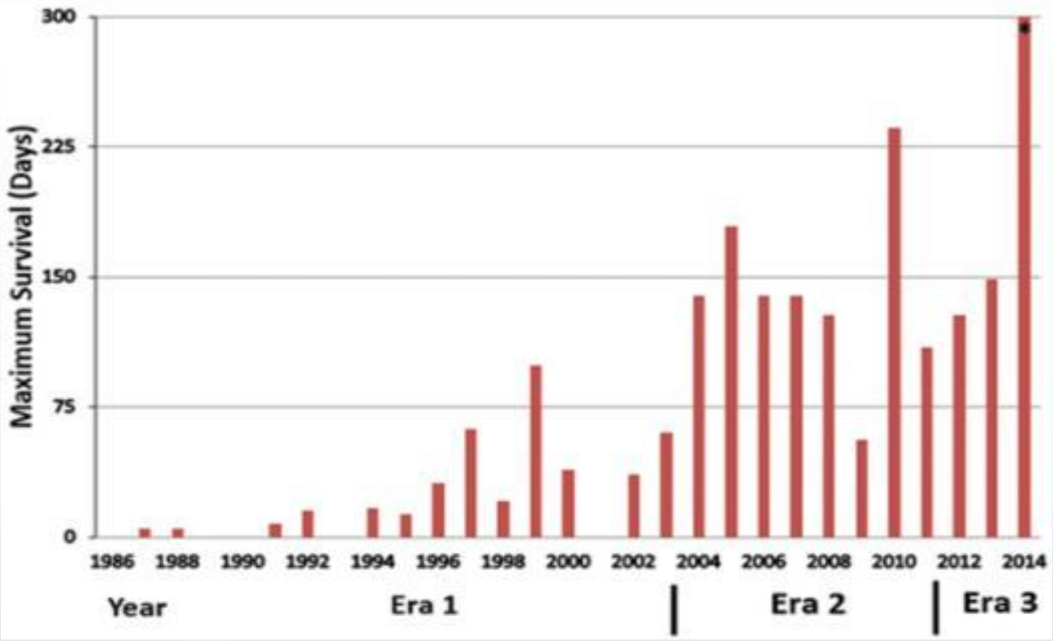
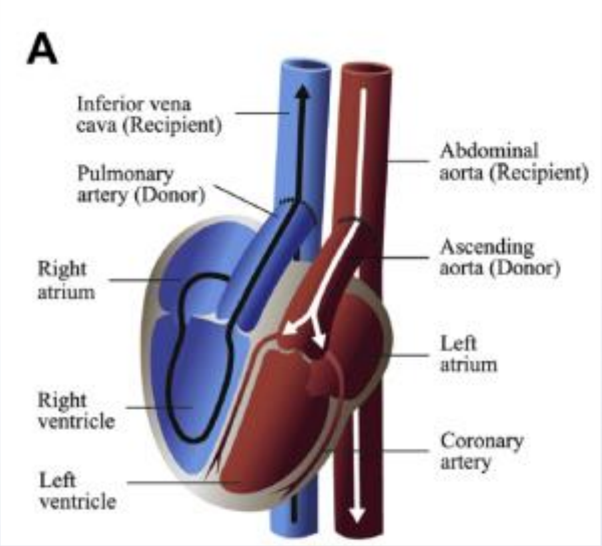
Vascularized organ Xenotransplantation

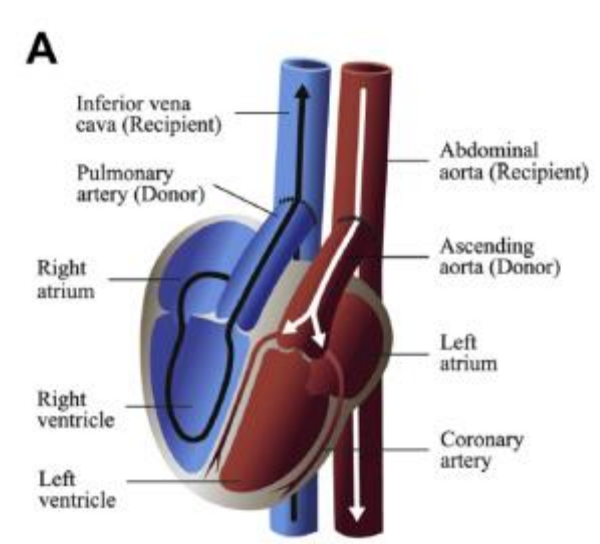
Pig to NHP heart xenotransplantation

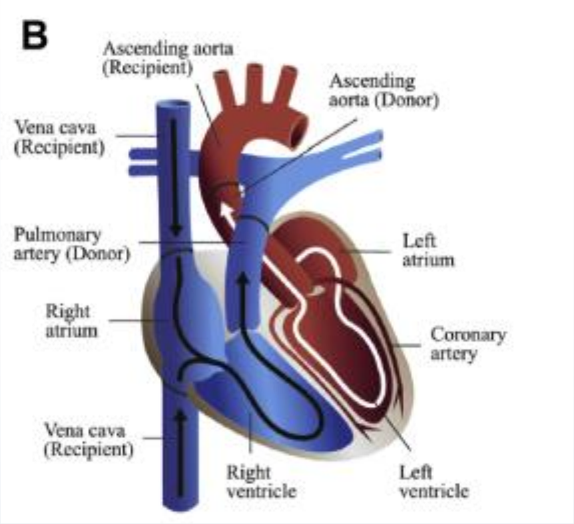




- Maximum pig nonworking heterotopic heart graft survival by year
  - Era 1— before α1,3-galactosyltransferase gene knockout (GTKO) (1986–2003)
  - Era 2—GTKO ± hCRP (2004–2011)
  - Era 3—GTKO/hCRP/human coagulation regulatory transgene (2012–present)

Pig to NHP heart xenotransplantation





Heterotopic intraabdominal: >2 years

Regimen

- GTKO:hCD46:hTM
- ATG+Rituximab+aCD40(50mg/kg) + MMF
- CVF, Heparin

(Nature Comm. 2016 Apr; 7:1113.)

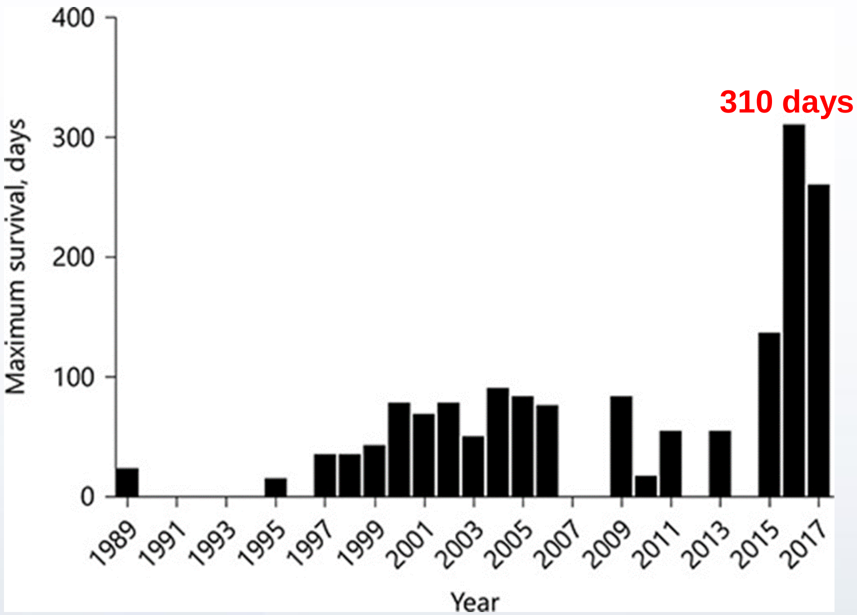
Orthotopic: >195 days

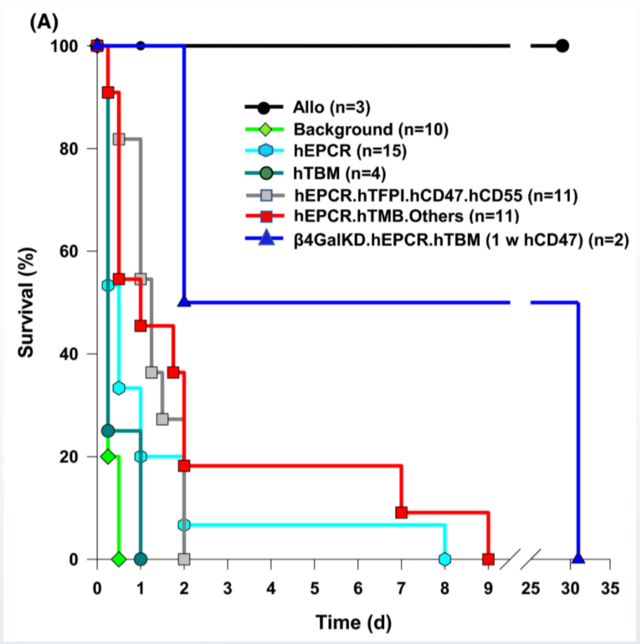
Regimen

- GTKO: hCD46:hTM
- ATG+Rituximab+aCD40(50mg/kg) + MMF
- IL-6R antagonist, anti-TNF, anti-IL1R

(Nature. 2018 Dec;564:430-3.)

Pig to NHP kidney / lung xenotransplantation





GTKO:hCD55

Regimen

- anti-CD4 and anti-CD8 (induction)
- anti-CD154mAb, MMF, corticosteroids (maintenance)

(Am J Transplant. 2016;16 (suppl 3))

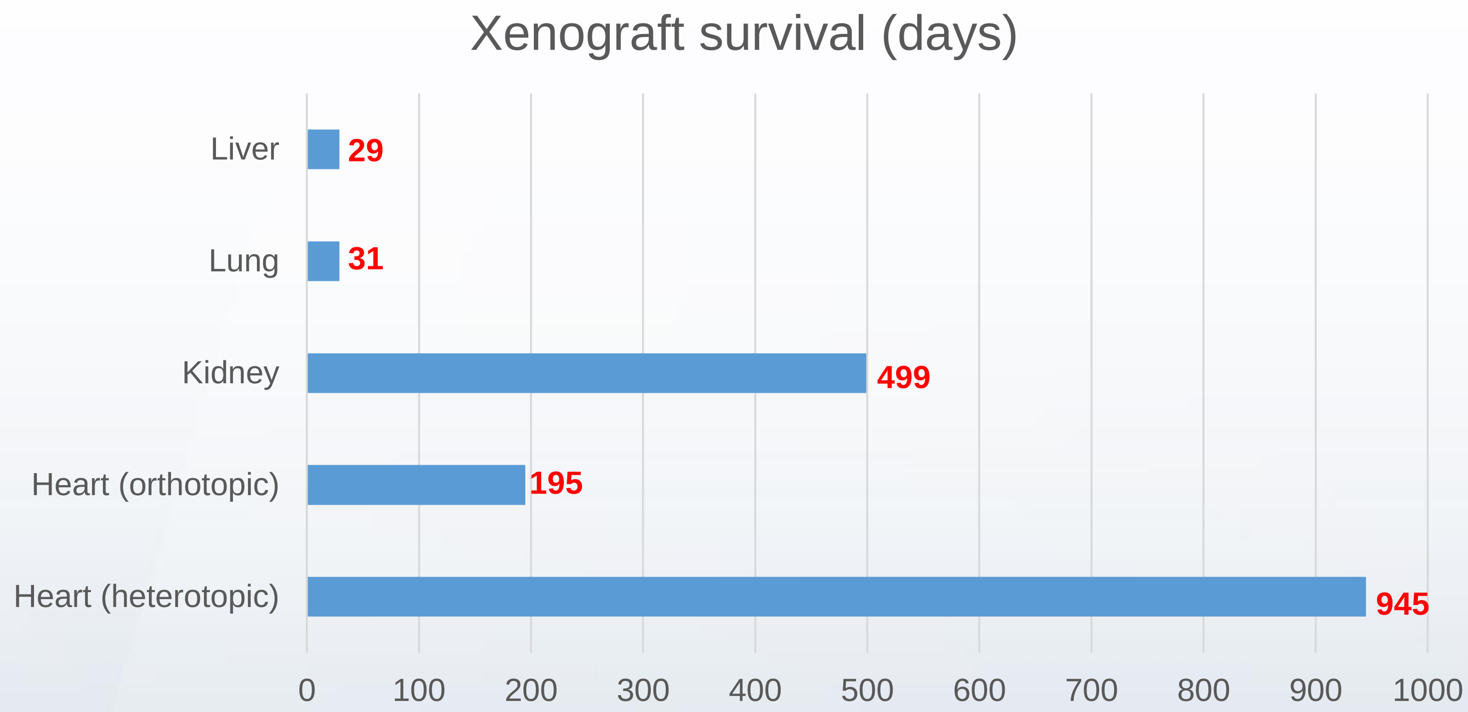
β4GalKD:hTBM:hEPCR:(hCD47)

