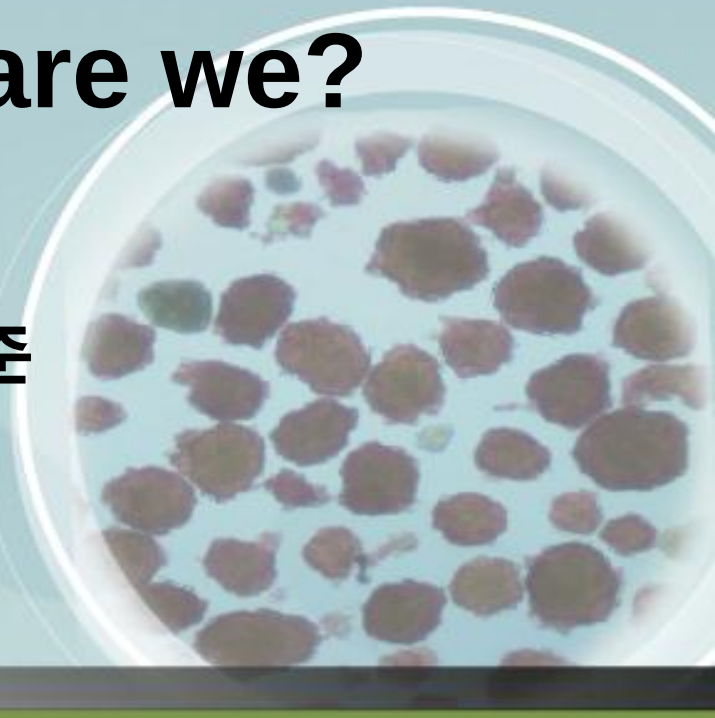
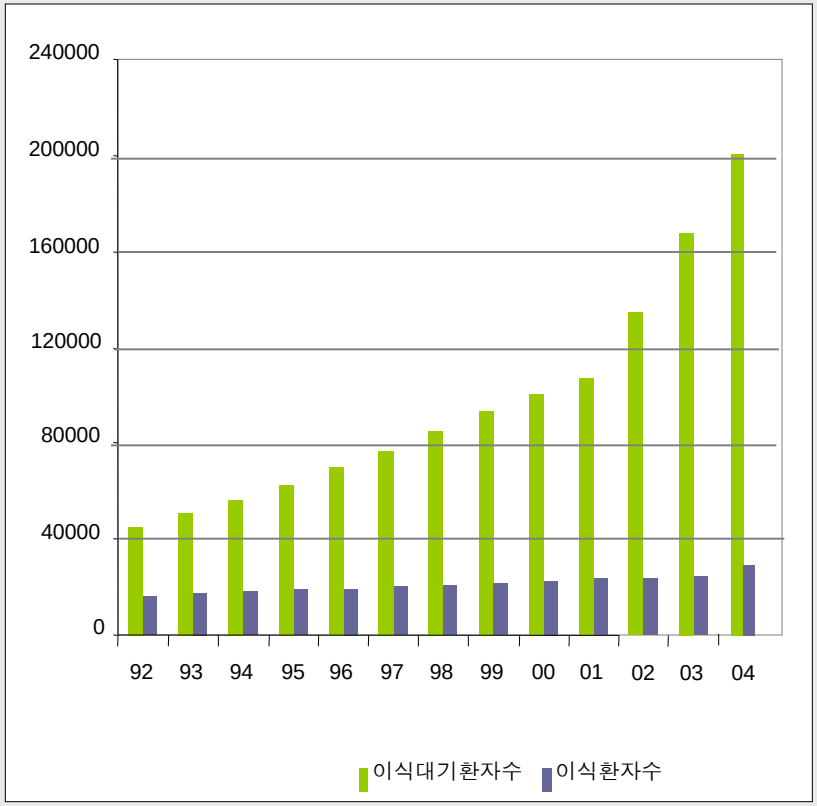
Xenotransplantation:

Where are we?



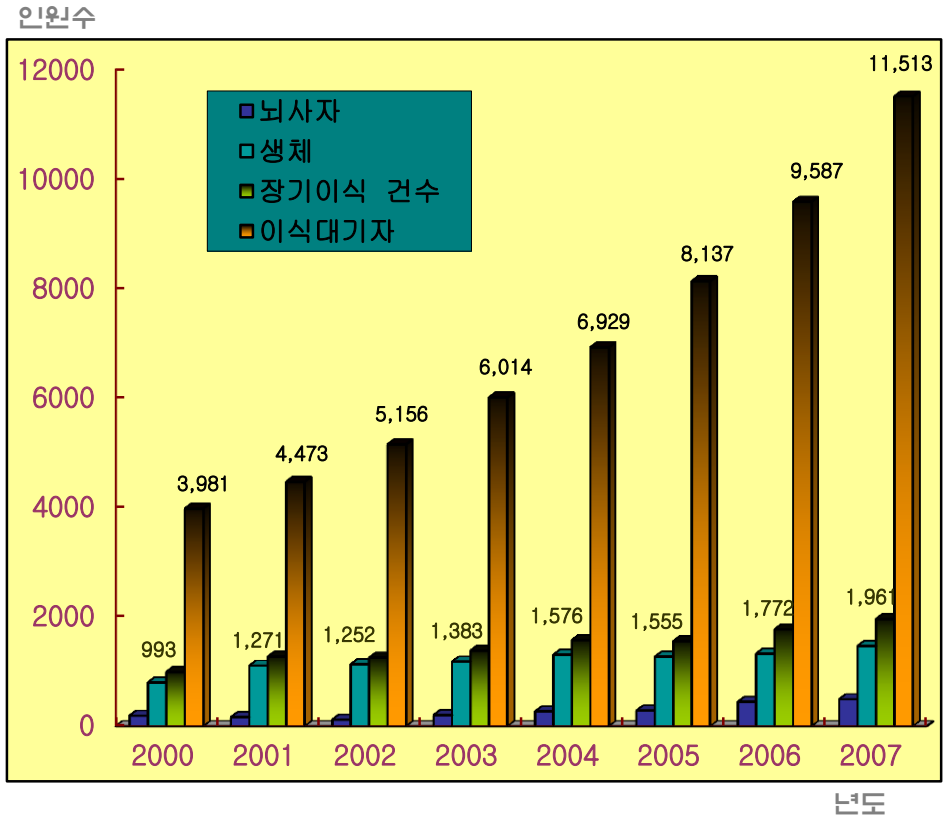
서울의대 김 상 준

장기 수급 현황



Number of Kidney Transplantation Patients

United Network for Organ Sharing (2004)



고형장기 국내이식 현황

(2007 KONOS 자료)

부족장기를 대체할 수 있는 방법

	적용 장기	현 황
이종이식(돼지)	대부분의 장기 : 간 (향후 개발이 필요)	전임상시험중 (돼지 췌도는 일부 임상적용)
인공장기	대부분 심장 환자	동종이식시까지 임시 방편으로 사용
바이오 인공신장	급성신부전증	체외투석
줄기세포를 이용한 조직재생	모든 장기	세포치료는 실험단계 (일부 임상시험) 고형장기는 초기단계

이종이식의 역사

년 도	보고자	공여동물 및 조직 또는 장기	생존기간
1682	(러시아)	동물의 뼈	
1800 말	(영국)	개구리 피부	
1905	Princeteau	토끼신장	16일
1906	Jaboulay	돼지, 양의 신장	3일
1910	Unger	원숭이 신장	2일
1923	Neuhof	양 신장	9일
1963-64	Reemtsma	원숭이 신장	11일-9개월
1964	Hardy	침판지 심장	1일
1966	Starzl	침판지 간	9일
1984	Baily	바분원숭이 심장	20일
1992	Starzl	바분원숭이 간	70일

이종이식을 성공적으로 받은 환자들

Maribeth Cook

- 34/여
- 뇌졸중으로 좌측 마비
- $3x10^7$ 개의 돼지 신경세포를 손상받은 뇌에 이식받음. (1994)

⇒ 보조기를 차고 마라톤에 출전할 정도로 호전됨.

Amanda Davis

- 21/여
- 좌측 반신마비
- 돼지 신경세포를 손상받은 뇌에 이식받음. (1999)

⇒ 보조기 없이 보행 가능할 정도로 호전됨.

Jim Finn

- 파킨슨병
- 돼지 신경세포를 손상받은 뇌에 이식받음. (1997)

⇒ 혼자 걷고 앉거나 서는 행동이 가능할 정도로 호전됨.

Robert Pennington

- 20/남
- 급성 간부전
- 체외에 돼지 간을 연결함. (1997)

⇒ 공여자 간이 준비될 때까지 3일간 돼지 간으로 성공적으로 연명하였음.

어떤 동물의 장기 사용?

- 멸종위기종
- 번식난이
- 윤리적 문제
- 높은 감염 위험성

- 적절한 장기 크기
- 낮은 감염 위험성
- 번식용이
- 형질전환 무균사육용이

Xenotransplantation: Key issues

- Immunology
- Physiology
- Biosafety
- Ethics and regulations

알파- 갈 형질전환 돼지 제작,
혈청 여과 기술

혈청보체 조절인자 형질전환 돼지 제작
혈관내피세포 안정화
내피세포유래 면역 매개물질 조절
혈소판 기능조절

면역 억제, 면역 관용

Genetic engineering of the pig : Possible targets of intervention

- Complement regulation (CD55, CD46, CD59)
- Immunogenicity (αGALT-KO)
- Coagulation (CD39, TM, TFPI, TF-KO, TM...)
- Immunomodulation (CTLA4Ig, HLA-G)
- Ischemia/IRI (CD39, A20, VEGF...)

[adapted from D'Apice et al, Xenotransplantation 2008]

Production of genetically engineered animals

- Immunology
- Physiology
- Biosafety
- Ethics and regulations

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]*

In Korea

Xeno 1, born on April 4, 2009

Xeno 2, born on June 25, 2009

GalT-KO 돼지 장기를 영장류에 이식한 실험 결과

NATURE MEDICINE VOLUME 11 | NUMBER 1 | JANUARY 2005

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

장기 생존율 > 180일

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

장기 생존율 > 80일

결론

- GalT-KO 돼지 장기를 사용하여 초급성 거부반응을 극복할 수 있다는 사실을 확인함
- 급성혈관성거부반응: 형질전환 돼지 생산으로 극복 가능함
- 세포매개성 거부반응: 현재 사람에게 적용되는 방법을 사용하여 극복 가능함

Potential microbial problems in xenotransplantation

Potentially problematic microbes are those:

- difficult to eliminate
  - Porcine endogenous retrovirus (**PERV**)
- maintained in a latent or intracellular state in an asymptomatic host
  - Herpesviruses
  - Circovirus etc.
- **as yet unidentified**

PERV 감염

- 현재까지 돼지장기를 이식 받은 영장류 및 사람에서 PERV 감염이 확인되지 않고 있음
  - 돼지 장기를 영장류에 이식한 경우
    - Nat Med. 2006 Mar;12(3):304-6.
    - Nat Med. 2006 Mar;12(3):301-3
    - J Virology. 2008 Dec;82(24):12441-8
  - 돼지 췌도를 사람에 이식한 경우
    - 1994년 Groth 등; 2000년, 2005년 Elliott 등; 2005년 Wang 등

PERV 감염에 대한 consensus

- PERV 감염 모니터링을 국제 기준에 따라 시행할 경우 돼지 장기를 이용한 이종장기이식은 충분히 가능함

Longest survival of primates transplanted with porcine organs or cells

- 179 days for a heterotopic heart xenograft
- 56 days for an orthotopic heart xenograft
- 90 days for a life supporting kidney xenograft

- >180 days in at least five series of **islet** xenografts
- >180 days in one series of **neural** xenografts

어떤 장기를 먼저 임상 적용하나?

- Cell Transplantation
  (islet, neuroblasts)
- Corneal xenotransplantation
- Vascularized organ

Porcine islet xenotransplantation

- It is not as technically demanding as vascularized organ Xtx
- Ex vivo manipulation is possible
- Many patients who could potentially benefit from the procedure
- Porcine insulin has been used clinically for years
- Failure demands not so much pain

Clinical Pig islet Transplantation

American Journal of Transplantation 2006; 6: 1269–1274

Table 1: Experience with clinical pig islet transplantation

First author (reference #)	Source pig islets	Recipients (n)	Site/number of islets (ICC/NPI) (when stated)	Immunosuppressive regimen	Outcome
Groth (3) [1994]	WT fetal	*Group A:* 2	*A:* Kidney capsule, 200,000 and 410,000	*A and B:* CyA (n = 10), prednisolone (n = 10)	*A:* No plasma C-pep. Mononuclear and eosinophilic infiltrates at day 21 (on kidney biopsy).
		Group B: 8	*B:* Intraportal, 330,000–1,020,000	ATG (n = 5) 15-deoxyspergualin (n = 5)	*B:* Urine C-pep documented for up to 460 days (n = 4.)
Elliott (4,5) [2000/2005]	WT neonatal	2	1 million encapsulated in peritoneal cavity	Non-immunosuppressed (n = 1) CyA, AZA, prednisone (n = 1)	Decreased exogenous insulin requirement (up to 34%), documented urinary C-pep production, and decreased glycosylated Hb for between14–27 months. Nine years post Tx: viable islet cells identified in capsules (n = 1).
Valdes-Gonzalez (6) [2005]	WT neonatal + Sertoli cells	12	14,000–21,000/kg subcutaneously, in collagen tubes in steel/teflon stents. Retransplants after 6 months (n = 11) and 3 years (n = 4)	Non-immunosuppressed	Decreased exogenous insulin requirement for up to 4 years (n = 6). No serum C-pep. Glucose-stimulated serum porcine insulin (n = 3).
Wang (7) [2005]	WT neonatal	*Group A*: 15	*Groups A, B and C*: Intra-hepatic artery, 5–7 million	*A:* CyA, MMF, prednisolone	*A and B:* Decreased exogenous insulin requirement (33–62%).
		Group B: 3		*B:* OKT-3, tacrolimus, sirolimus, prednisolone	C-pep for 1 year.
		Group C: 2		*C:* CyA, MMF	*C*: No improvement.

ATG = Anti-thymocyte globulin, AZA = azathioprine, C-pep = porcine C-peptide, CyA = cyclosporine, ICC = islet-like cell clusters, MMF = Mycophenolate mofetil, NPI = neonatal pig islet, WT = wild-type.