Regimen

- anti-CD40mAb, MMF
- A1AT, Selectin integrin blockade

(Am J Transplant. 2022: 22: 28–45)

Summary



- **a graft from a specific genetically-engineered pig**
- **an effective immunosuppressive regimen with anti-inflammatory therapy**

Pancreatic islet Xenotransplantation

Type 1 Diabetes

- **15 to 20 million people worldwide suffer from type 1 diabetes (T1D).** *WHO. Diabetes. 2010*

- **12.5 % of patients with T1D for >20 years are "impaired awareness of hypoglycemia"** *Pedersen-Bjergaard et al., DMRR 2009*

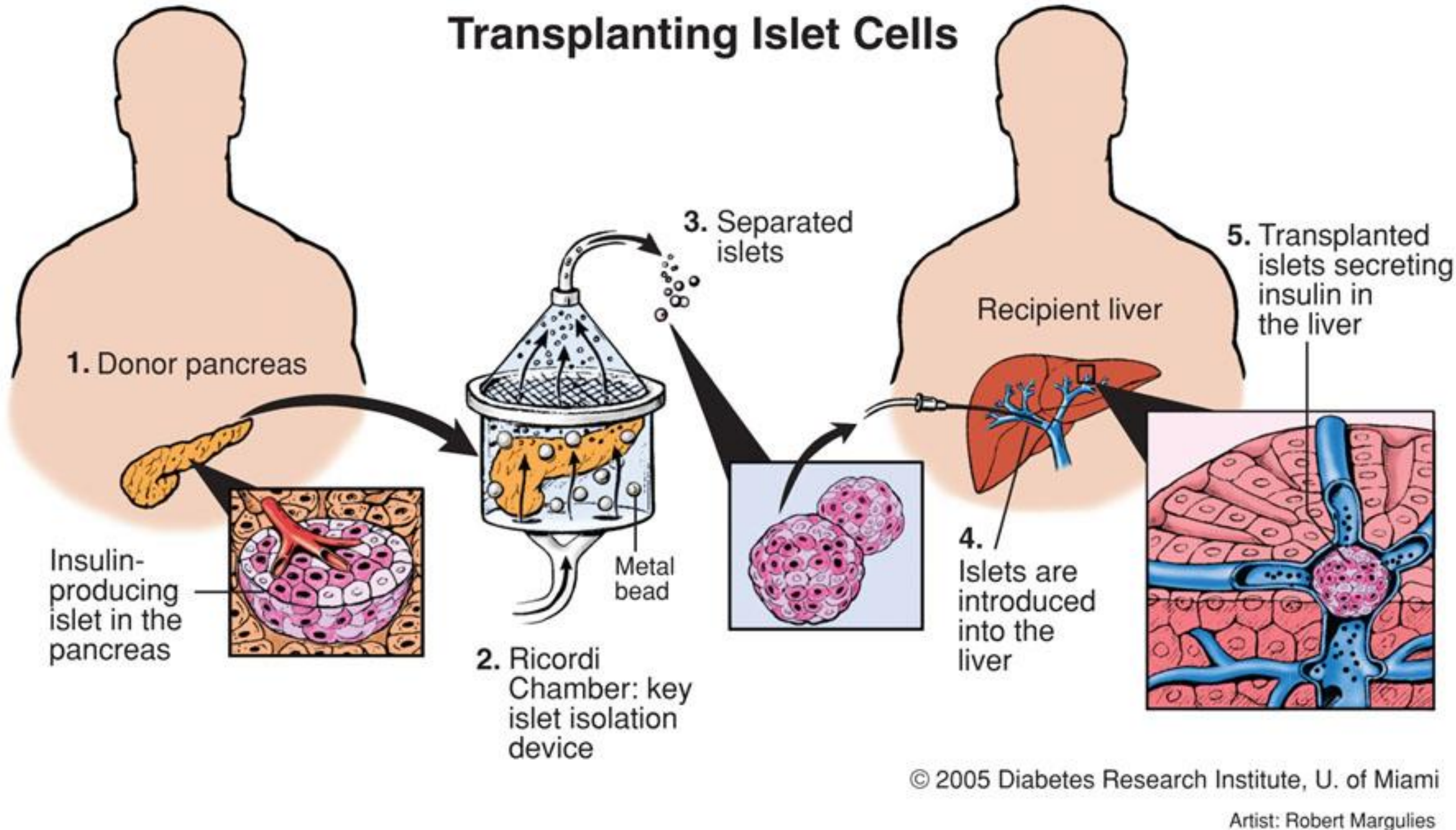
- **4-10% of all deaths in patients with type 1 diabetes caused by "impaired awareness of hypoglycemia"** *American Diabetes Association 2015*

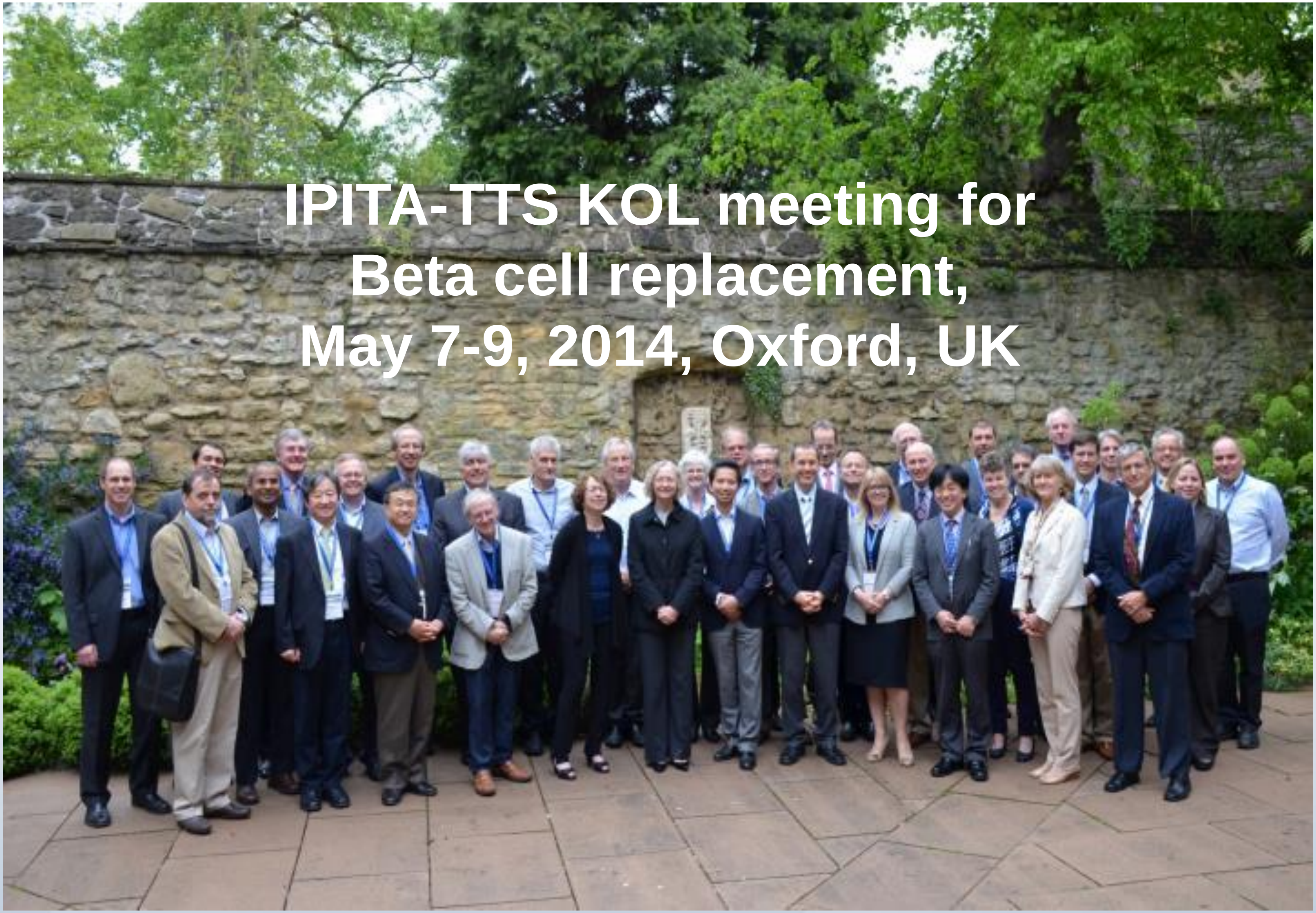
Pancreatic Islet TPL as a cure for SH

The effectiveness of human **pancreas and islet transplantation** in restoring protection from **severe hypoglycemia** is unmatched by any other therapy.

However, these therapies are at best available to 0.1% of patients with T1D.

Current status of Xenotransplantation




IPITA-TTS KOL meeting for
Beta cell replacement,
May 7-9, 2014, Oxford, UK

KOL Participants

Work Group	Team Leader	Team Member		
Allogeneic pancreas transplantation	Raja Kandaswamy	Steve Bartlett	Peter Stock	Peter Friend
Allogeneic islet transplantation	Olle Korsgren	Paul Johnson	Mitch Gotoh	Nancy Bridges
Artificial pancreas	Thomas Kay	Pratik Choudhary	Michael R. Rickels	Boris Kovatchev
Stem cells	Jon Odorico	Albert Hwa	Ed Stanley	Mark Zimmerman
Xenogeneic islets	Bernhard Hering	David Cooper	Chung-Gyu Park	Philip O'Connell
Beta cell regeneration-proliferation	Gordon Weir	Timo Otonkoski	Eduard Montanya	Julia Greenstein
Encapsulation	David Scharp	Cherie Stabler	Barbara Ludwig, Shinichi Matsumoto	Jim Markmann
Immune tolerance	Jonathan Bromberg	Kristy Kraemer	Megan Sykes	Kathryn Wood

Rationale for Pig Islets

Availability of Plenty, Potent, Safe Islets

- Unlimited and on-demand supply
- Consistently high quality of islets from healthy pigs
- Less likely susceptible to gradual graft loss caused by non-immune, amyloid-mediated toxicity
- Designated pathogen-free pigs are safer islet donors than deceased human donors

Possibility of Less Immunosuppression

- Pig islets could be less susceptible to autoreactive, MHC-restricted CD8 Tm cells
- Offer potential for pre-tx administration of donor antigen aimed at mitigating anti-xenodonor immunity

Clinically translatable ideas with 5-year horizon in islet xenotransplantation

Idea #1:
Intraportal transplantation of wild-type adult porcine islets in immunosuppressed T1D recipients

Idea #2:
Intraportal tx of genetically-modified neonatal porcine islets in immunosuppressed T1D recipients

Idea #3:
Intraperitoneal tx of encapsulated wild-type NICC in T1D with and w/o immunosuppression

Clinical Islet xenotransplantation

- Efficacy and safety from Nonhuman primate study is required.

  - The International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes. executive summary. Xenotransplantation. 2016; 23(1):46-52.

    - ≥ 6 months normoglycemia or significant reduction of insulin in 4 of 6 (5 of 8) consecutive experiments

Long-term survival of pig islets in NHPs

Reference	Pig islet source (Recipient)	Max. Graft Survival	Immunosuppressive Regimen
Hering et al.[8]	WT adult (C)	> 187 days	Anti-IL-2R+anti-CD154+FTY720+rapamycin
Cardona et al.[9]	WT neonatal (R)	> 260 days	Anti-IL-2R+anti-CD154+CTLA4-Ig+rapamycin
van der Windt et al.[11]	hCD46-Tg adult (C)	396 days	ATG+anti-CD154+MMF
Thompson et al.[12]	GTKO neonatal (R)	249 days	Anti-CD154+anti-LFA-1+CTLA-4-Ig+MMF
Bottino et al.[13]	Multi-Tg adult (C)	365 days	ATG+anti-CD154+MMF
Thompson et al.[15]	WT neonatal (R)	114 days	Anti-IL-2R+anti-LFA-1+CTLA-4-Ig+MMF+LFA-3-Ig
Thompson et al.[17]	WT neonatal (R)	> 203 days	Anti-IL-2R+anti-CD40+CTLA-4-Ig+rapamycin

Int J Surg. 2015 Aug 4. S1743-9191(15)01075-4.

Long-term survival of pig islets in NHPs

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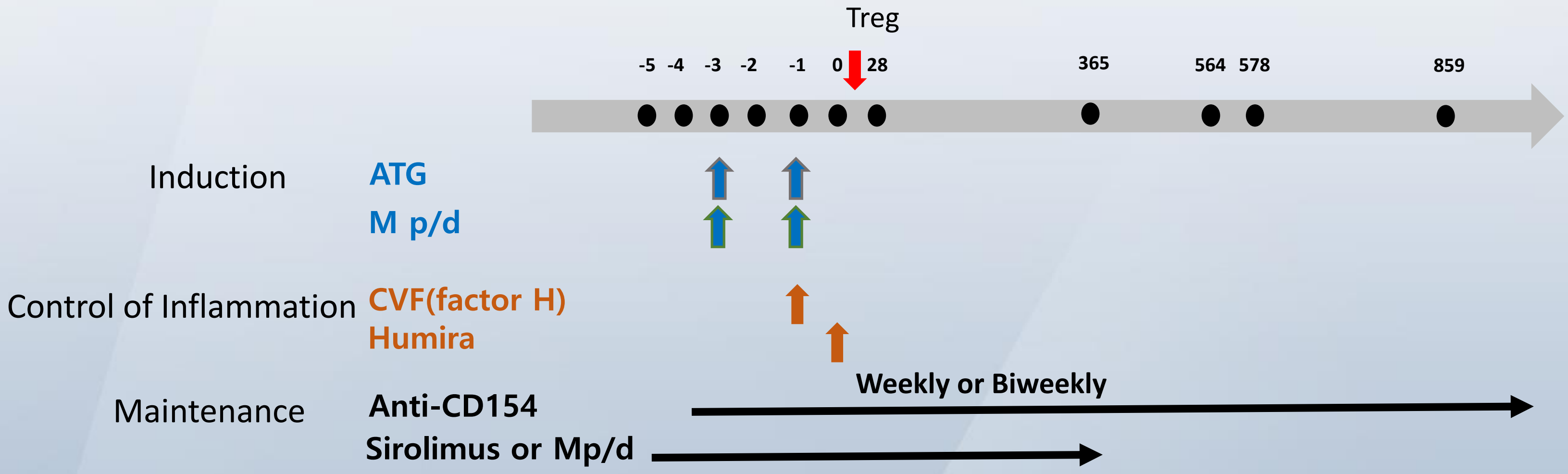
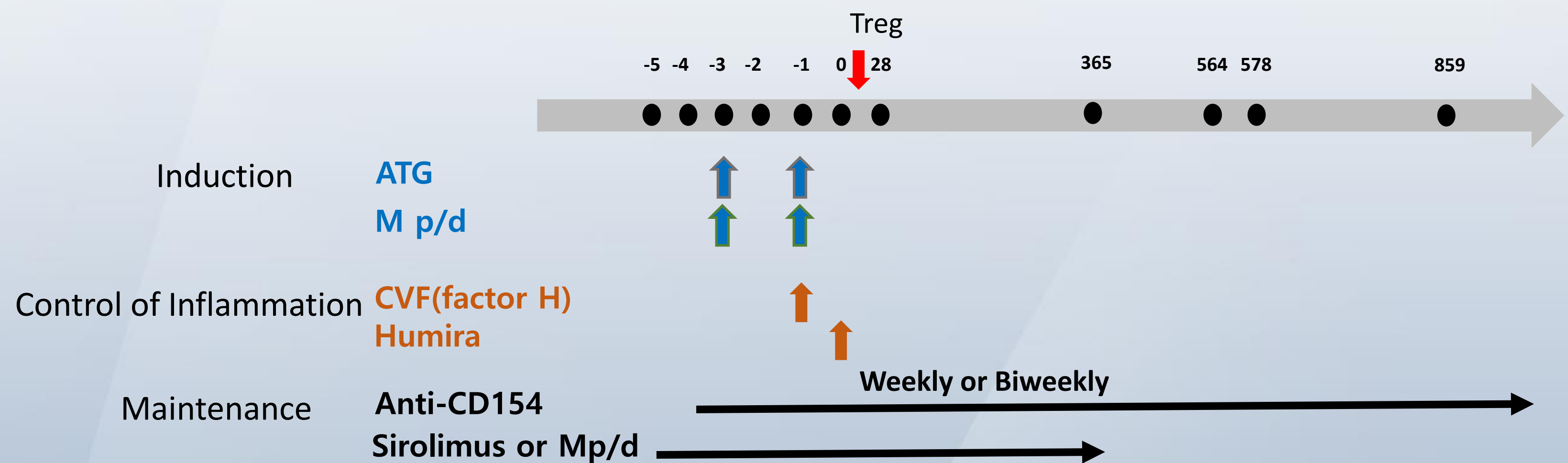
Thompson et al.[17]	WT neonatal (R)	> 203 days	Anti-IL-2R+anti-CD40+CTLA-4-Ig+rapamycin
Shin et al.[10]	WT adult (R)	> 960 days	ATG+anti-CD154+rapamycin±Treg

Recipient			Donor pigs				Islet infusion	
ID	Sex	Bwt (kg)	No. of pigs used	Bwt (kg)	Age (months)	Pancreas weight (g)	Total islet (IEQ)	Islet mass (IEQ/kg)
R051	M	7	3 (2M/1F)	71, 68, 84	25, 23, 23	36, 55, 52	530,000	81,000
R080	F	4.3	3 (2M/1F)	86, 78, 75	45, 31, 27	46, 53, 45	430,000	100,000
R082	F	5.1	2 (2M)	62, 82	19, 91	32, 46	510,000	100,000
R084	F	5.3	2 (2F)	98, 95	44, 44	48, 56	530,000	100,000
R089	F	5.4	2 (2M)	72, 63	33, 33	53, 44	540,000	100,000
Mean±SD			2.4±0.5	77.8±11.6	36.5±19.3	47.1±7.4	508,000±45,000	96,200±8,500



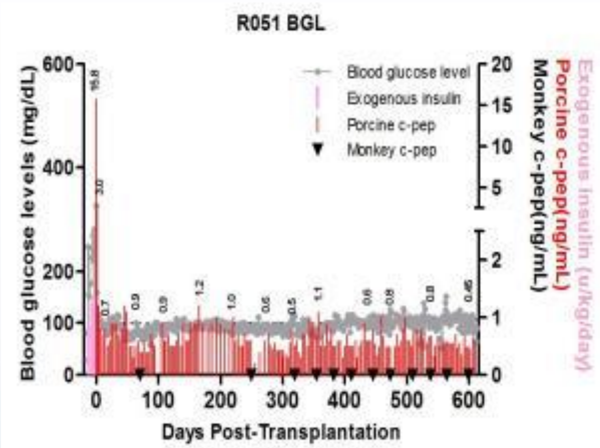
Shin et al., *American Journal of Transplantation*,15(11):2837-50, 2015

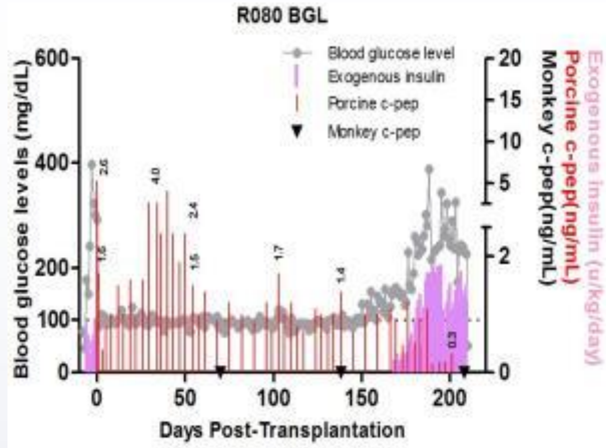
Recipient			Donor pigs				Islet infusion		Insulin independence	Graft survival*
ID	Sex	Bwt (kg)	No. of pigs used	Bwt (kg)	Age (months)	Pancreas weight (g)	Total islet (IEQ)	Islet mass (IEQ/kg)	Day	Day
R051	M	7	3 (2M/1F)	71, 68, 84	25, 23, 23	36, 55, 52	530,000	81,000	>960	>960
R080	F	4.3	3 (2M/1F)	86, 78, 75	45, 31, 27	46, 53, 45	430,000	100,000	167	180
R082	F	5.1	2 (2M)	62, 82	19, 91	32, 46	510,000	100,000	512	513
R084	F	5.3	2 (2F)	98, 95	44, 44	48, 56	530,000	100,000	303	503
R089	F	5.4	2 (2M)	72, 63	33, 33	53, 44	540,000	100,000	180	180
Mean±SD			2.4±0.5	77.8±11.6	36.5±19.3	47.1±7.4	508,000±45,000	96,200±8,500		