Minnesota: BSM + α-CD154 + RAD + FTY720 + LFM

Emory/AB: BSM + α-CD154 + Rapa + LEA29Y

Prolonged diabetes reversal after intraportal xenotransplantation of wild-type porcine islets in immunosuppressed nonhuman primates

Bernhard J Hering[1], Martin Wijkstrom[1], Melanie L Graham[1], Maria Hårdstedt[1], Tor C Aasheim[1], Tun Jie[1], Jeffrey D Ansite[1], Masahiko Nakano[1], Jane Cheng[2], Wei Li[2], Kathleen Moran[2], Uwe Christians[3], Colleen Finnegan[4], Charles D Mills[1], David E Sutherland[1], Pratima Bansal-Pakala[1], Michael P Murtaugh[4], Nicole Kirchhof[5] & Henk-Jan Schuurman[2]

Long-term survival of neonatal porcine islets in nonhuman primates by targeting costimulation pathways

Kenneth Cardona[1,4], Gregory S Korbutt[2,4], Zvonimir Milas[1], James Lyon[2], Jose Cano[1], Wanhong Jiang[1], Hameeda Bello-Laborn[1], Brad Hacquoil[2], Elizabeth Strobert[3], Shivaprakash Gangappa[1], Collin J Weber[1], Thomas C Pearson[1], Ray V Rajotte[2] & Christian P Larsen[1]

XRC

Six-month survival in islet pig-to-primate xenotransplantation

- Hering et al. [nature Med. 2006]
- Cardona et al. [Nature Med. 2006]
- Larsen et al. [ATC, San Francisco 2007]
- Cooper et al. [Nature Med. 2009]
- Gianello et al. [Bull Mem Acad R Med Belg. 2007]

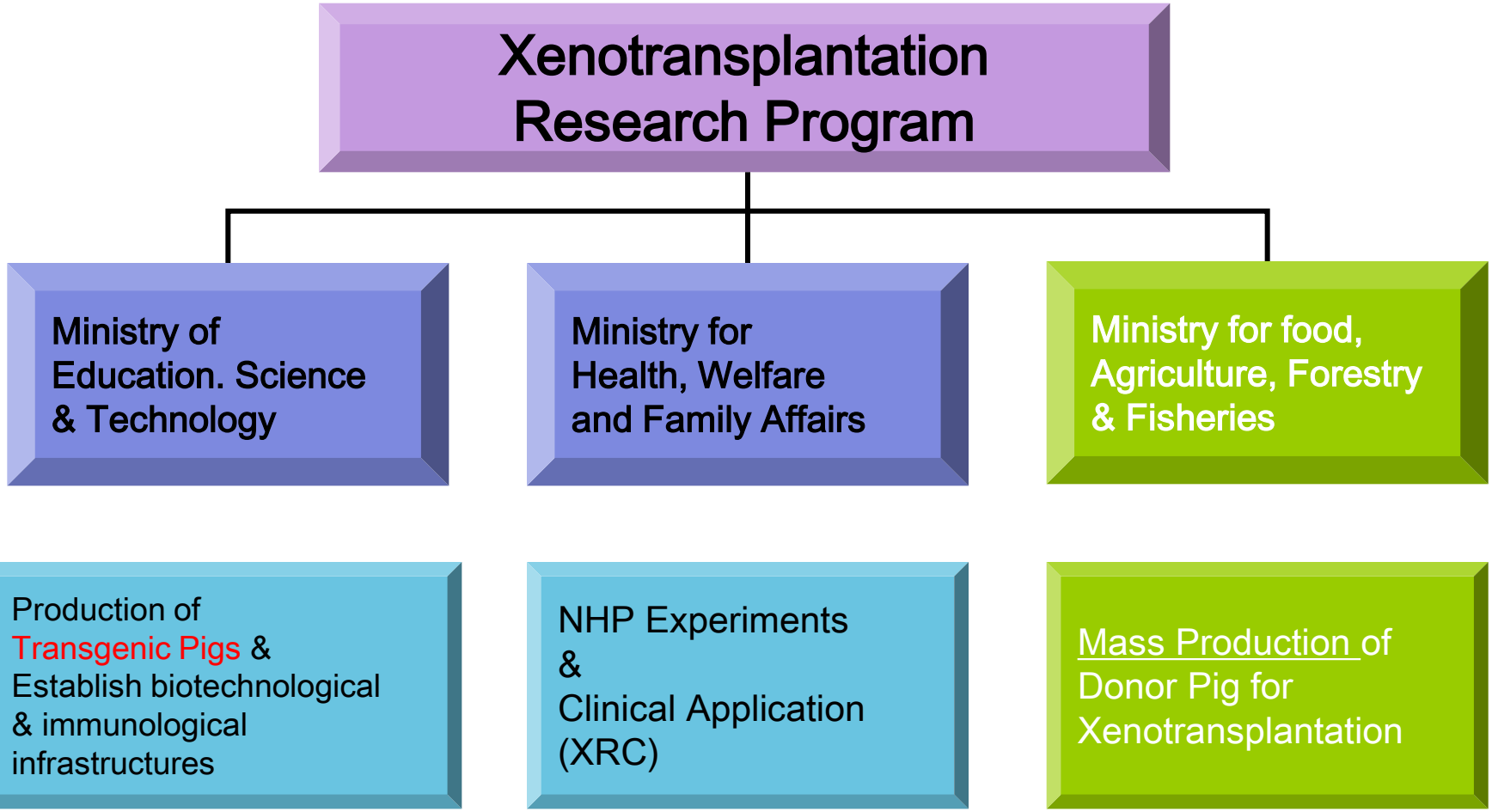
Encapsulation with alginate, **no immunosuppression**

뉴질랜드의 DIABET CELL 2009년 임상시험 허가

이종 이식 임상 시험을 위한 국제적 consensus의 마련

- 2008년 11월 중국 Changsha: 국제보건기구 (WHO) 주관으로 우리나라를 포함한 19개국을 소집하여 ”이종이식의 임상적용에 대한 규제“ 회의를 개최하였고 ”이종이식 임상적용 규제“에 관한 의정서를 채택함

한국에서의 이종이식 : 2004년~



바이오이종장기개발사업

- Research Period : 2004.05.01 ~ 2013.03.31
  (for 8 yrs & 11 months)

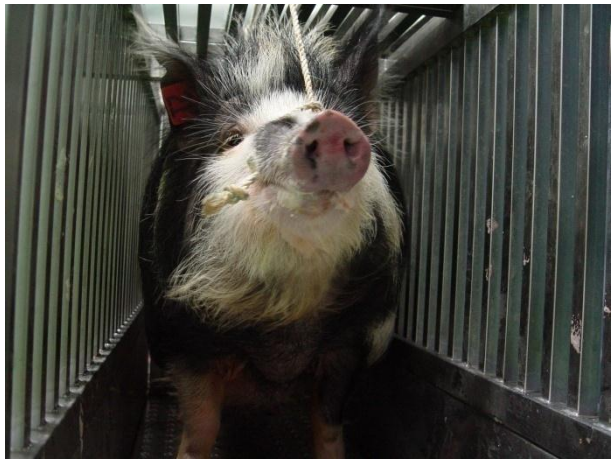
1st Stage (2004.05.01 ~2006.03.31)
: Establish infrastructures

2nd Stage (2007.04.01 ~ 2010.03.31)
: Non-human primate experiments

3rd Stage : (2010.04.01 ~ 2013.03.31)
: Clinical application

Structure of Program

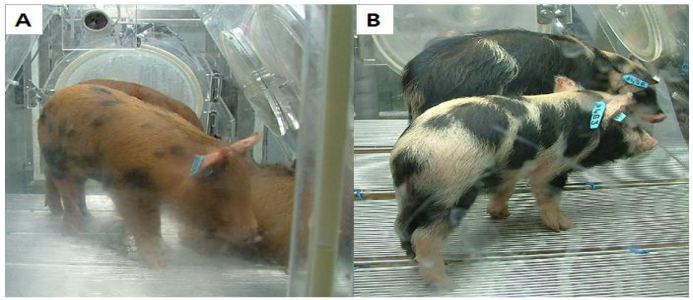
SNU miniature pig as a source for Xenotransplantation



Origin & History

Under SPF grade for more than 35 years !!

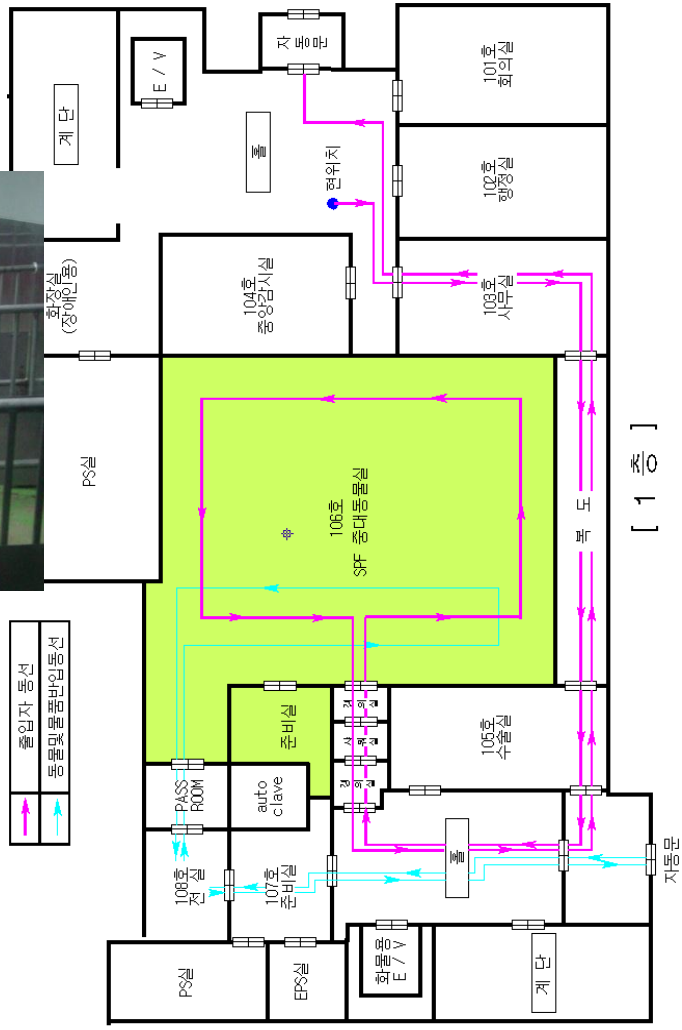
2004 Transportation (USA → KOR)



1973 Established SPF miniature pig, Dr. Yoon-Berm Kim

1949 Developed Minnesota miniature pig in Hormel institute, USA

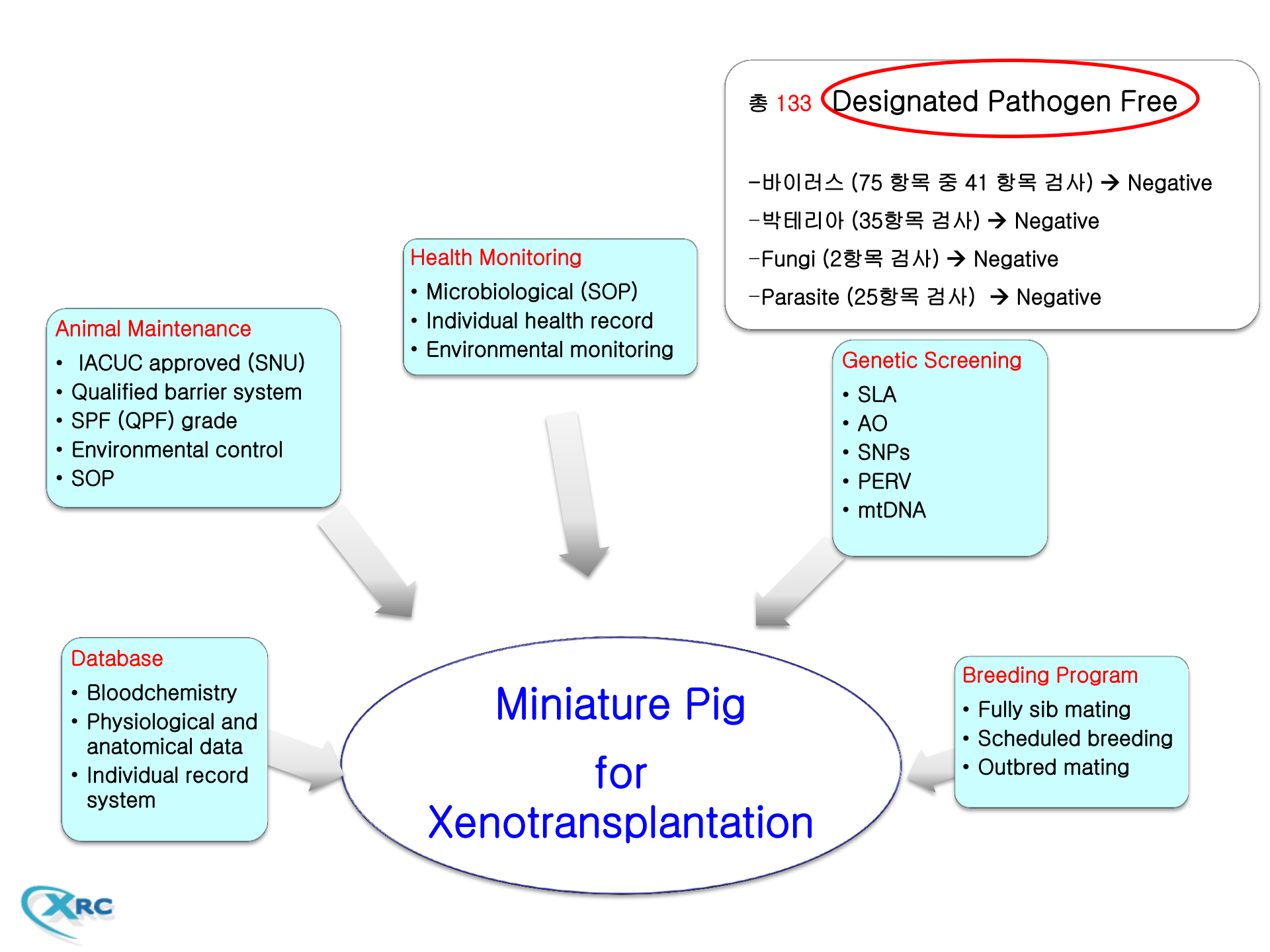
Biosecure barrier pig facility (SNU)



CARD

(Center for Animal Resource Development)

- **BSL-2**
- **Capacity: 80 pigs**


총 133 Designated Pathogen Free
-바이러스 (75 항목 중 41 항목 검사) → Negative
-박테리아 (35항목 검사) → Negative
-Fungi (2항목 검사) → Negative
-Parasite (25항목 검사) → Negative
Health Monitoring
• Microbiological (SOP)
• Individual health record
• Environmental monitoring
Animal Maintenance
• IACUC approved (SNU)
• Qualified barrier system
• SPF (QPF) grade
• Environmental control
• SOP
Genetic Screening
• SLA
• AO
• SNPs
• PERV
• mtDNA
Database
• Bloodchemistry
• Physiological and anatomical data
• Individual record system
Miniature Pig for Xenotransplantation
Breeding Program
• Fully sib mating
• Scheduled breeding
• Outbred mating

Non-human primate research center

★ 2009년 7월 3일 설립 ★ 2010년 7월 9일 국제실험동물시설인증협회(AAALAC international) 인증

★ 2011년 상반기 식품의약품안전청 KGLP 인증 예정

Laboratoy

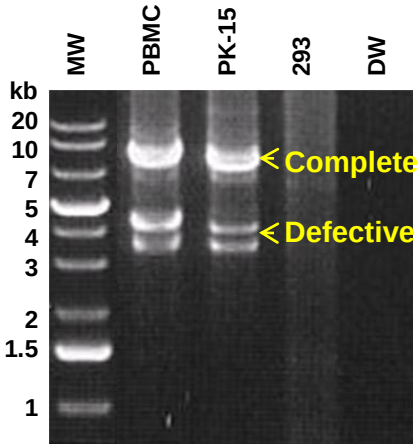
Intensive Care Unit

Operating room

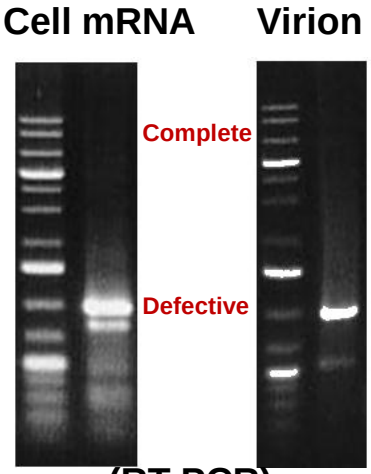
3 breeding rooms – Capacity : 50 Rhesus monkeys

Genomic construct of PERV derived from SNU miniature pig

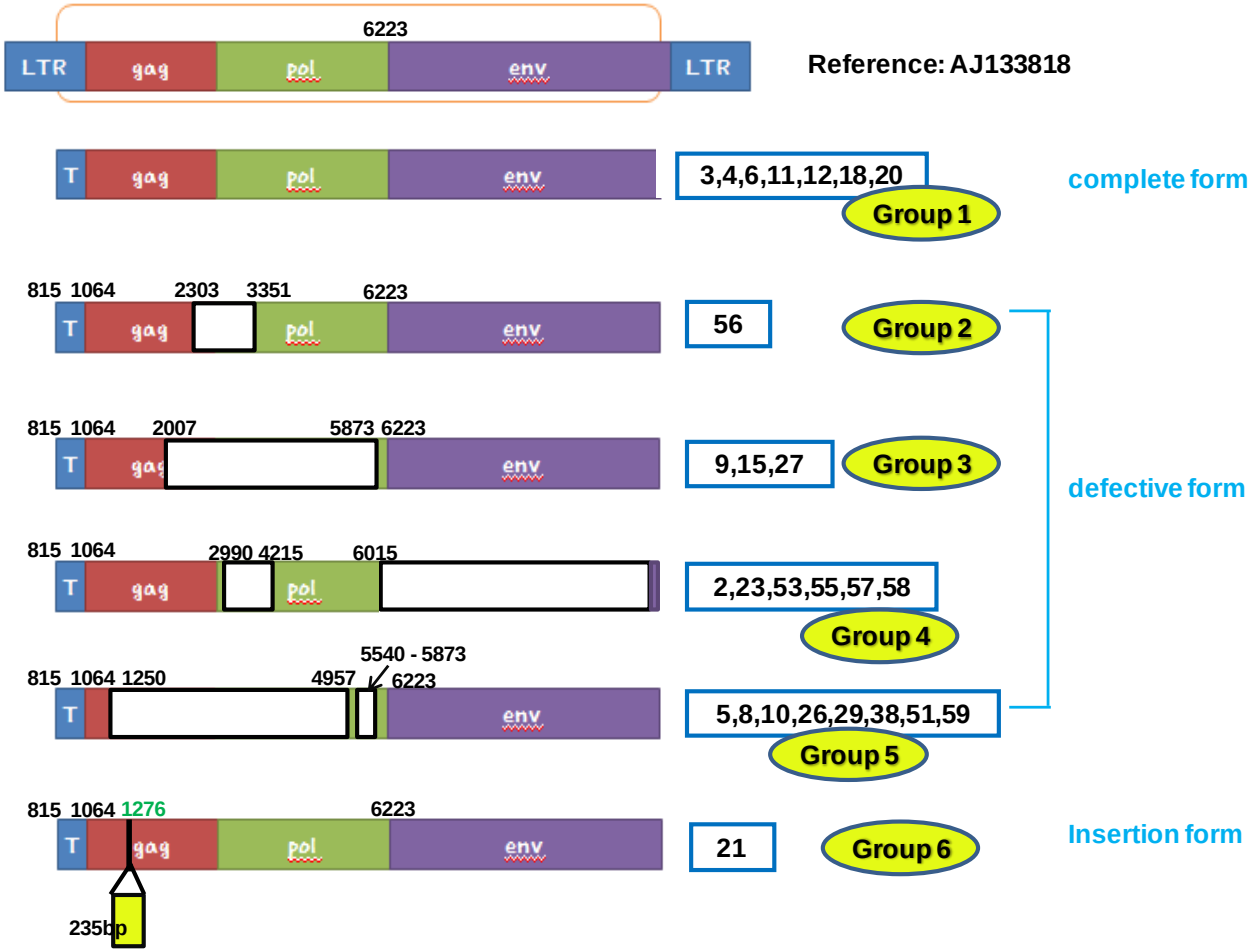
- Genomic level



(PCR)



(RT-PCR)



Detection of PERV in HEK-293 cells co-cultured with stimulator-activated islet cells from SNU miniature pig

EXP-1	Day 5	Day 13	Day 22	Day 41	Day 62
Media	+/+	+/+	+/+	-/-	-/-
PMA	+/+	+/+	+/+	-/-	-/-
PHA	+/+	+/+	+/+	-/-	-/-
LPS	+/+	+/+	+/+	-/-	-/-
PGE2	+/+	+/+	+/+	-/-	-/-
EXP-2	Day 30	Day 40	Day 58	Day 69	Day 90
Media	+/+	-/+	-/-	-/-	-/-
PMA	ND	+/+	-/-	-/+	-/-
PHA	+/+	-/+	-/-	-/-	-/-
LPS	-/+	-/+	-/-	-/-	-/-
PGE2	+/+	-/+	-/-	-/-	-/-

+/+: *pol*(+)/COII(+), -/+: *pol*(-)/COII(+), -/-: *pol*(-)/COII(-), ND: not tested

Ethics

Inducing "Public Consensus" on Xenotransplantation: Concensus Conference (April-September 2007)

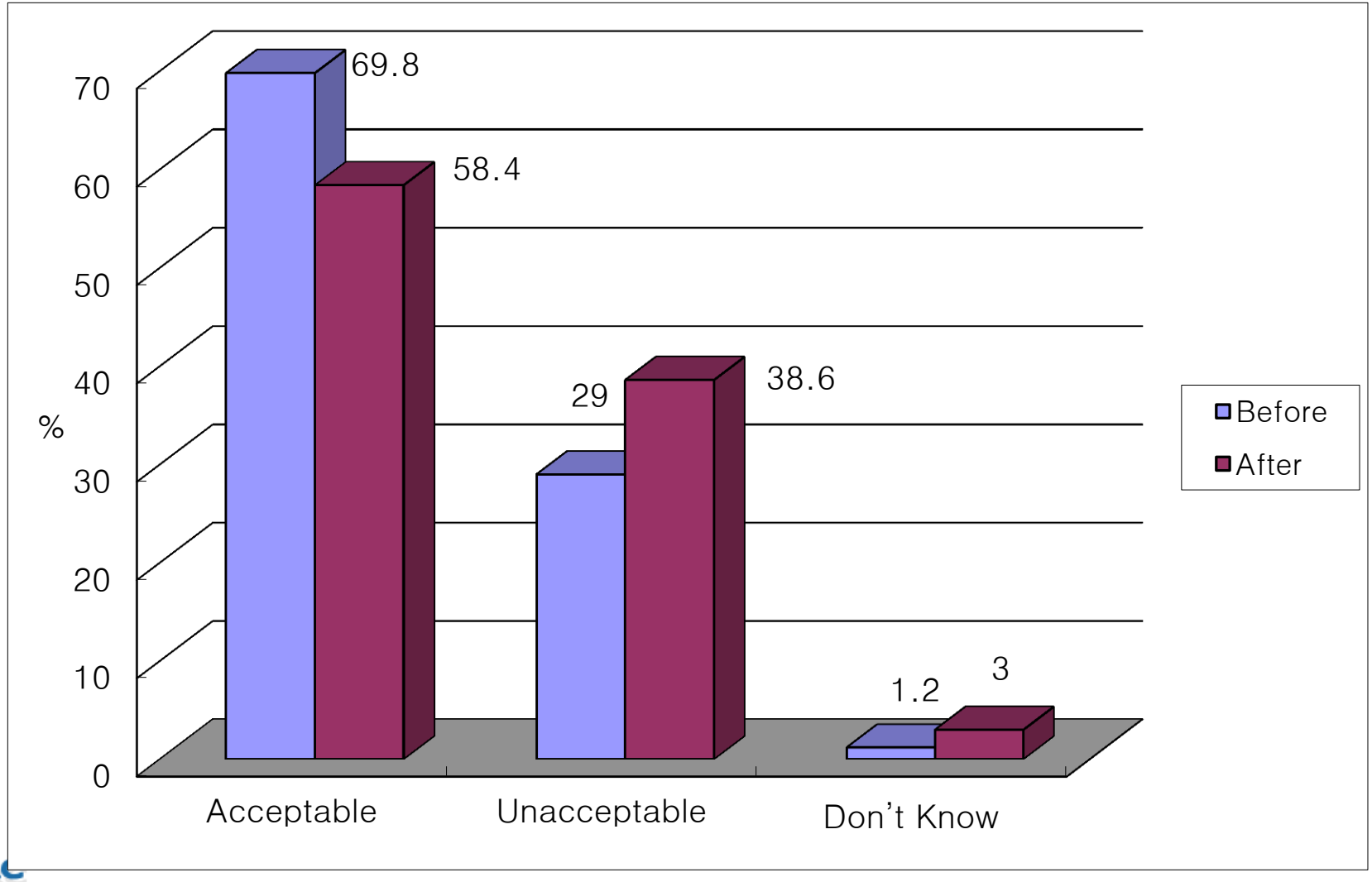
이종이식에 관한 시민합의회의

- 2007년 4월~9월
- 총 14명의 시민패널과 18명의 전문가패널 참여
- 2007 동물장기이식에 관한 시민합의종합보고서 발간

: 이종이식 연구의 필요성은 인정하나 잠재적인 공중보건의 위험성을 대비해야 하며 이종이식 임상시험이 실현되기 전에 관리 체계나 법규가 마련되어야 함

Public Audit with National Assembly for "Xeno-Regulation" (February 2010)

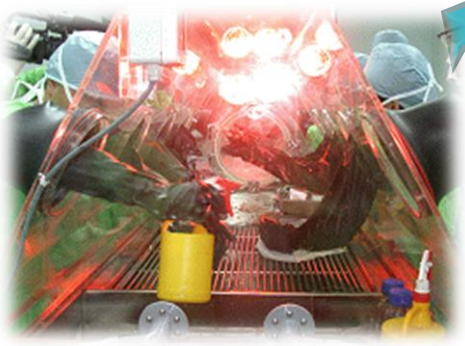
Korean people's attitude to XT before/after informing the related benefit/risk



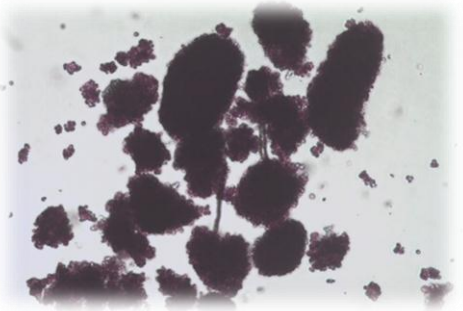
Porcine Islets Xenotransplantation

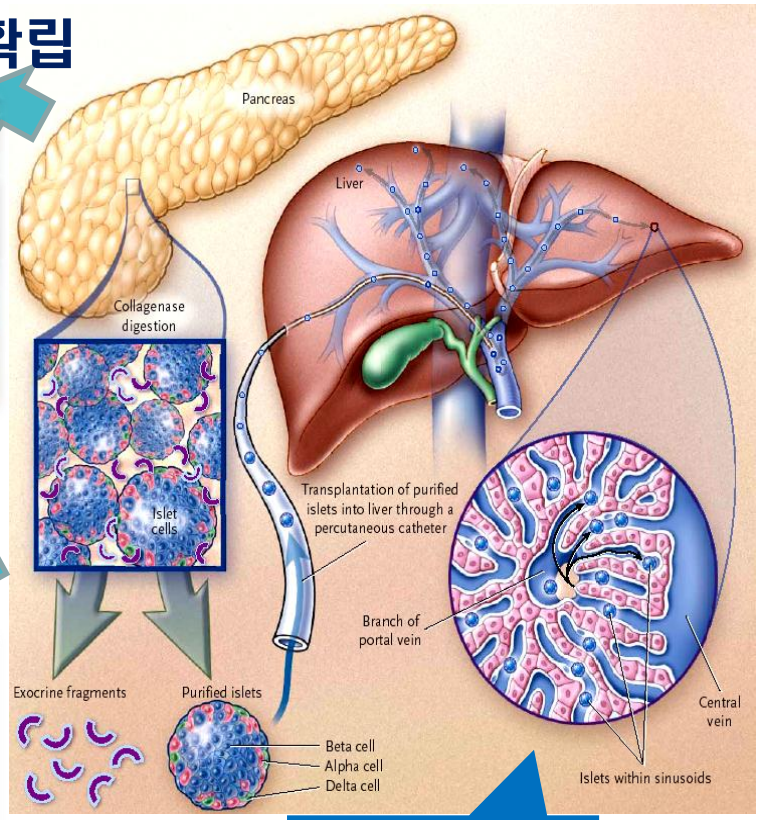
돼지췌도이식기술개발

돼지 췌장 공급원 확립



췌도 분리, 보존, 기능평가





췌도 생착유도

면역거부반응 극복

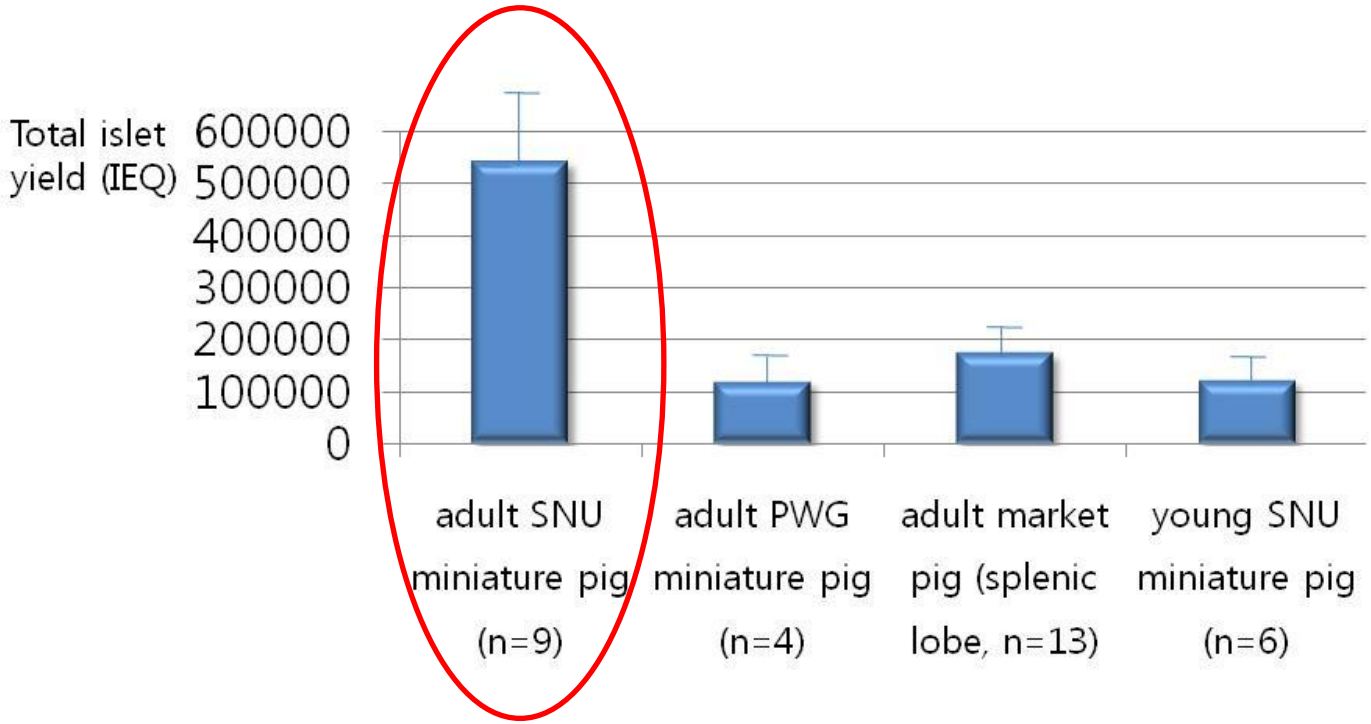
국지적
면역억제요법개발

전신면역억제요법개발
면역관용유도

캡슐화를 이용한
면역회피

Production of Porcine Islets

Kim JH, Kim H-I, Lee K-W, Yu JE, Kim SH, Park HS, Park C-G, Ihm S-H, Ha J, Kim SJ, Lee HK, Ahn C, Park KS. Influence of strain and age differences on the yields of porcine islet isolation: extremely high islet yields from SPF CMS miniature pigs. Xenotransplantation 2007; 14: 60–66.