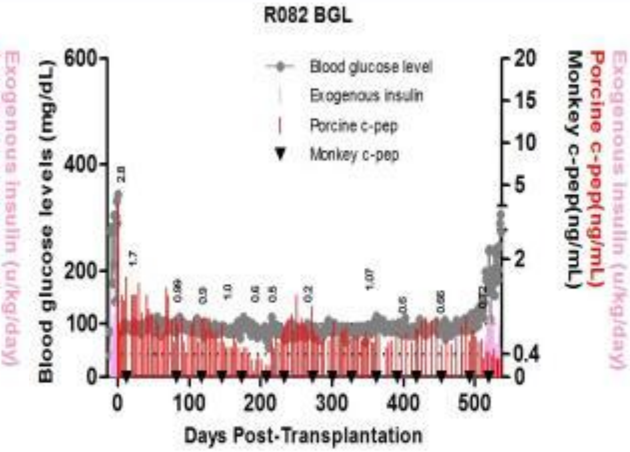


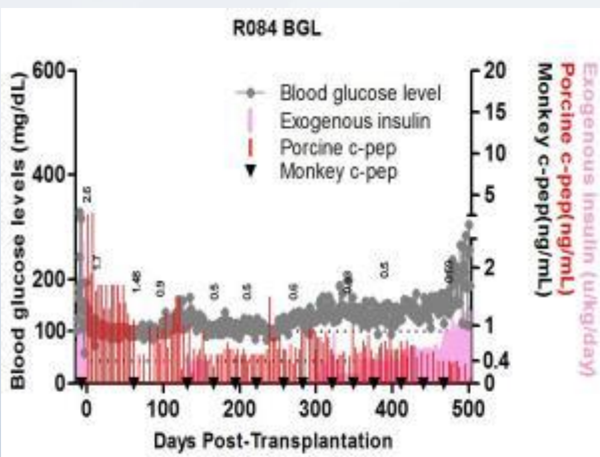
Shin et al., *American Journal of Transplantation*,15(11):2837-50, 2015

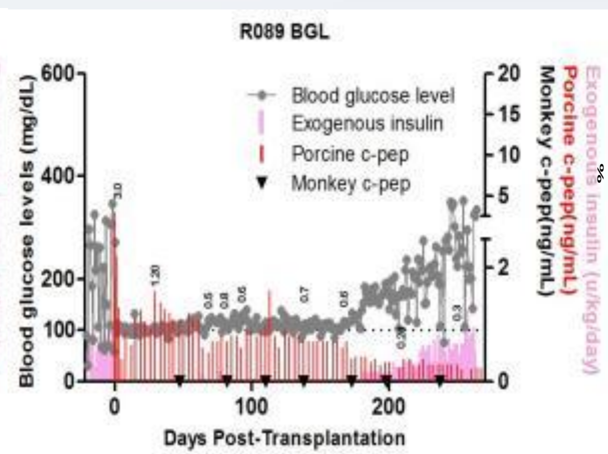
Regimen: ATG + aCD154 + Siro ± Treg

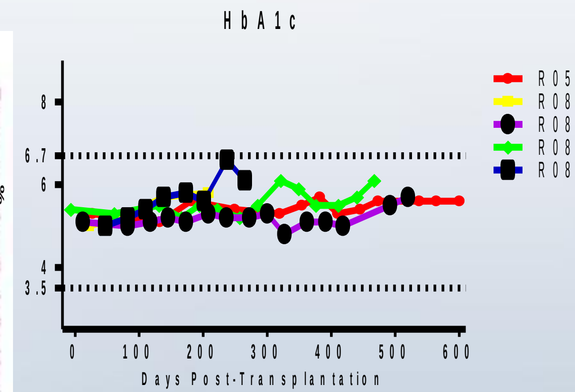






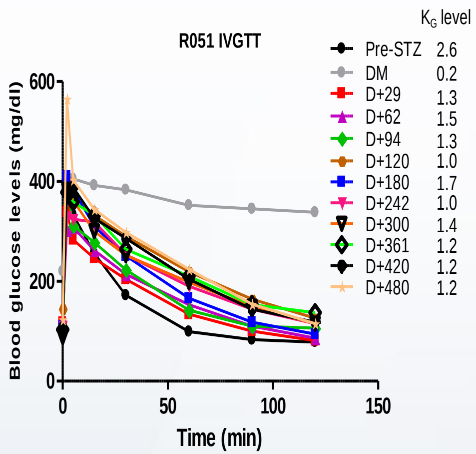


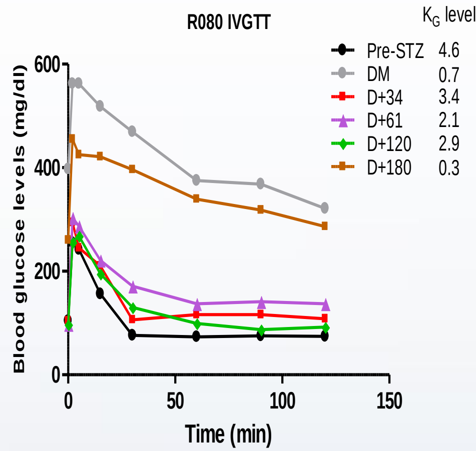


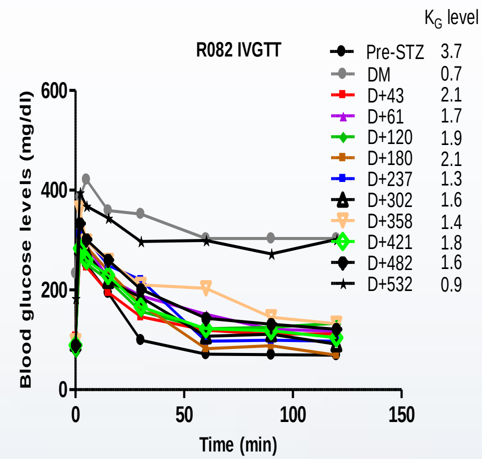


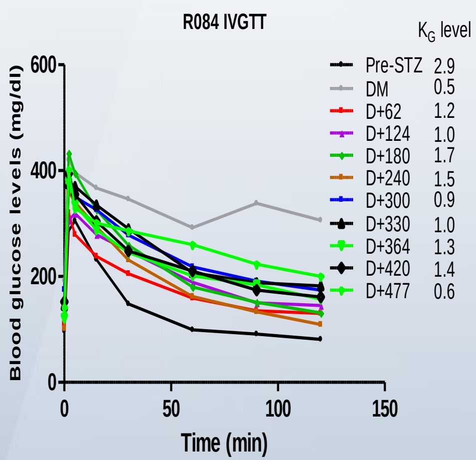
American Journal of Transplantation, 2015

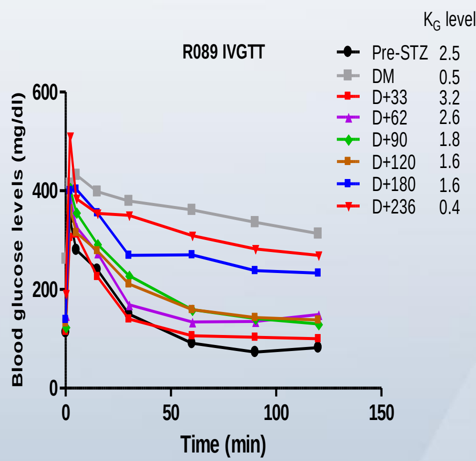
Regimen: ATG + aCD154 + Siro ± Treg





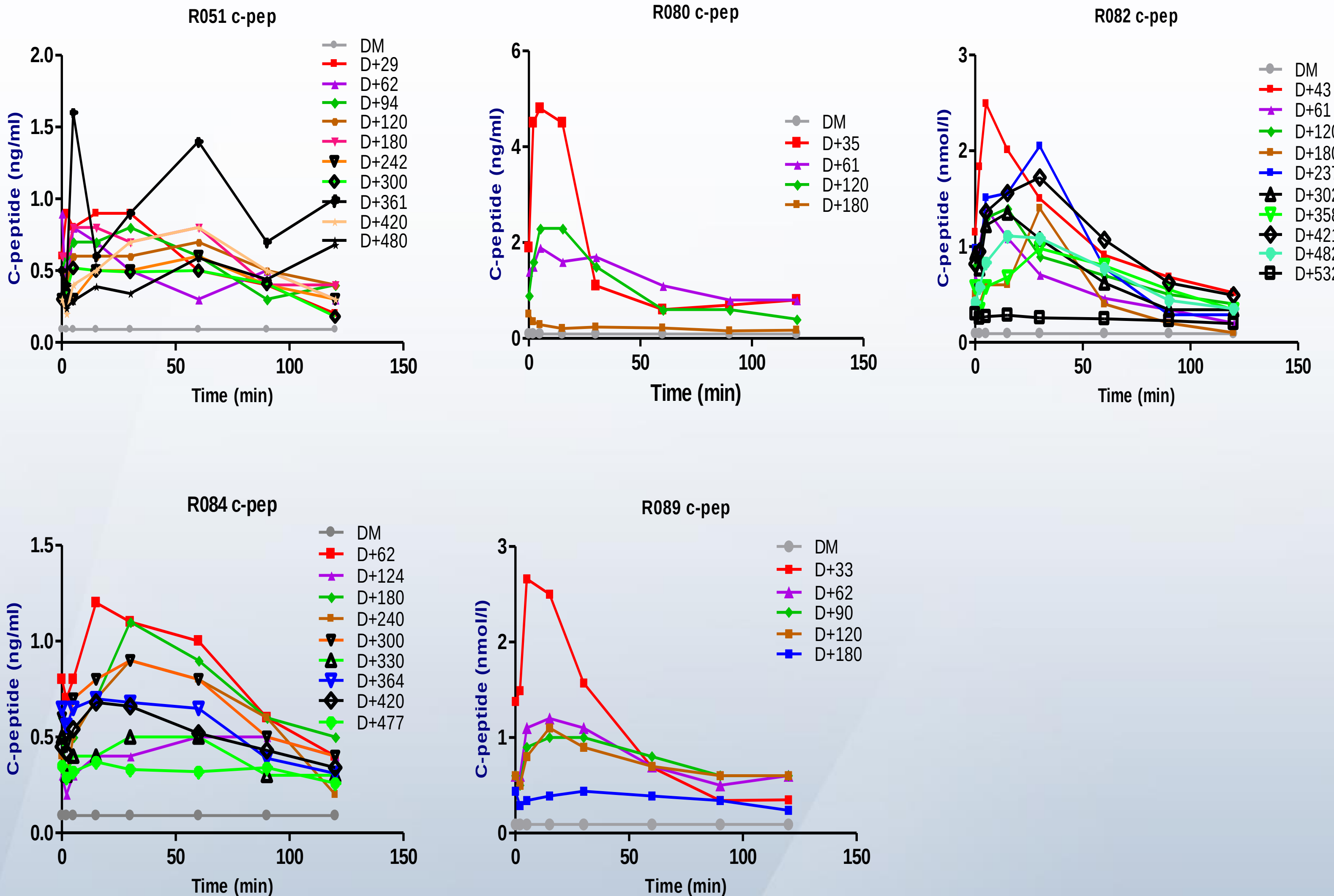






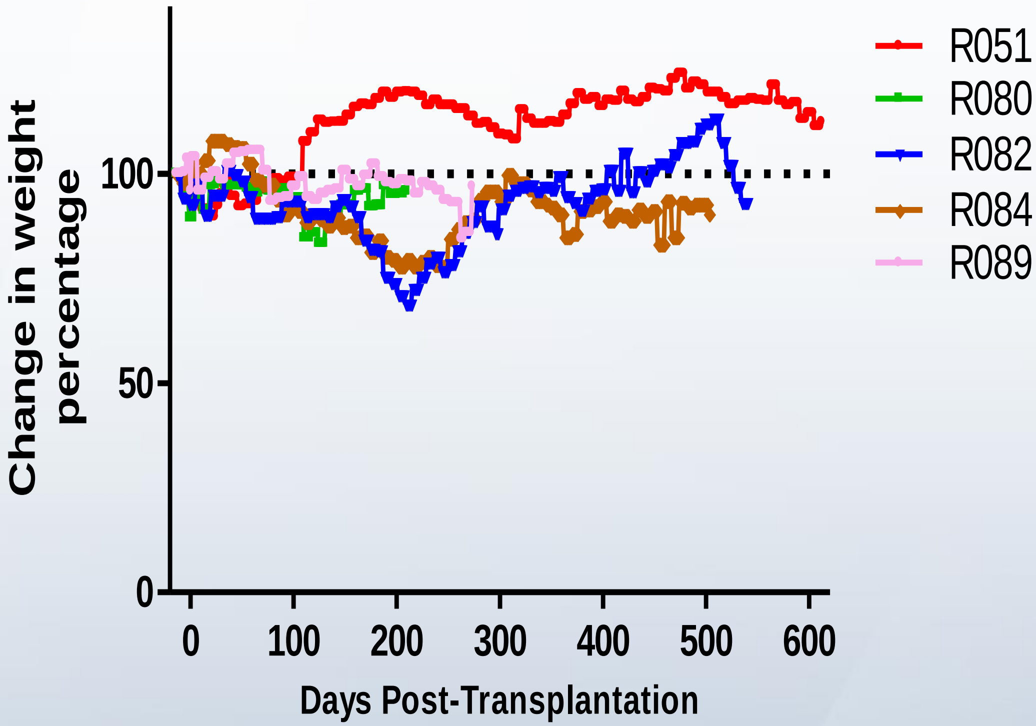
Shin et al., *American Journal of Transplantation*,15(11):2837-50, 2015