Parameters for successful pig islet isolation as determined using 68 specific-pathogen-free miniature pigs

Kim H-I, Lee S-Y, Jin SM, Kim KS, Yu JE, Yeom S-C, Yoon TW, Kim JH, Ha J, Park C-G, Kim S-J. Parameters for successful pig islet isolation as determined using 68 specific-pathogen-free miniature pigs.

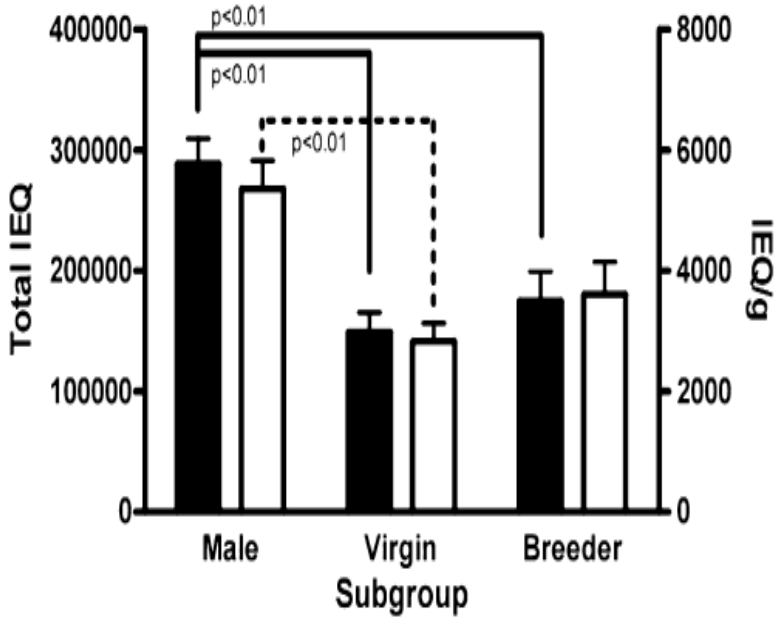


Table 5. Predictors of high islet isolation yield

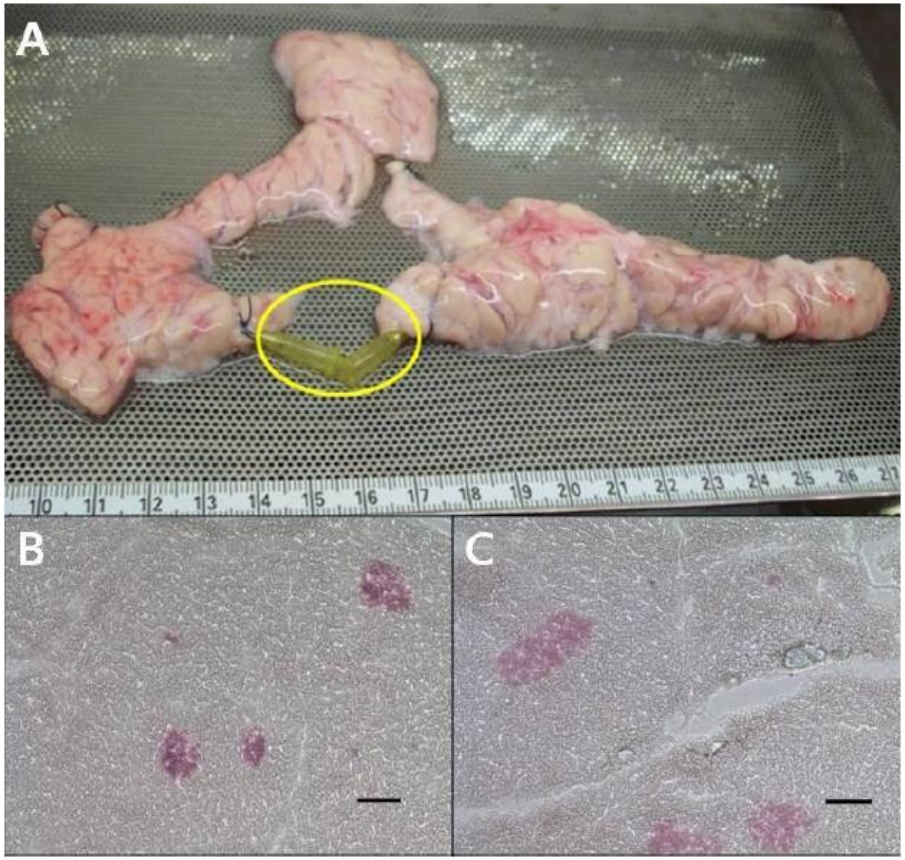
	Odds ratio	95% Confidence interval	P-value
Parameters for higher IEQ/g			
Distension (moderate vs. poor)	40.06	3.21–500.36	0.004**
Distension (good vs. poor)	18.71	1.99–176.17	0.010*
Male	5.35	1.49–19.12	0.010*
Parameters for higher Total IEQ			
Older age group (>2 yr)	25.60	2.22–294.73	0.009**
Distension (moderate vs. poor)	70.15	2.51–1958.62	0.012**
Male	7.55	1.35–42.31	0.022*
Good decapsulation	10.18	0.78–132.79	0.077
Distension (good vs. poor)	14.05	0.73–270.26	0.080

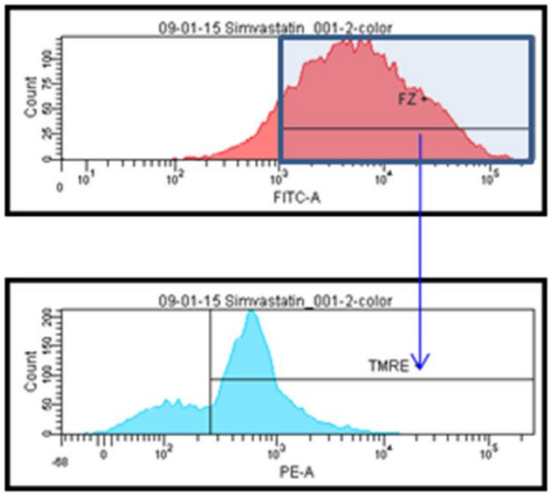
Binary logistic regression analysis; *P < 0.05, **P < 0.01.

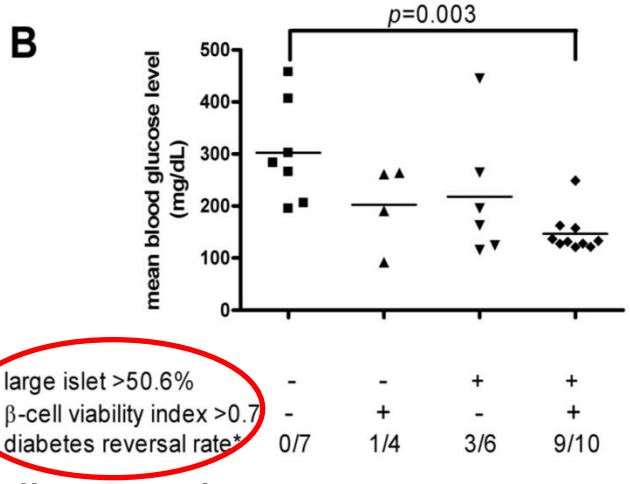
Xenotransplantation 2009; 16(1):11-8

Enhanced prediction of porcine islet yield and posttransplant outcome using a combination of quantitative histomorphometric parameters and flow cytometry

Jin SM, Kim KS, Lee SY, Gong CH, Park SK, Yu JE, Yeom SC, Yoon TW, Ha J, Park CG, Kim SJ.





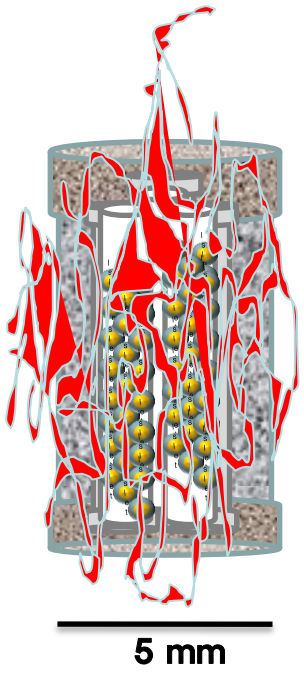


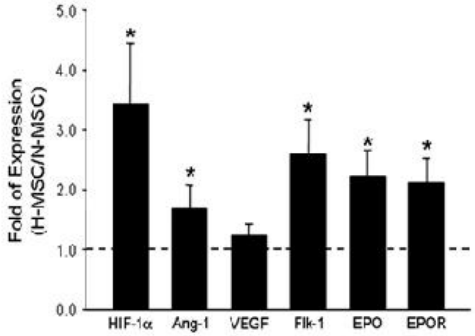
Cell Transplant 2010; 19:299-311

Enhanced Islets Engraftment

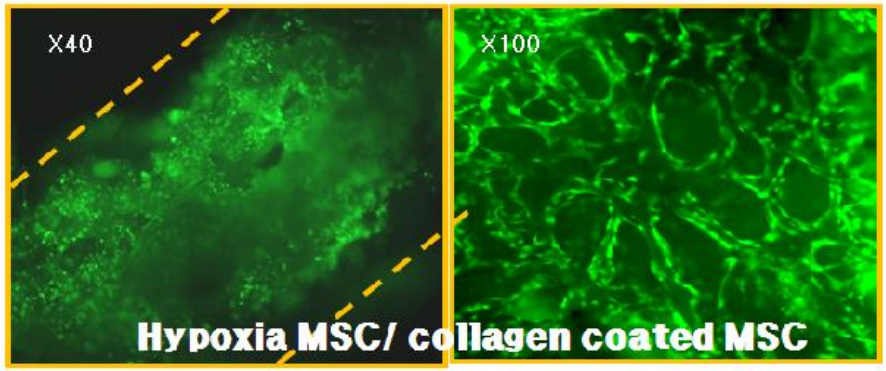
이식된 췌도의 생착유도

- Prevascularized PLGA device: its application to clinical islet transplantation





Hypoxia Preconditoned MSC
(2010. stem cell)



Hypoxia MSC

Normoxia MSC (#2)
3% 0_2 Hypoxic condition culture

7 Days

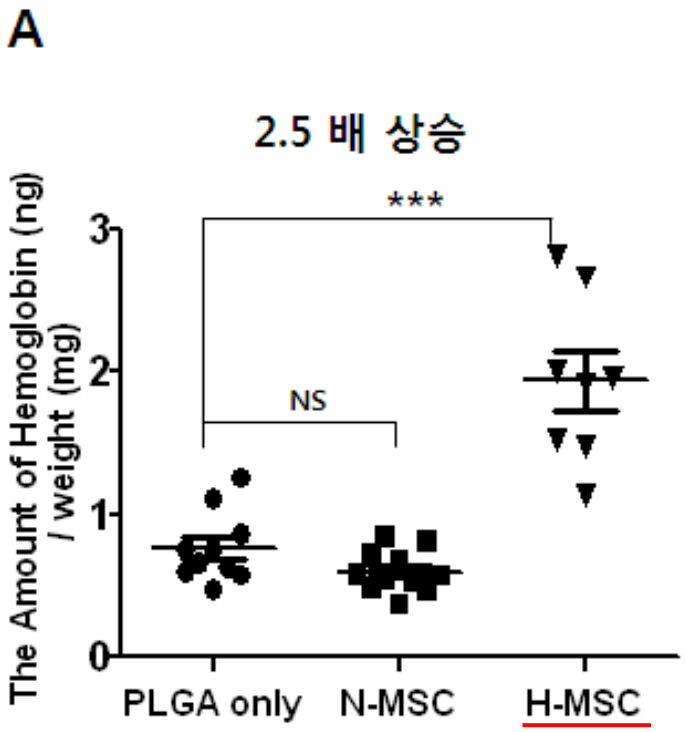
MSC loading/Collagen coated PLGA
3% 0_2 Hypoxic condition culture

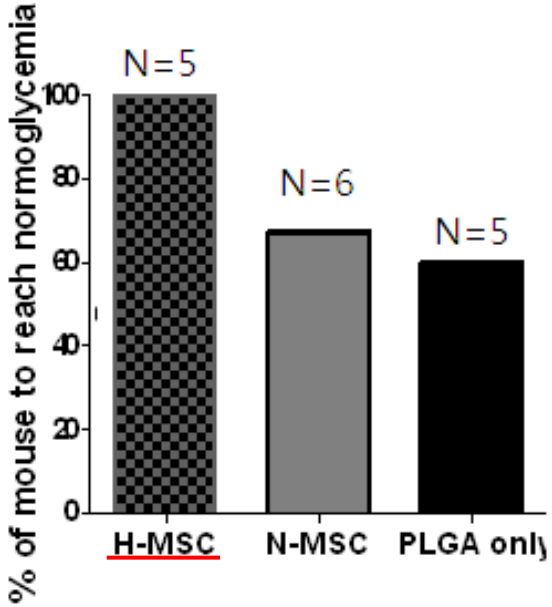
1 Days

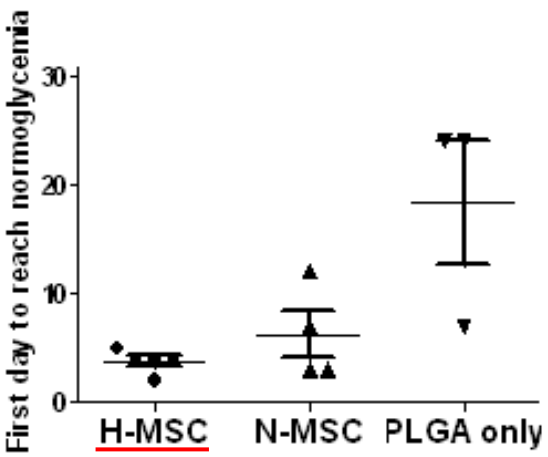
Transplantation

이식된 췌도의 생착유도

- Prevascularized PLGA device: its application to clinical islet transplantation







A : PLGA 이식 후 혈관 분석 (Total Hemoglobin 정량 (90% PLGA 이식후 8주)

B : Porcine islet (5,000IEQ) 이식후 혈당조절 비율 (% of mouse)

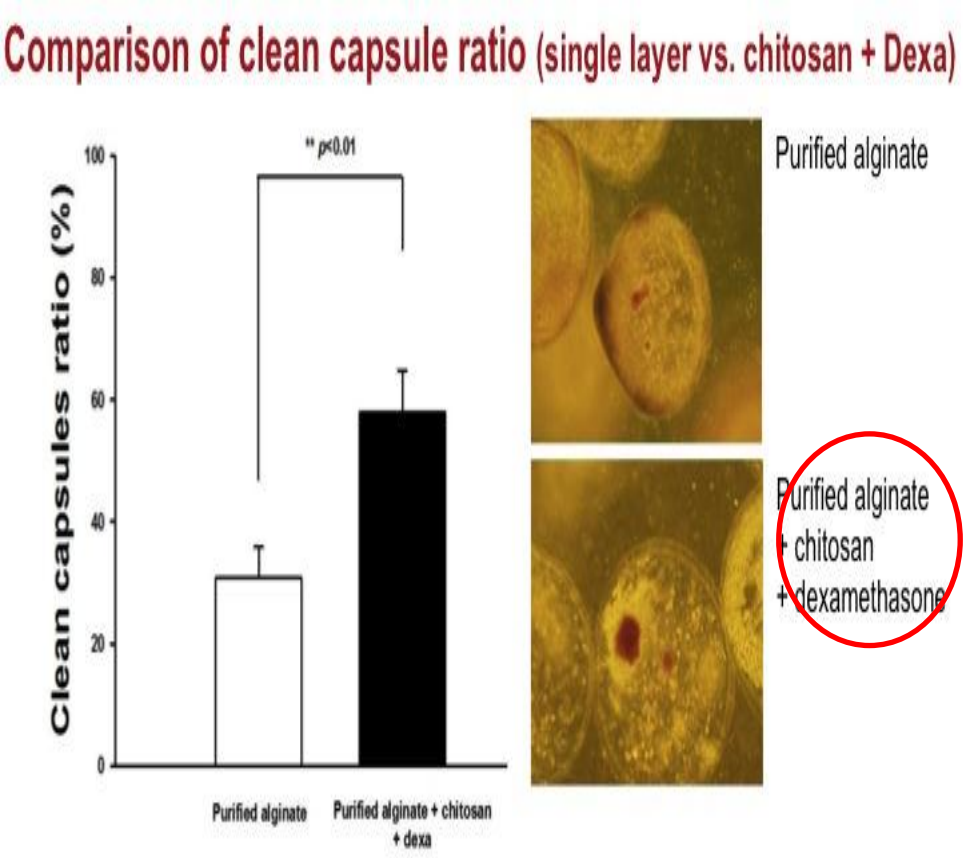
C: Porcine islet 이식 후 최초 혈당조절시간 (day)

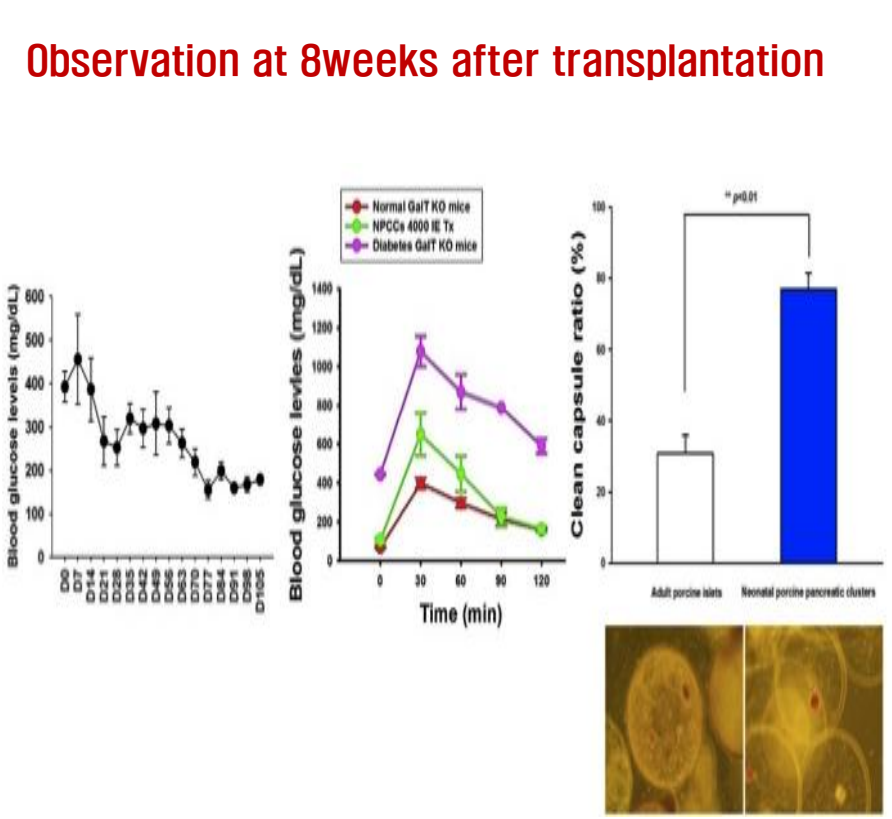
이식된 췌도의 생착유도

- **피하이식 시 생착유도법 확립**

- **임상적용 가능한 PLGA device 이용**
  - **중배엽줄기세포 이용 혈관형성 유도**
  - **돼지→생쥐 췌도이식 모델에서 효능 확인**
  - **임상적용 위해 KFDA 승인 필요**

Encasulated Porcine Islets

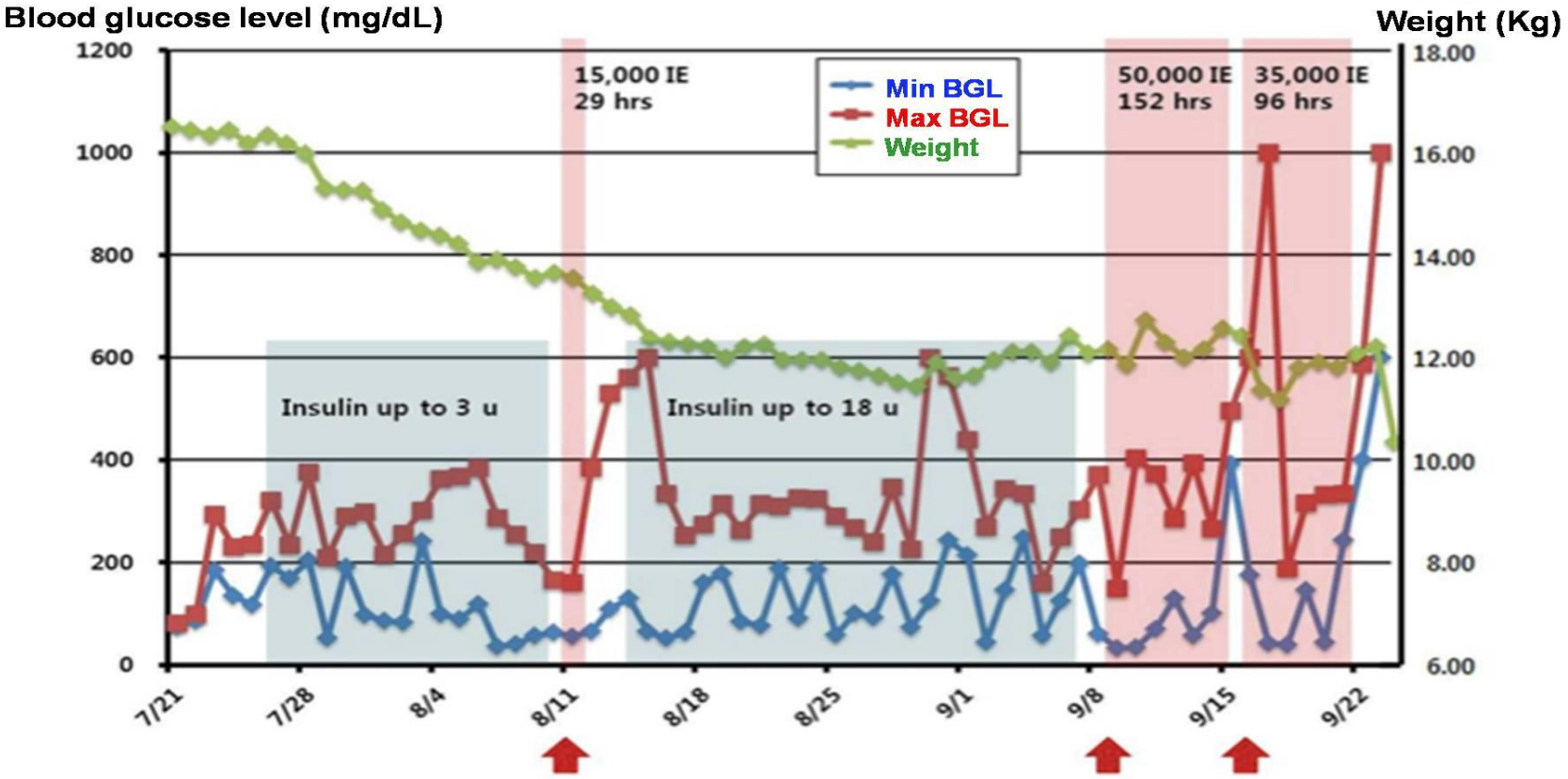




A : 성체돼지 췌도 이식 후 수거한 캡슐의 염증성 세포 침착율은 chitosan+dexamethasone 이중 코팅한 경우 유의하게 감소함을 확인함.

B : 신생돼지 췌장 세포 이식 후 8주까지 혈당 조절능력이 서서히 회복되는 것을 확인함. 또한 염증성 세포 침착율은 성체돼지 세포 캡슐에 비해 유의하게 적음을 확인함.

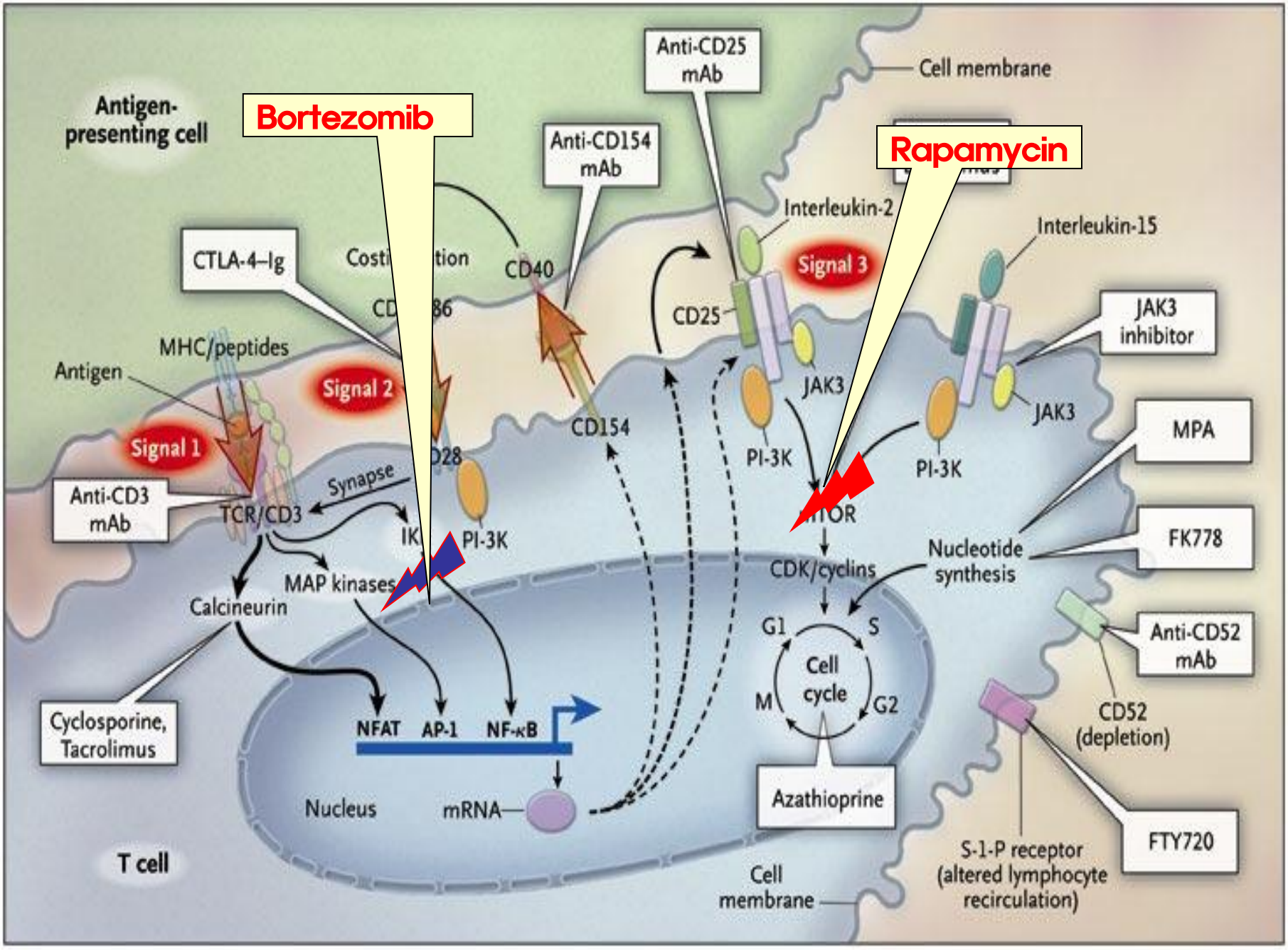
Observation at 8 weeks after transplantation



Chitosan + Dexamethasone 코팅을 추가한 순도 높인 알긴산캡슐(성체돼지 췌도)를 췌장 절제 된 비글개 이식 후 혈당 변화 관찰 결과

: 이식된 췌도 수에 따라 혈당 조절 능력 기간이 증가하는 것을 관찰함. 수거된 캡슐 중 clean capsule 비율(84.38%)이 높음을 확인.