Regimen: ATG + aCD154 + Siro ± Treg



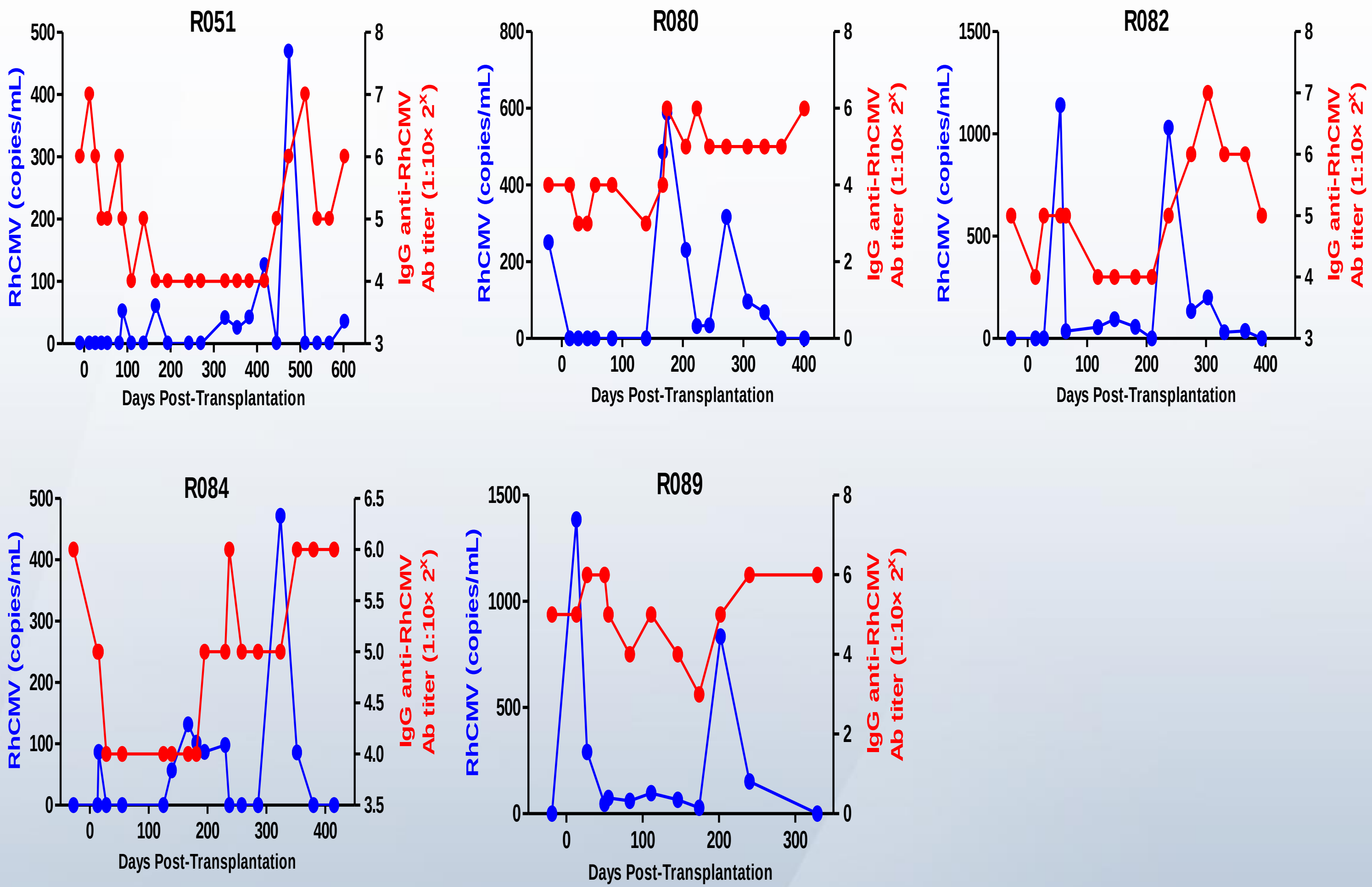
Shin et al., *American Journal of Transplantation*,15(11):2837-50, 2015

Regimen: ATG + aCD154 + Siro ± Treg



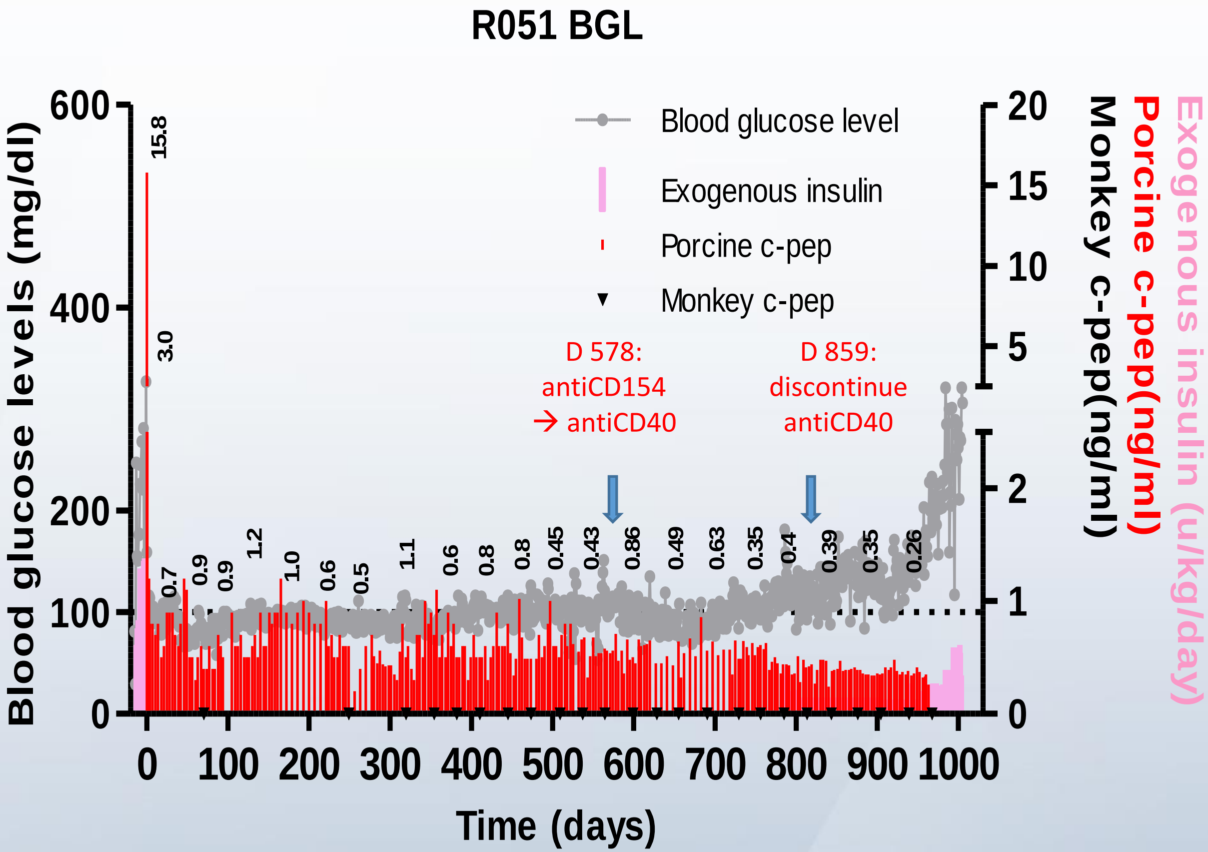
Shin et al., *American Journal of Transplantation*,15(11):2837-50, 2015

Regimen: ATG + aCD154 + Siro ± Treg



Shin et al., *American Journal of Transplantation*,15(11):2837-50, 2015

Regimen: ATG + aCD154 + Siro + Treg (on day 6)
Islet mass: 530,000 IEQ (81,000 IEQ/kg)



Shin et al., *American Journal of Transplantation*,15(11):2837-50, 2015

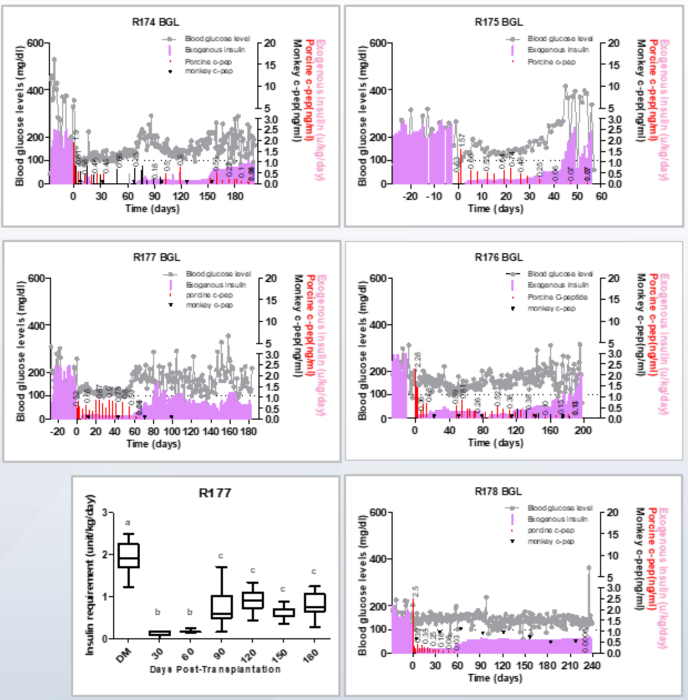
Summary

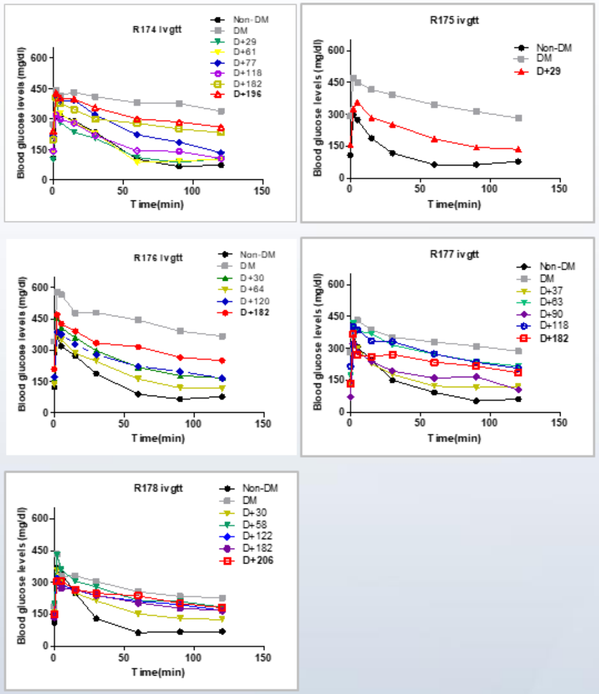
- Anti-CD154 based regimen achieved consistent long-term control of diabetes in pig to NHP islet xenotransplantation model meeting the requirement for clinical application from IXA guideline.

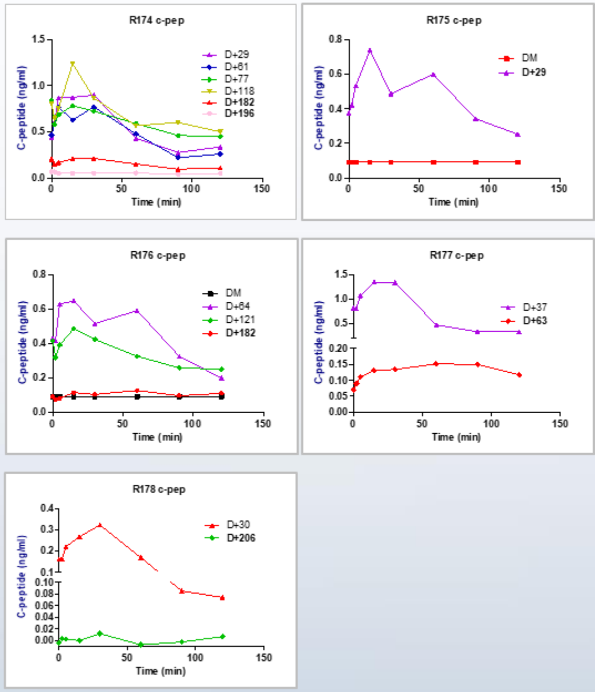
- Addition of Treg infusion should be considered for the control of both initial inflammatory reaction and adaptive immune response.

Clinically applicable regimen

Blood glucose levels (BGL)	BGL in IVGTT	Porcine C-peptide in IVGTT





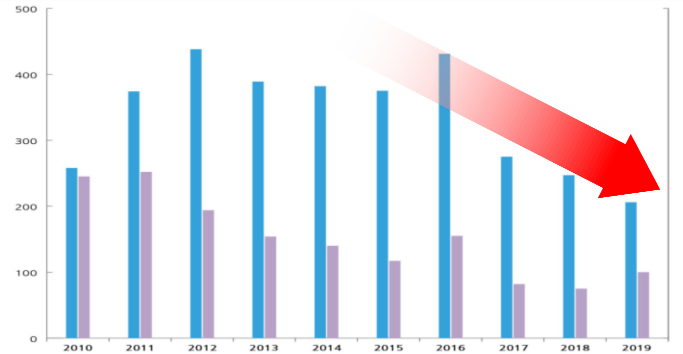


Regimen: ATG + Rituximab + Tacrolimus +JAK3 inhibitor + Siro

Corneal Xenotransplantation

Background

- 국내 각막이식 수술 건수 및 이식대기자 (출처: 질병관리본부, 2019년도 장기이식 통계연보)



구분	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	전년대비 증감율 (%)
뇌사	258	374	438	389	382	375	431	275	248	206	▼ 16.60
사후	245	252	194	154	140	117	155	82	75	100	▲ 33.33
계	503	626	632	543	522	492	586	357	323	306	▼ 4.97

각막 이식 "7년 이상 대기"

2016년
2,371일

2019년
2,939일

Corneal allotransplantation in 2008*

Country	Estimated number of cases per year	Waiting List
United States	41,652	0
United Kingdom	2,711	500
South Africa	330	1884
India	15,000	300,000
China	101	4,000,000
Taiwan	263	637
Korea	480	3,630
Japan	1,634	2,769
Australia	1,096	0

*Based on Eye Bank data in individual countries and personal communications

Adopted from *Hara H, Cooper DK. Xenotransplantation––the future of corneal transplantation? Cornea. 2011 Apr;30(4):371–8.*

Anatomical and surgical considerations for corneal xenotransplantation

- Diameter
- Thickness: 853μm (SNU pig) vs 536μm (Human) vs 490μm (NHP)
  - Porcine: swell more quickly in storage solution
  - 850 μm or less is ok.

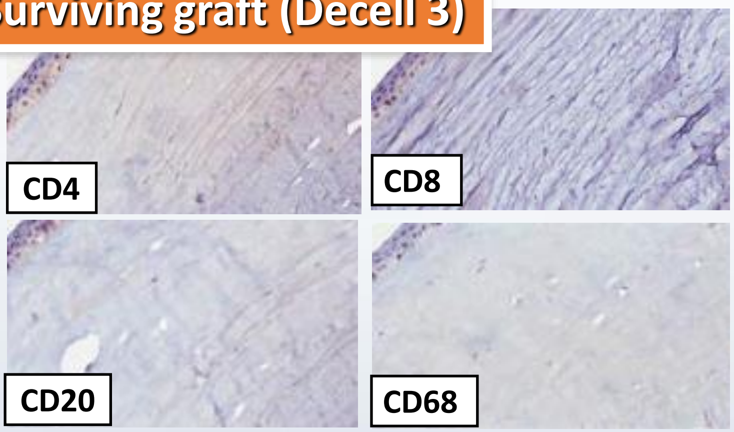
- Tensile strength: Less stiff but no problem
- Cellular behaviors (Endothelial cell density):
  - Similar
  - To maintain transparency: >2200-2400/mm^2(Human donor)
  - WT: 2130.2 in 42months, GE: 1714 in 20months → limit pig age

- Optical: refractive power:
  - 40D (pig) vs 44D (Human): Tolerable

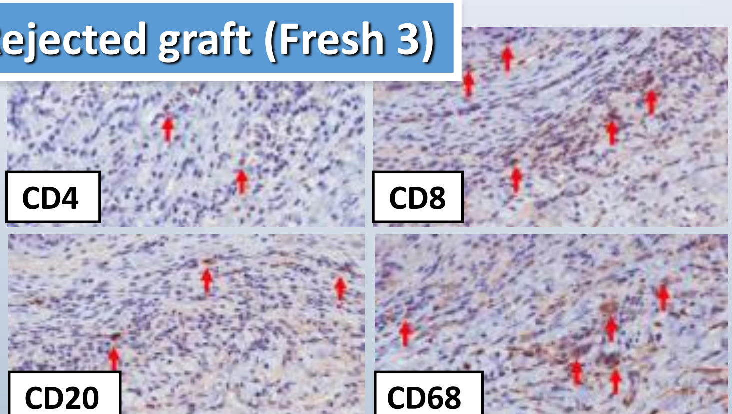
Partial thickness xenocorneal transplantation study in NHP

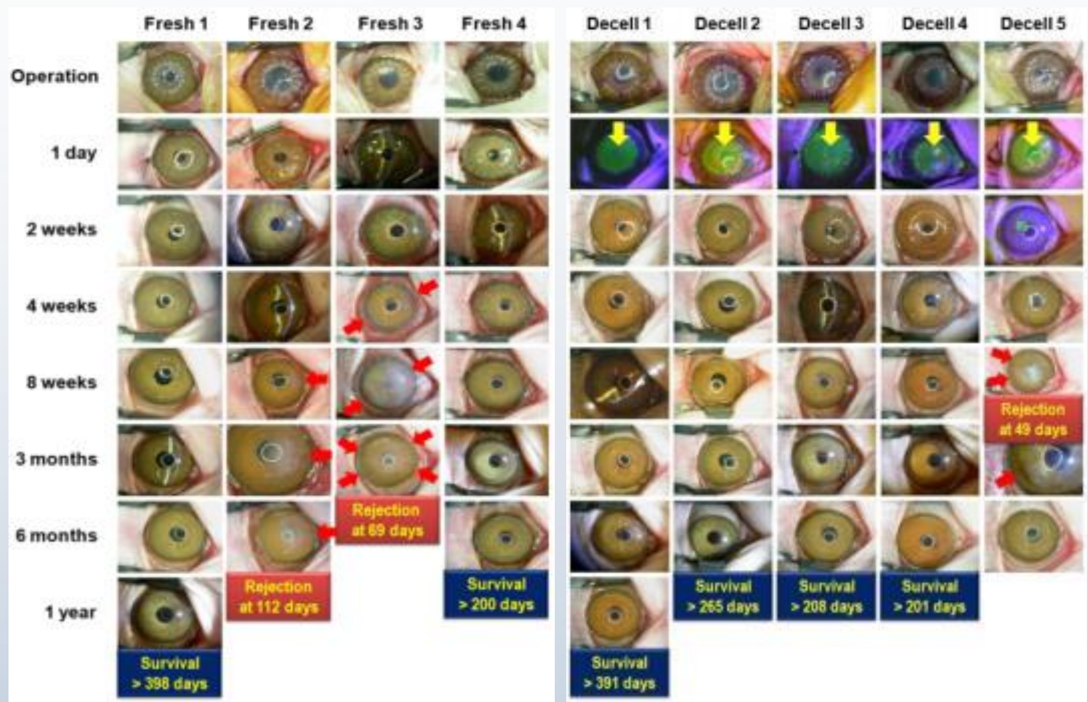
- Under systemic & topical steroid
  - Decellularized porcine cornea: promising substitute
  - Fresh porcine cornea : need more powerful immunosuppression

Surviving graft (Decell 3)



Rejected graft (Fresh 3)



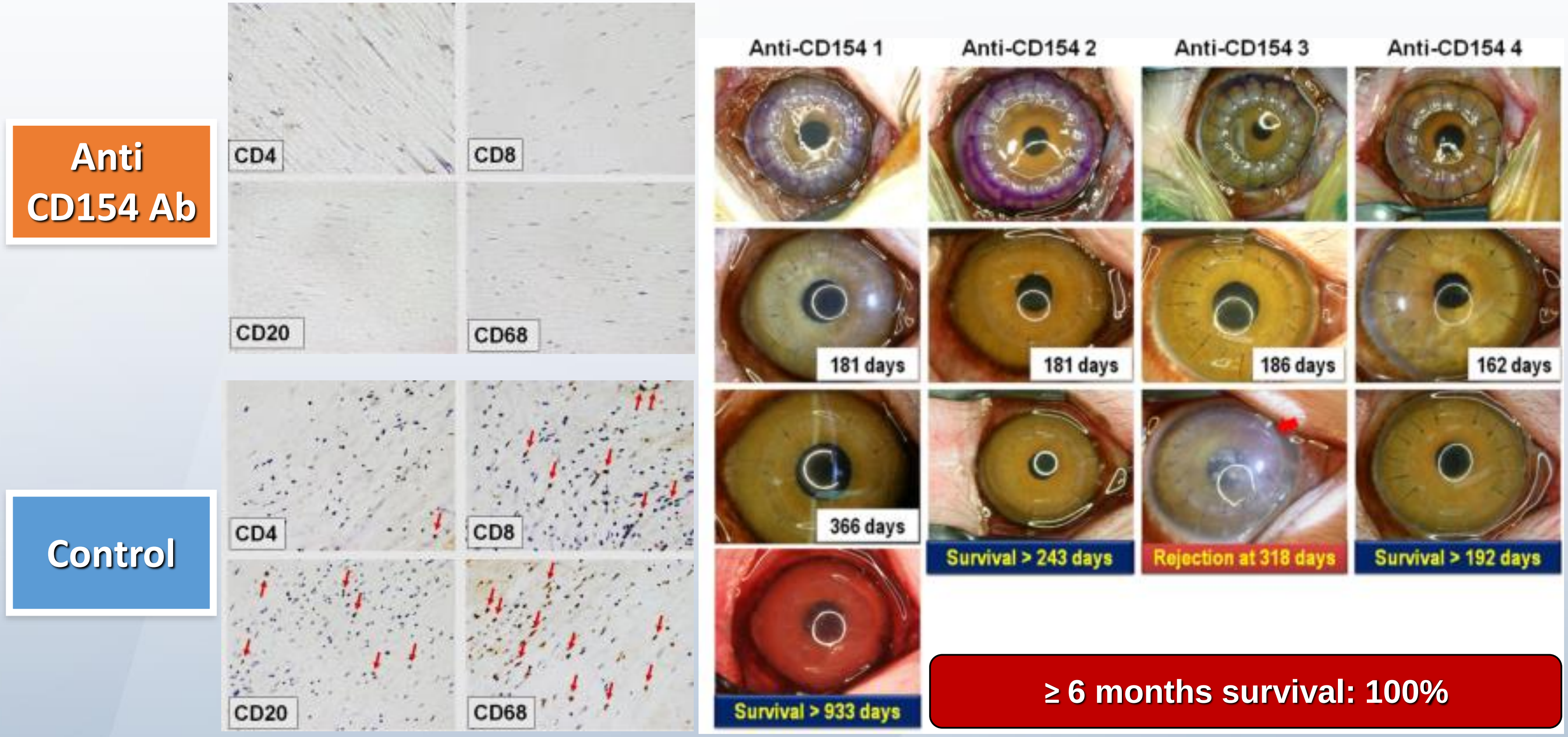


≥ 6 months survival: Fresh (50%) vs. Decell (80%)