Development of Immunosuppressive Regimen


Antigen-presenting cell
Bortezomib
Anti-CD25 mAb
Cell membrane
Anti-CD154 mAb
Rapamycin
Interleukin-2
Interleukin-15
CTLA-4–Ig
Costi tion
CD40
Signal 3
CD25
JAK3 inhibitor
MHC/peptides
Antigen
Signal 2
JAK3
JAK3
CD154
MPA
Signal 1
PI-3K
PI-3K
Synapse
Anti-CD3 mAb
TCR/CD3
PI-3K
FK778
MAP kinases
CDK/cyclins
Nucleotide synthesis
Calcineurin
G1
S
Anti-CD52 mAb
Cell cycle
M
G2
Cyclosporine, Tacrolimus
NFAT
AP-1
NF-κB
CD52 (depletion)
Azathioprine
Nucleus
mRNA
FTY720
T cell
S-1-P receptor (altered lymphocyte recirculation)
Cell membrane

Bortezomib Can Suppress Activation of Rapamycin-Resistant Memory T Cells Without Affecting Regulatory T-Cell Viability in Non-Human Primates

Jung-Sik Kim,[1,2,3,4] Jae-Il Lee,[2] Jin-Young Shin,[1,2,3,4] Su-Young Kim,[1,2,3,4] Jun-Seop Shin,[1,2,3,4] Jong-Hyung Lim,[1,2,3,4] Hyoung-Soo Cho,[1,2,3,4] Il-Hee Yoon,[1,2,3,4] Ki-Hyun Kim,[6] Sang-Joon Kim,[2,5] and Chung-Gyu Park[1,2,3,4,7]

Background. Memory T cells specific for donor antigens are currently recognized as a significant barrier for maintaining a successful transplant. Furthermore, it has been shown that commonly used immunosuppressive drugs do not alleviate this memory response. Here, we report that rapamycin allows significant proliferation of memory T cells and bortezomib can abrogate the proliferation of rapamycin-resistant memory T cells when preserving the survival of regulatory T cells.
Methods. Peripheral blood mononuclear cells freshly isolated from non-human primates were stimulated with anti-CD3/CD28 antibodies, and inhibitory and apoptotic effects of rapamycin and bortezomib on memory T-cell proliferation were investigated. The CD95 marker in $CD3^+$ T cells was used for the separate enrichment of memory T cells and naïve T cells.
Results. Rapamycin at the level even higher than therapeutic concentration could not suppress the proliferation of a significant proportion of memory T cells. However, the combined administration of bortezomib and rapamycin abrogated the proliferation of rapamycin-resistant memory T cells. Furthermore, bortezomib preserved the survival of preexisting $CD4^+FoxP3^+$ regulatory T cells, while inducing apoptosis of $CD4^+FoxP3^-$ conventional T cells. The combined administration of low doses of rapamycin and bortezomib also exerted an additive effect on suppressing T-cell proliferation. Cytokine analysis demonstrated that bortezomib could not only suppress rapamycin-permissive interleukin (IL)-6 production, but also production of interferon (IFN)-γ, IL-4, and IL-10.
Conclusions. This article provides in vitro data from which immunosuppressive regimens for the effective control of memory T cells in non-human preclinical experiments and in clinical trials are selected.

Keywords: Rapamycin, Bortezomib, Memory T cell, Proteasomal inhibitor, Regulatory T cell.

(*Transplantation* 2009;88: 1349–1359)

Porcine Islets Xenotransplantation to Non-human Primate

Strategic Plan

영장류 전임상시험

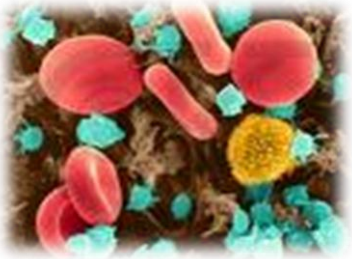
윤리
안전성

실험적
췌도이식

임상시험을 위한 준비

췌도이식
임상시험

영장류에서
면역 반응 조절



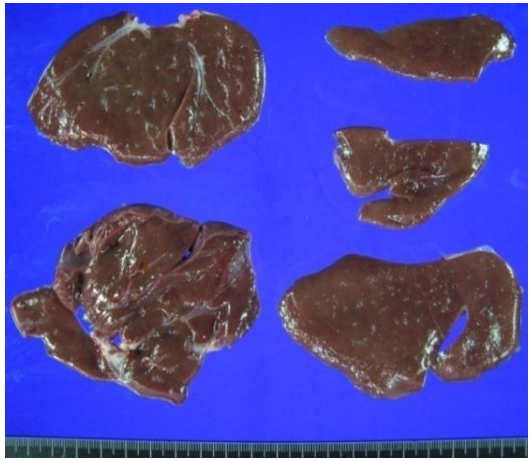
임상시험을 위한
이식 대상자 파악

췌도 이식의 최적 장소 연구와 이식 기술 확립

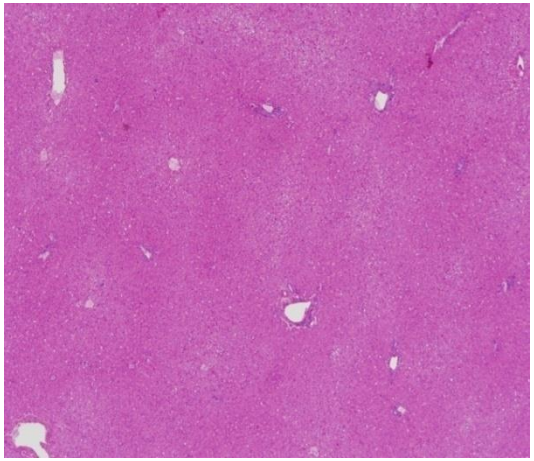
영장류의 간내 이식법 확립

- 전신 마취 후 개복하여 공장 정맥을 노출하고 24G 카테터를 삽입 후 M199 20ml에 부유시킨 췌도를 heparin(70 u/kg)과 함께 중력을 이용해서 5분 이상에 걸쳐서 주입하고 생리 식염수 5ml를 더 주입함

(A)



(B)



(C)

(그림 A) 원숭이 공장 정맥에 돼지 췌도를 이식하는 과정

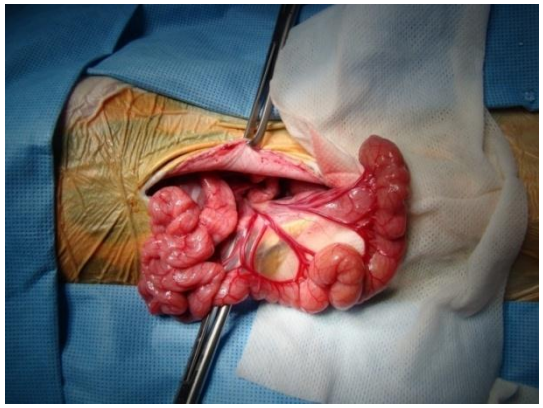
(그림 B, C) 간내 이식 2달 후 간 생검 사진(B)과 조직 슬라이드 사진(C)

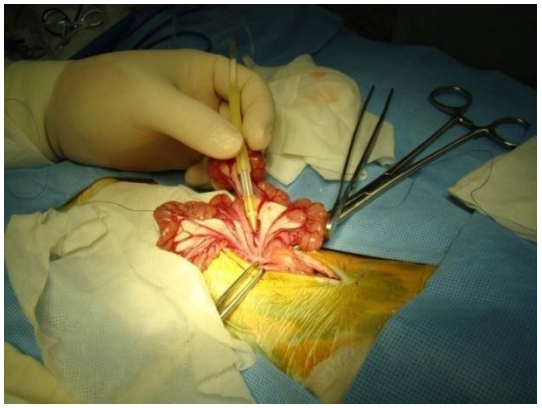
Primate Experiments

Recipient / Age (y)	Donor	Sex / Wt. (kg)	TPL site	IEQ/kg	Immunosuppressive regimen
R001 (4.5)	SNU pig	F / 4.42	Kidney	22,600	None
R002 (7.1)	SNU pig SNU pig	F / 4.64	Muscle Liver	51,000 43,000	ATG, Rituximab, Rapa, MMF None
R003 (6.2)	SNU pig Farm pig	F / 6.06	Peritoneal cavity Liver	54,000 30,000	None (alginate coated islets) Bortezomib, Rapa, CVF
R013 (6.5)	SNU pig	F / 4.12	Liver	48,500	rATG, Rapa, Lef, MMF, Bortezomib
R004 (3.5)	SNU pig Farm pig	F / 4.40	Liver Omentum	47,700 24,324	Basiliximab, Rituximab, Tac, Lef, MMF None (Islet + MSC)
R006 (8.2)	SNU pig Farm pig	F / 5.10	Liver Omentum	21,800 25,380	Campath-1H, Tac, Lef, MMF None (Islet only)
R006	SNU pig	F / 4.95	SC	38,000	Campath-1H, Tac, Lef, MMF, Bortezomib
R014 (7.1)	SNU pig Farm pig	F / 4.86	Liver Omentum	24,000 30,232	rATG, Lef, MMF, Bortezomib Lef, MMF (Islet + fibrin)
R012 (3.2)	Farm pig	F / 3.80	Omentum	35,294	None (Islet + fibrin + MSC)

췌도 이식의 최적 장소 연구와 이식 기술 확립

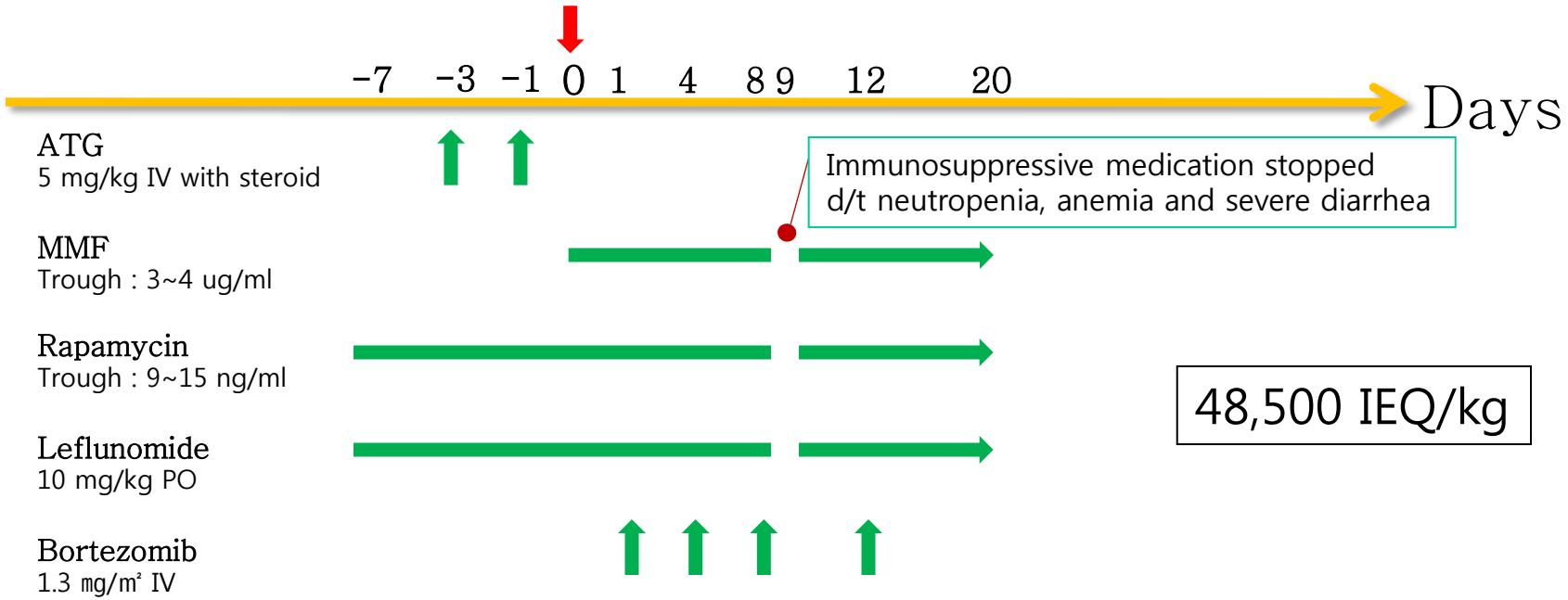
간내 이식



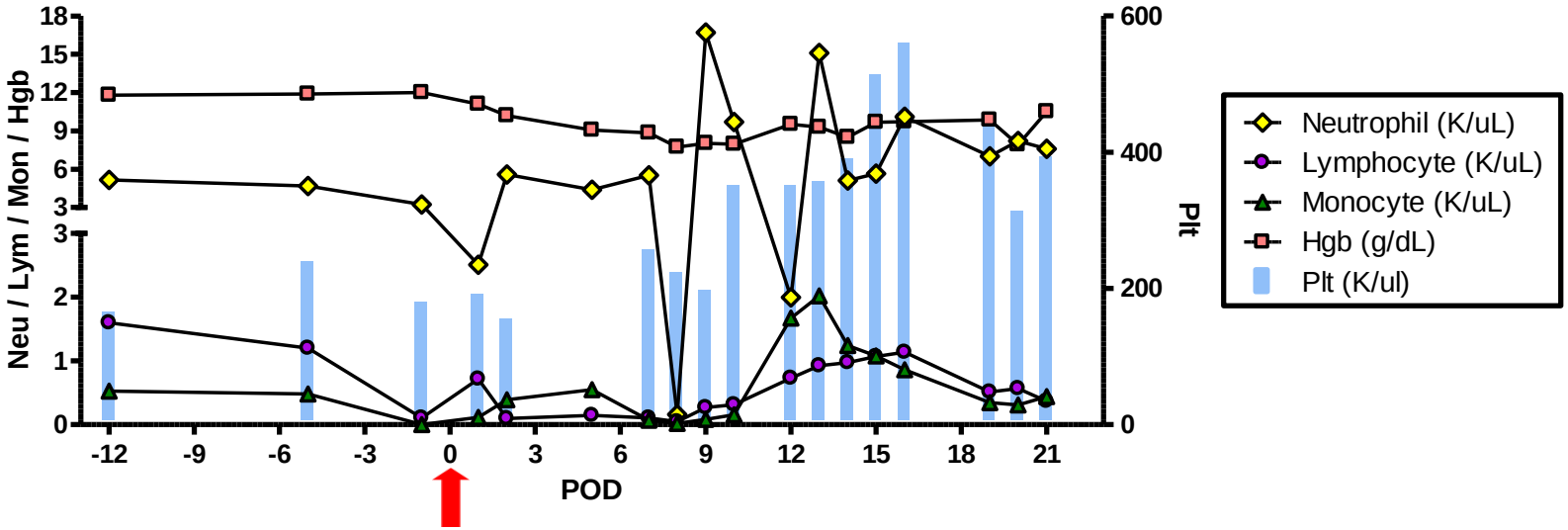


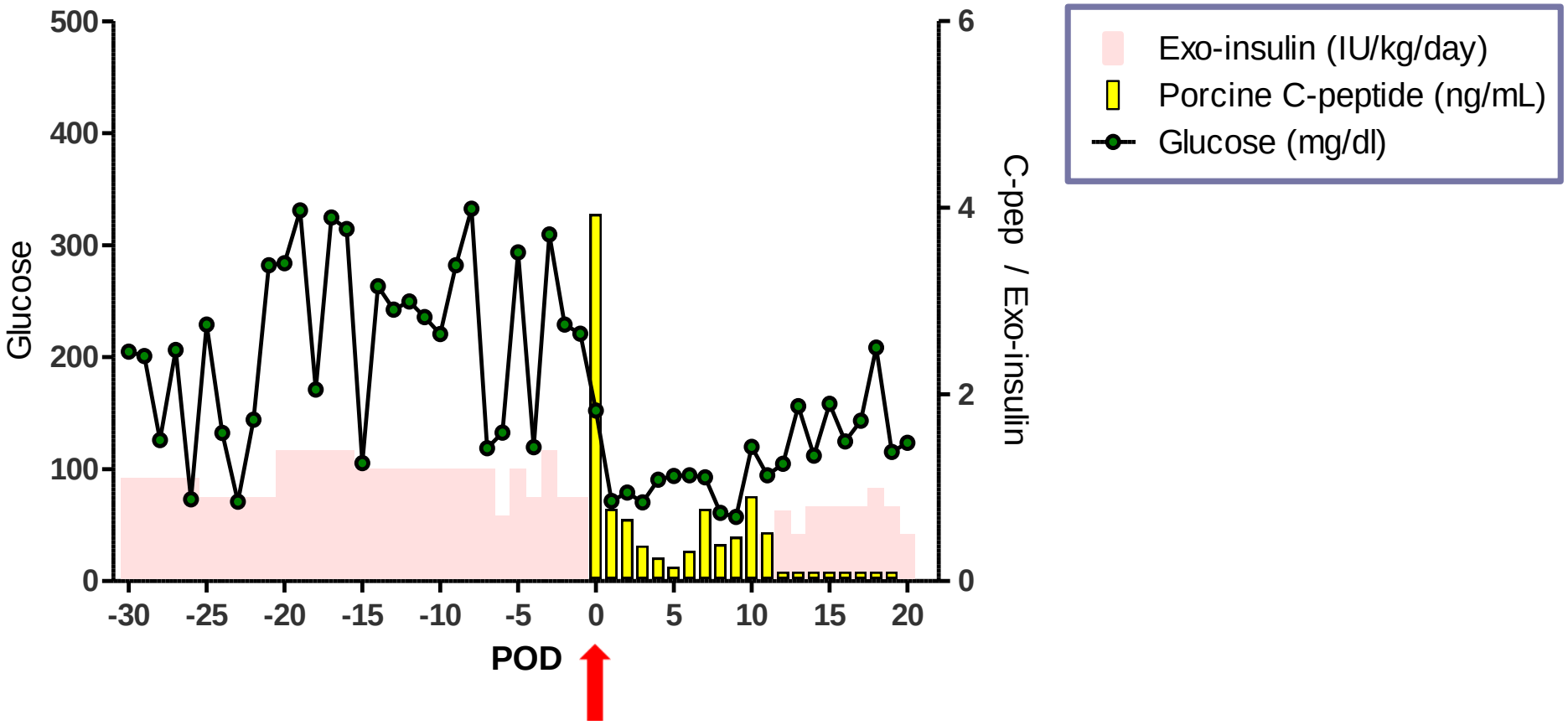
Date	Feb. 04, 2009
Donor	Miniature pig 43 months / female / 97Kg
Recipient	Rhesus monkey, 6.5 years / female / 4.12Kg
Infused islet (IEQ)	200,000 (43,000 IEq/Kg) Naked islets
Immunosuppressant	ATG, RAPA, LFM, MMF, BTZM
Graft survival of islets	12 day