Invest Ophthalmol Vis Sci. 2011 Aug 22;52(9):6643-50

Full thickness xenocorneal transplantation study in NHP

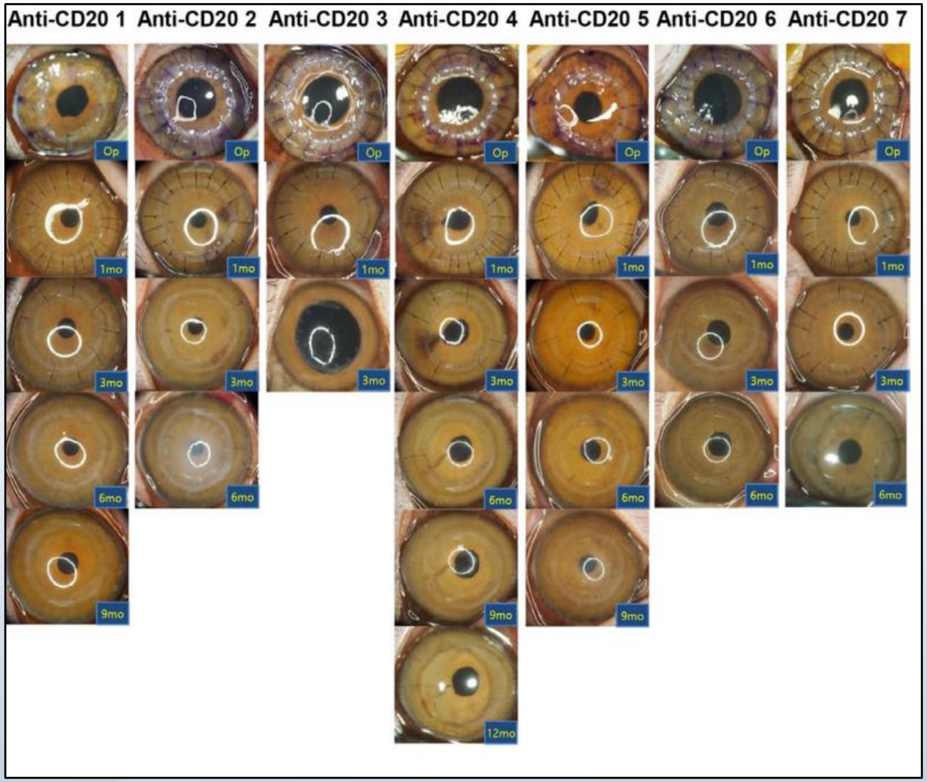
- Under Anti CD154 (CD40L) Ab + steroid
  - 100% survival more than 6 months

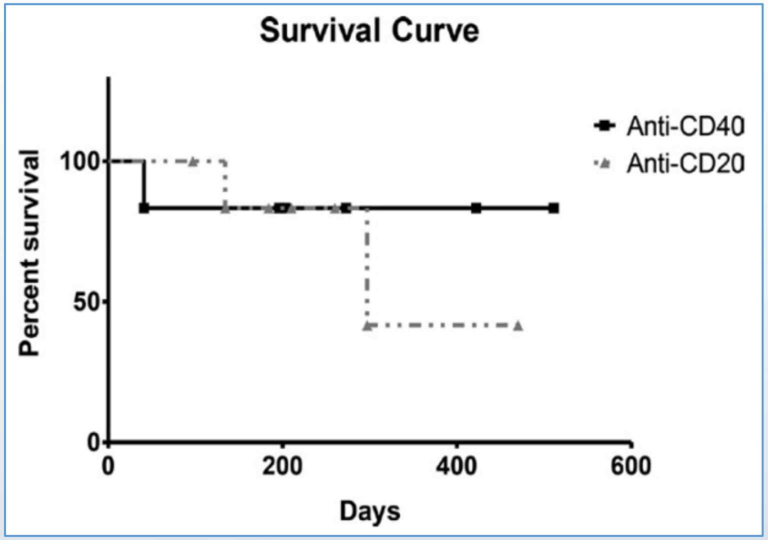


Am J Transplant. 2015 Mar;15(3):628-41

Full thickness xenocorneal transplantation study in NHP

- Under Anti CD20 Ab + Tacrolimus
  - 5 out of 7 survived over 6 months





Summary

- The results of NHP preclinical study confirm the potential of porcine islet and corneal xenotransplantation as a clinical treatment modality, satisfactorily meeting the international guidelines on safety and efficacy.

- For the vascularized organ xenotransplantation, the development of genetically engineered pigs are essential, especially to control consumptive coagulopathy.

Clinical trial of Kidney and Heart Xenotransplantation

Kidney xenotransplantation

- Sep 2021, NYU Langone Health led by Dr. Robert Montgomery.
- The kidney was attached to the blood vessels in the upper leg, outside the abdomen
- Kidney tissue sampling over the 54-hour period of study.
- Urine production and creatinine levels were normal and equivalent to what is seen from a human kidney transplant.
- No signs of rejection were detected.

Heart xenotransplantation

- Jan 7, 2022, University of Maryland Medical Center led by Dr. Bartley Griffith.
- Orthotopic cardiac xenotransplantation
- Survived for 2 months (died on 8th March, 2022)

Preparation of Clinical Trial for Pancreatic islet/Cornea

Designated pathogen-free (DPF) SNU mini-pig

- General information
  - Origin: Sinclaire bred in the University of Minnesota
  - Nomenclature: SNU mini-pig (CMS mini-pig)
  - Appearance: SNU mini-pigs have a brown and white-black spots color
  - Weight: Adult pigs weighing about 80~120 kg.

- History
  - SNU mini-pigs were originally purchased from the Hormel Institute, the University of Minnesota
  - They were derived by aseptic hysterectomy, were maintained in a germ-free isolator and entered the SPF facility.
  - The initial SPF SNU mini-pig stock was established at the Sloan Kettering Institute for Cancer Research, Rye, New York. In 1984
  - In 2004, 24 CMS mini-pigs were transferred using a germ-free isolator to the Center for Animal Resource Development (CARD) at Seoul National University (SNU).

Preparation of Clinical Trial for Pancreatic islet/Cornea

DPF status was confirmed by both internal and outside examinations
(Bacteria 40, Parasites 27, Fungi 6, Virus 73 : Total 146 pathogens)

4.1 Bacteria (40)

No	Pathogens	Results
1	*Actinobacillus equuli*	Negative
2	*Actinobacillus pleuropneumoniae (APP)*	Negative
3	*Actinobacillus suis*	Negative
4	*Actinomyces (Eubacterium) suis*	Negative
5	*Arcobacter spp.*	Negative
6	*Bacillus anthracis*	Negative
7	*Bordetella bronchiseptica*	Negative
8	*Brucella suis (Bru)*	Negative
9	*Campylobacter spp. (e.g., coli, jejuni)*	Negative
10	*Chlamydia spp.*	Negative
11	*Clostridium difficile*	Negative
12	*Coxiella burnetii*	Negative
13	*Erysipelothrix rhusiopathiae*	Negative
14	*Escherichia coli (E. coli) (verotoxigenic)*	Negative
15	*Haemophilus parasuis (HP)*	Negative
16	*Klebsiella species (e.g., pneumoniae)*	Negative
17	*Lawsonia intracellularis*	Negative
18	*Legionella pneumophila*	Negative
19	*Leptospira spp. (hardjo, pomona, tarrasovi, interogans)*	Negative
20	*Listeria monocytogenes / Listeria spp.*	Negative
21	*Methicillin-resistant Staphylococcus aureus*	Negative
22	*Mycobacterium sp. (bovis, tuberculosis, non-tuberculosis mycobacteria)*	Negative
23	*Mycoplasma hyopneumoniae (MH)*	Negative
24	*Mycoplasma hyorhinis*	Negative
25	*Ureaplasma parvum*	Negative
26	*Mycoplasma suis (Eperythrozoon suis)*	Negative
27	*Nocardia species*	Negative
28	*Pasteurella multocida (PM) / Pasteurella spp.*	Negative
29	*Vancomycin-resistant enterococci (VRE)*	Negative
30	*Pseudomonas species / Pseudomonas pseudomallei*	Negative
31	*Rhodococcus equi*	Negative
32	*Salmonella spp. (Sal)*	Negative
33	*Brachyspira hyodysenteriae*	Negative
34	*Brachyspira pilosicoli*	Negative
35	*Shigella*	Negative
36	*Staphylococcus hyicus*	Negative
37	*Streptococcus spp.*	Negative
38	*Streptococcus suis*	Negative
39	*Vancomycin intermediate-resistant Staphylococcus aureus (VISA)*	Negative
40	*Yersinia spp.*	Negative

4.2 Fungi (6)

No	Pathogens	Results
41	*Aspergillus spp. (colonized or lesions)*	Negative
42	*Blastomyces spp.*	Negative
43	*Candida spp.*	Negative
44	*Cryptococcus spp. / Cryptococcus neoformans*	Negative
47	*Histoplasma spp. /Histoplasma capsulatum*	Negative
48	*Systemic mycoses*	Negative

4.3 Parasites (27)

No	Pathogens	Results
49	Arthopods	Negative
50	*Babesia spp.*	Negative
51	*Balantidium coli*	Negative
52	Blood parasites	Negative
53	*Cryptosporidium parvum / Cryptosporidium spp.*	Negative
54	*Eimeria spp.*	Negative
55	*Entamoeba suis*	Negative
56	*Oesophagostomum spp.*	Negative
57	*Giardia spp.*	Negative
58	*Ascaris suum*	Negative
59	*Echinococcus spp. / Echinococcus granulosus)*	Negative
60	*Strongyloides spp*	Negative
61	*Trichinella spiralis*	Negative
62	*Hyostrongylus rubidus*	Negative
63	*Isospora spp.*	Negative
64	*Macrocanthorhynchus hirudinaceous*	Negative
65	*Metastrongylus spp*	Negative
66	*Neospora caninum*	Negative
67	*Sarcocystis miescheriana*	Negative
68	*Sarcocystis suihominis*	Negative
69	*Stephanurus dentatus*	Negative
70	*Taenia solium*	Negative
71	*Toxocara spp*	Negative
72	*Toxoplasma spp. / Toxoplasma gondii*	Negative
73	*Trichostrongylus spp*	Negative
74	*Trichuris suis*	Negative
75	*Trypanosoma cruzi*	Negative