Immunosuppressant protocol (R013)



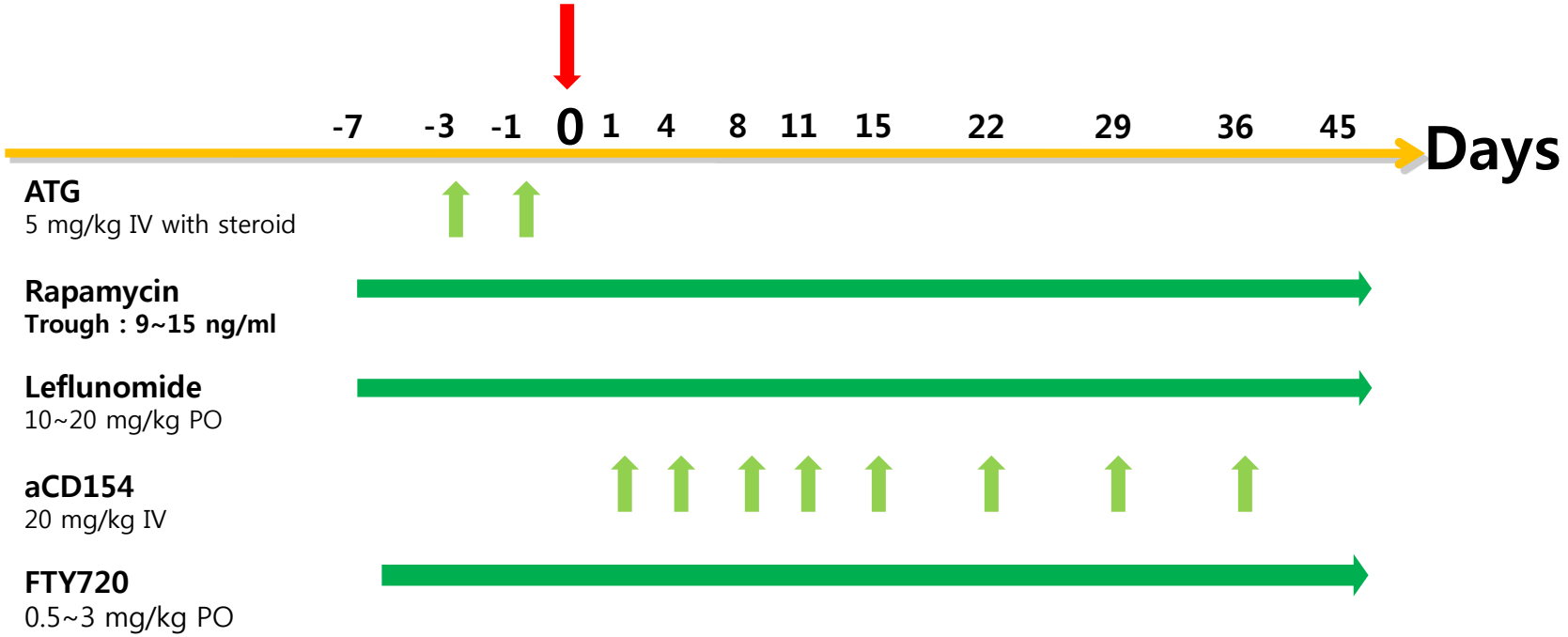
48,500 IEQ/kg

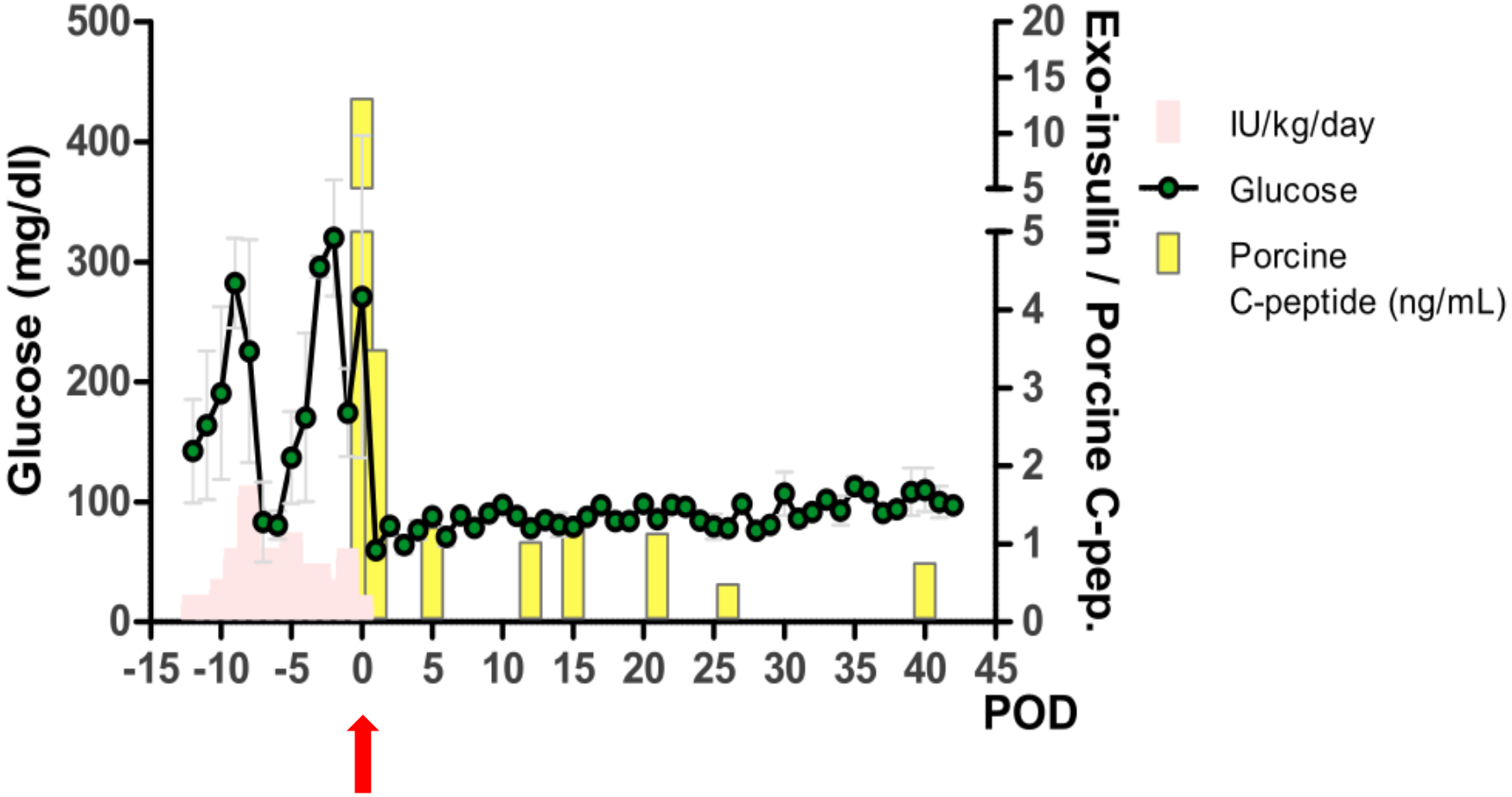



Exo-insulin (IU/kg/day)
Porcine C-peptide (ng/mL)
Glucose (mg/dl)
Glucose
C-pep / Exo-insulin
500
400
300
200
100
0
6
4
2
0
-30
-25
-20
-15
-10
-5
0
5
10
15
20
POD

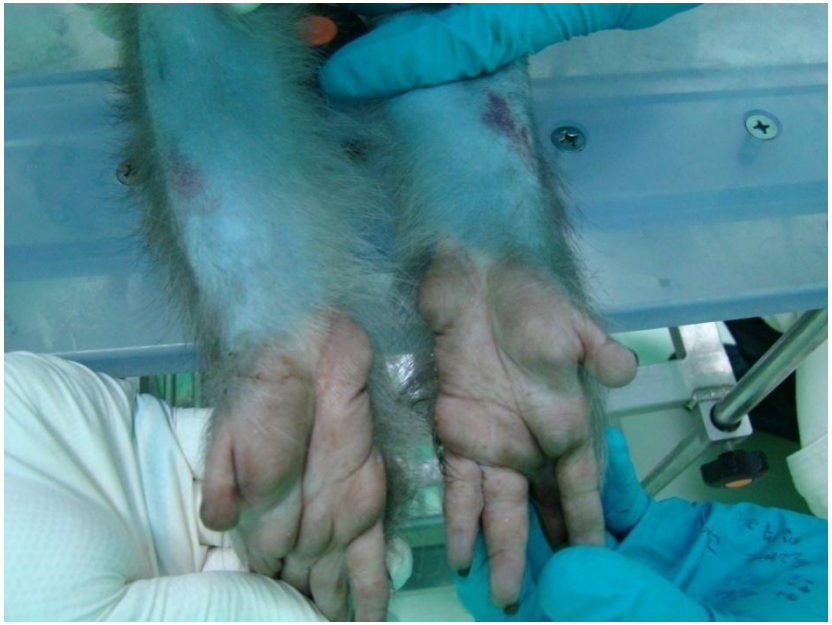
Pig to NHP islet TPL

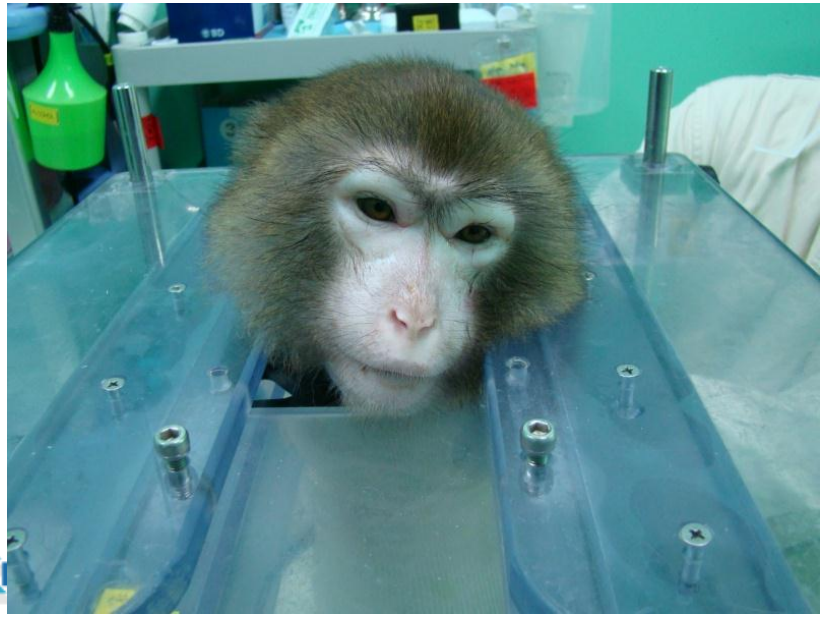
Recipient	Sex / Wt (kg)	TPL site	IEQ/kg	Immunosuppressive regimen
R041 (Diabetes induced by STZ)	F / 5.5	Liver	46,300	rATG, Rapamycin Leflunomide FTY720 antiCD154Ab

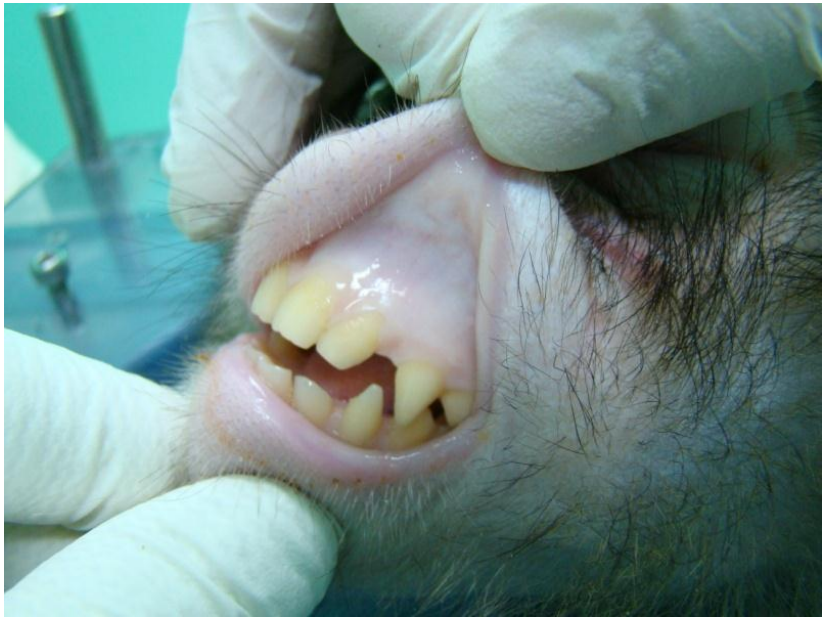



Glucose (mg/dl)
500
400
300
200
100
0
-15 -10 -5 0 5 10 15 20 25 30 35 40 45
POD
Exo-insulin / Porcine C-pep.
20
15
10
5
5
4
3
2
1
0
IU/kg/day
Glucose
Porcine
C-peptide (ng/mL)