4.4 Virus (73)

No	Pathogens	Results
76	Akabane virus	Negative
77	Alphatorquevirus (Torque teno virus)	Negative
78	Apoi	Negative
79	Batai	Negative
80	Border disease	Negative
81	Bornavirus	
82	Bovine virus di	
83	Cache Valley	
84	Chikungunya	
85	Classical swine	
87	Dengue fever	
88	Desoxyvirus (A	
89	Eastern equine	
90	Encephalomyoc	
91	Enteric calicivi	
96	Foot and mouth	
97	Getah virus	
98	Hantavirus	
99	Hemagglutinati	
100	Hepatitis B viru	
101	Hepatitis E viru	
102	Hepatitis A viru	
103	Human enterov	
104	Rhinovirus (hu	
105	Influenza A/B/C	
106	Japanese encep	
107	Louping III/TB	
108	Lymphocytic cl	
109	Menangle virus	
110	Measles virus	
112	Nipah (Hendra-	
113	Ibaraki	
115	Orthopoxvirus (	
116	Parainfluenza 2	
117	Parainfluenza 3	
118	Parainfluenza 1	
120	Porcine adenov	
121	Porcine astrovi	
122	Porcine circovi	
123	Porcine circovi	

4.4 Virus (73)

No	Pathogens	Results
124	Porcine circovirus type 3 (PCV3)	Negative
125	Porcine cytomegalovirus (PCMV)	Negative
126	Porcine endogenous retrovirus (PERV)	Positive
127	Enterovirus G	Negative
128	Porcine epidemic diarrhea virus (PEDV)	Negative
129	Porcine genital papillomavirus	Negative
130	Porcine lymphotropic herpesvirus type 1 (PLHV1)	Negative
131	Porcine lymphotropic herpesvirus type 2 (PLHV2)	Negative
132	Porcine parvovirus (PPV)	Negative
133	Porcine picobirnavirus	Negative
134	Porcine polyomavirus	Negative
135	Porcine reproductive and respiratory syndrome virus (PRRSV)	Negative
136	Porcine respiratory coronavirus (PRCV)	Negative
137	Porcine rotavirus (Rota)	Negative
138	Porcine rubulavirus (la Piedad Michoacan)	Negative
139	Porcine teschovirus (PTV)	Negative
140	Porcine torovirus	Negative
141	Pseudorabies (Aujeszky's disease)	Negative
142	Rabies virus	Negative
143	Reovirus	Negative
144	Respiratory syncytial virus (RSV)	Negative
146	Suipoxvirus (Swinepox)	Negative
148	Swine vesicular disease	Negative
150	Transmissible gastroenteritis virus (TGEV)	Negative
152	Venezuelan encephalitis	Negative
154	Vesicular stomatitis virus	Negative
155	Wesselsbron disease	Negative
156	West Nile fever virus	Negative
157	Western equine encephalitis virus (WEEV)	Negative
158	Tioman virus	Negative
159	parvovirus PPARV-4 (bocavirus)	Negative
160	Kobuvirus	Negative
162	Ljungan-like virus	Negative

Preparation of Clinical Trial for Pancreatic islet/Cornea

신청확인서

접수번호 : 20200179580 신청일자 2020-08-24

민원명	임상시험계획승인-연구자(학술연구용) : KXITSW1.0		
민원인 (대표자 또는 대리인)	김광원		
처리주관부서	식 품 의 약 품 안 전 처 임상제도과	전 화 :	
교부(수령)기관	식 품 의 약 품 안 전 처 임상제도과		
수수료	※ 수수료는 전자지불을 통해 금 0원을 납부하였음을 증명합니다. (납부일 : 2020-08-24)		

청렴하고 깨끗한 식품의약품안전처가 되겠습니다.

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※ 민원처리 공직자가 금품을 요구 또는 불공정하게 업무를 처리하거나 불친절한 경우 우리 처 홈페이지(www.mfds.go.kr), 감사담당관 직통전화(043-719-1365) 또는 이메일(mfdsoig@korea.kr)로 알려주시기 바랍니다.
※ 모든 신고내용은 철저하게 관리하여 귀하의 개인정보가 외부로 유출되지 않도록 하겠으며, 청렴하고 공정한 식품의약품안전처를 만들기 위한 소중한 자료로 사용하겠습니다.

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식 품 의 약 품 안 전 처

신청확인서

접수번호 : 20200162369 신청일자 2020-07-29

민원명	임상시험계획승인-연구자(학술연구용) : SNU돼지이종각막		
민원인 (대표자 또는 대리인)	김미금		
처리주관부서	식 품 의 약 품 안 전 처 임상제도과	전 화 :	
교부(수령)기관	식 품 의 약 품 안 전 처 임상제도과		
수수료	※ 수수료는 전자지불을 통해 금 0원을 납부하였음을 증명합니다. (납부일 : 2020-07-29)		

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위 건명의 민원사안이 신청되었습니다.

식 품 의 약 품 안 전 처

Contents

Currently available genetically modified pig for organ source

Indication	Pathway
Complement regulation by human complement-regulatory gene expression	CD46 (membrane co-factor protein) CD55 (decay-accelerating factor) CD59 (protectin or membrane inhibitor of reactive lysis)
Gal or non-Gal antigen 'masking' or deletion	Human H-transferase gene expression (expression of blood type 0antigen) Endo-β-galactosidase C (reduction of Gal antigen expression) α1,3-galactosyltransferase gene-knockout (GTKO) Cytidine monophosphate-N-acetylneuraminic acid hydroxylase (CMAH)gene knockout (NeuGcKO) β1,4-N-acetylgalactosaminyltransferase (β4GalNT2) gene knockout (β4GalNT2KO)
Suppression of cellular immune response by gene expression or down-regulation	CIITA-DN (MHC class II transactivator knockdown, resulting in swine leukocyte antigen class II knockdown) Class I MHC-knockout (MHC-IKO) HLA-E/human β2-microglobulin (inhibits human natural killer cell cytotoxicity) Human FAS ligand (CD95L)Human N-acetylglucosaminyltransferase III (GnT-III) gene Porcine CTLA4-Ig (cytotoxic T lymphocyte antigen 4 or CD152) Human TRAIL (tumour necrosis factor-α-related apoptosis-inducingligand)
Anticoagulation and anti-inflammatory gene expression or deletion	von Willebrand factor (vWF)-deficient (natural mutant) Human tissue factor pathway inhibitor (TFPI) Human thrombomodulin Human endothelial protein C receptor (EPCR) Human ectonucleoside triphosphate diphosphohydrolase-1 (CD39)
Anticoagulation, anti-inflammatory and anti-apoptotic gene expression	Human tumour necrosis factor-α-induced protein 3 (A20) Human haemoxygenase-1 (HO-1) Human CD47 (species-specific interaction with SIRP-α inhibits phagocytosis) Porcine asialoglycoprotein receptor 1 gene-knockout (ASGR1-KO; decreases platelet phagocytosis) Human signal regulatory protein-α (SIRPα; decreases platelet phagocytosis by 'self' recognition)
Prevention of PERV activation	PERV siRNA PERV inactivation

Modified from Cooper DK, Ekser B, Ramsoondar J, Phelps C, Ayares D. J Pathol. 2016 ;238(2):288-99.

Timeline of Clinical xenotransplantation

Heart

Full-thickness cornea

Pancreatic islets

2020 2021 2022 2023 2030

- Development of clinically applicable immunosuppression regimen
- Safety assurance
- Regulatory framework

Generation of humanized organ

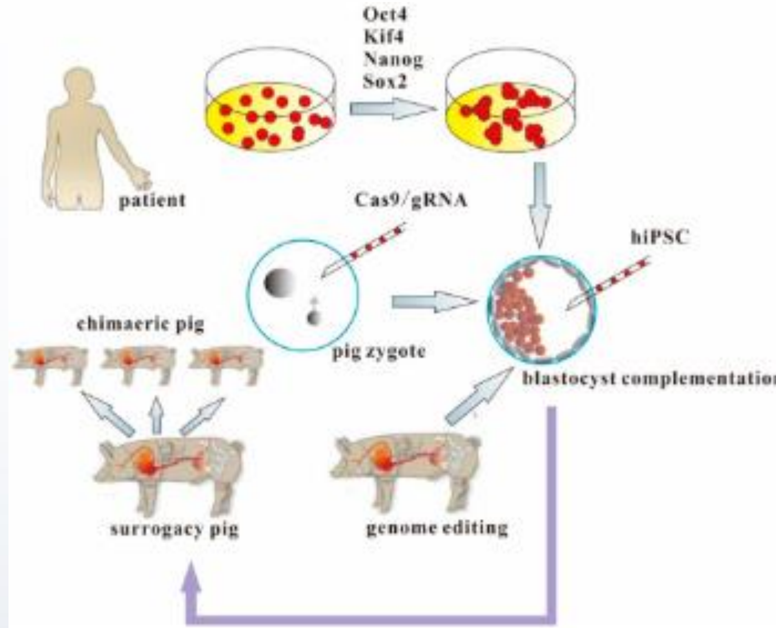
RESEARCH

GENOME ENGINEERING

Inactivation of porcine endogenous retrovirus in pigs using CRISPR-Cas9

Dong Niu,[1,2*] Hong-Jiang Wei,[3,4*] Lin Lin,[5*] Haydy George,[1*] Tao Wang,[1*] I-Hsiu Lee,[1*] Hong-Ye Zhao,[3] Yong Wang,[6] Yinan Kan,[1] Ellen Shrock,[7] Emal Lesha,[1] Gang Wang,[1] Yonglun Luo,[5] Yubo Qing,[3,4] Deling Jiao,[3,4] Heng Zhao,[3,4] Xiaoyang Zhou,[6] Shouqi Wang,[8] Hong Wei,[6] Marc Güell,[1†] George M. Church,[1,7,9†] Luhan Yang[1†‡]

Niu *et al.*, *Science* **357**, 1303–1307 (2017)



Genome editing and Blastocyst complementation

- **Inactivate 62 PERV genes**
- **Express more than 20 genes modifying immune response and coagulation**

Modified from Int. J. Mol. Sci. 2015, 16(3), 6545-6556

Acknowledgement

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Jun-Seop Shin
Byoung-Hoon Min
Su-Kyoung Park
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Seon Young Kim
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NHP Core
Jong Min Kim
Won Young Jeong
Ga Eul Lee
Min Sun Kim
Ju-Eun Kim

Immunology Core
Jung-Sik Kim
Il-Hee Yoon
Hyun-Je Kim
Bongi Kim
Yong-Hee Kim
Jin-Young Shin
Hye-Young Nam
Hyun-Joo Lim
Soo-Eon Kim
Sun-Ho Lee
NaRi Byun
Seung-Jun Kang

Coreneal XenoTPL
Jae Young Kim
Hyuck Jin Choi
Mee Kum Kim
Won Ryang Wee

Collaborators
Hee Jung Kang (Hallym University, XRC)
Tae-Hoon Cheon (Korea University, XRC)
Eung-Soo Hwang (SNU, XRC)
Ivo Kwon (Ehwa Woman's University, XRC)
Kwang-Won Kim (Gacheon University)
Keith A. Reimann (MassBiologics, University of Massachusetts Medical School)
Norma Kenyon (University of Miami)

Advisor
Sang-Joon Kim (Myongji Hospital)
David Cooper (University of Pittsburgh)
Bernhard Hering (University of Minnesota)

경청해 주셔서 감사합니다

XRC 2단계 바이오이종장기개발사업단
Xenotransplantation Research Center