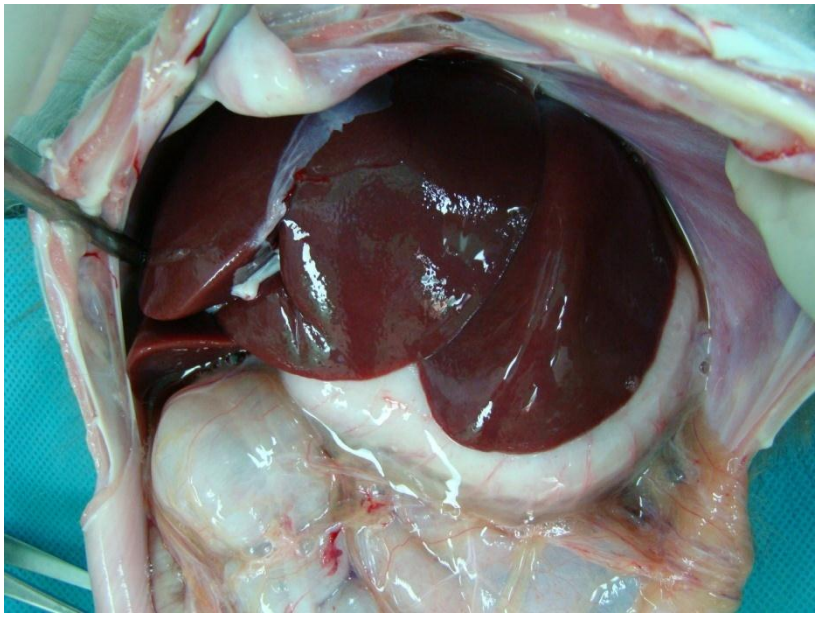
Appearance (D 40, 42)

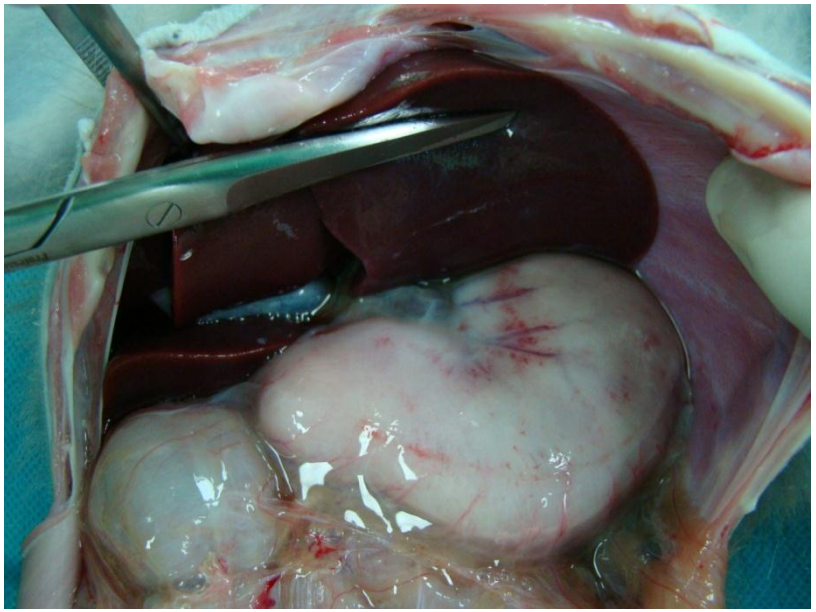


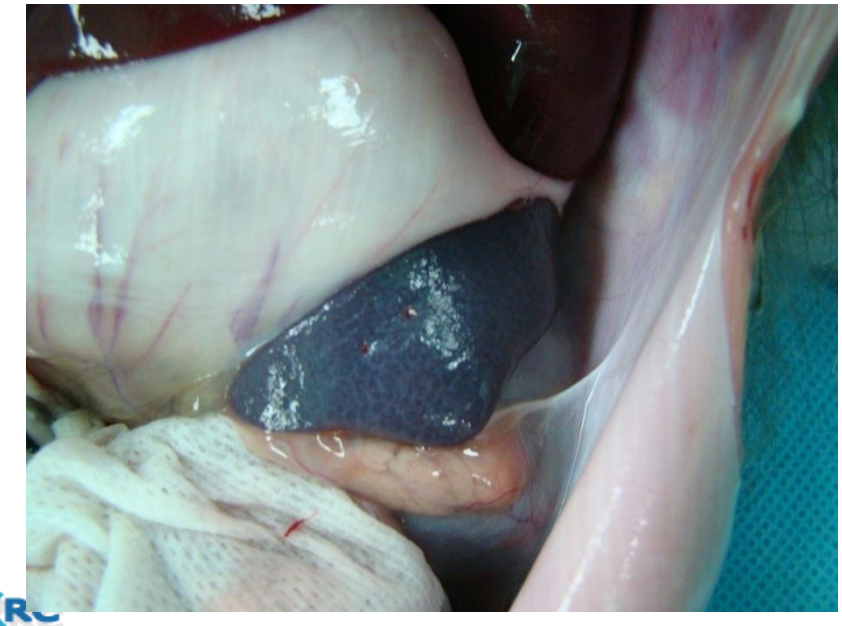


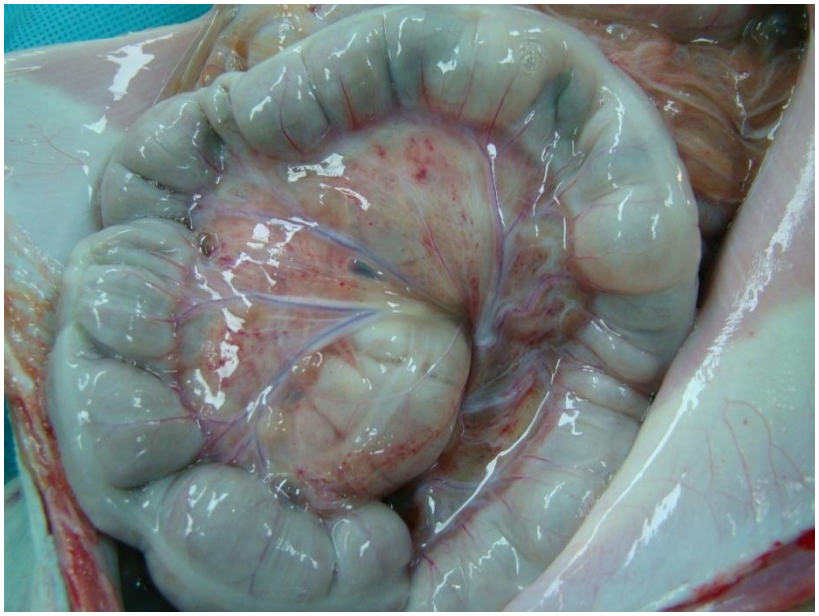


Autopsy findings : Abdomen

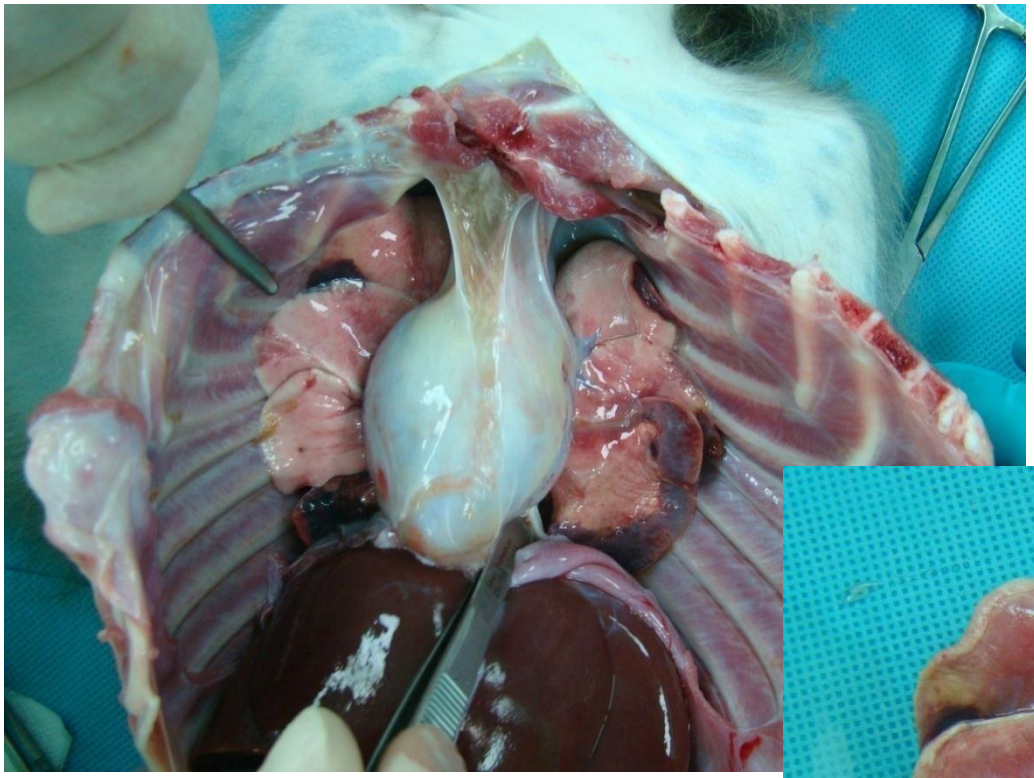


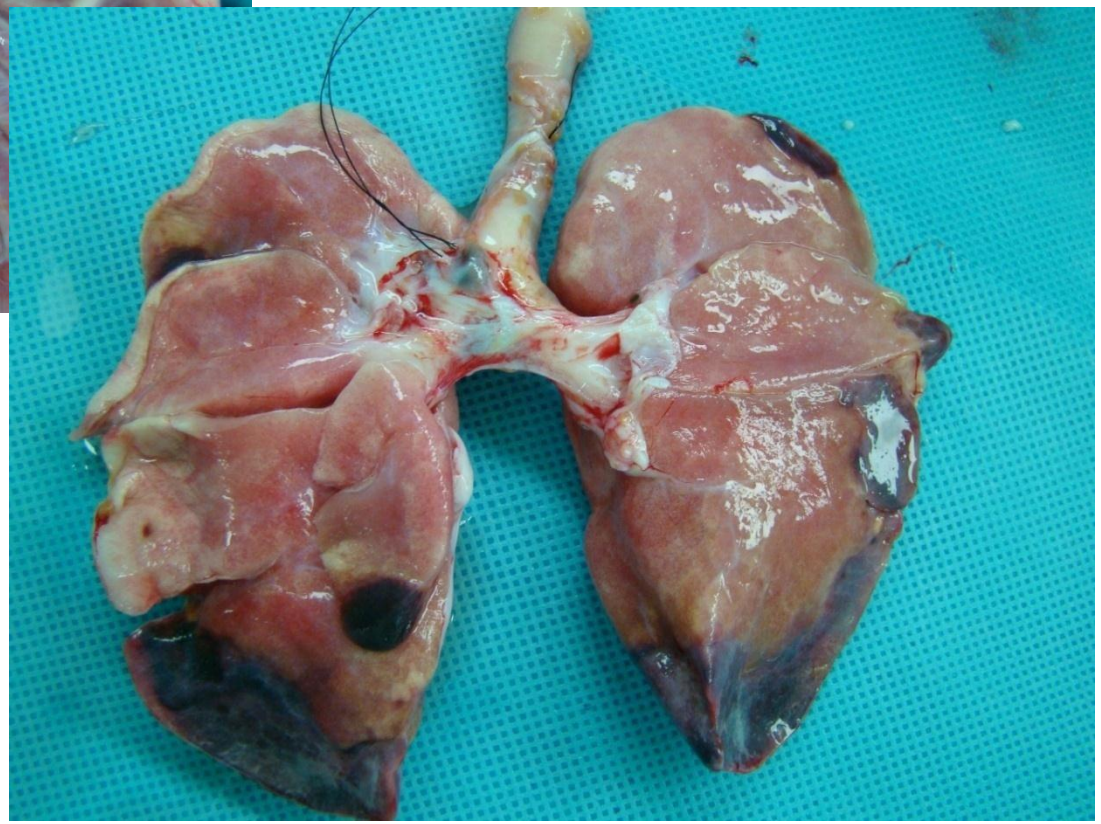




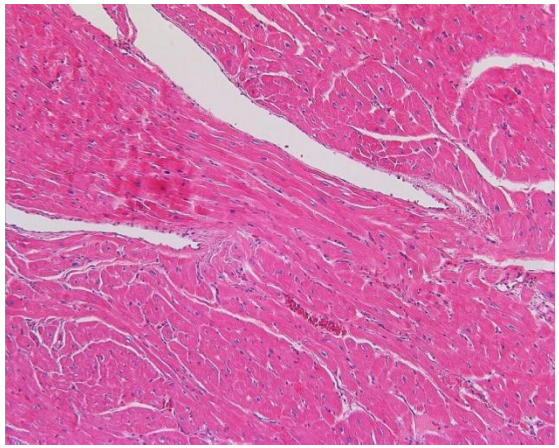


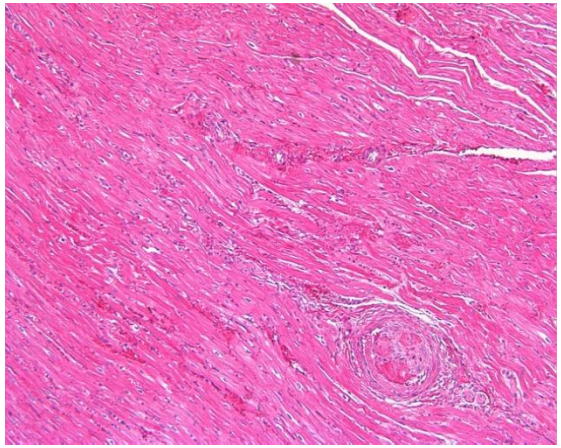
Lung



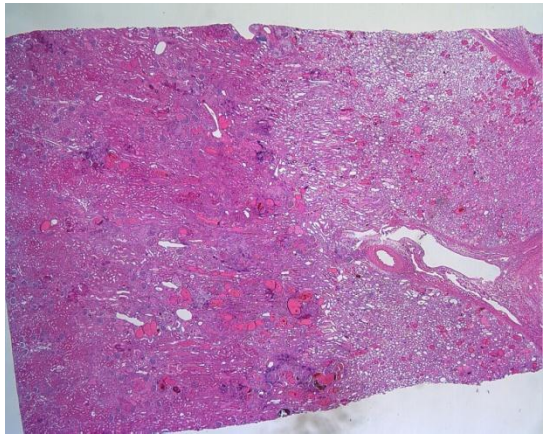


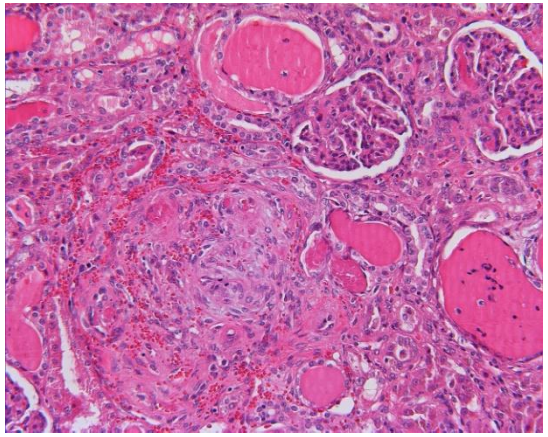
Heart



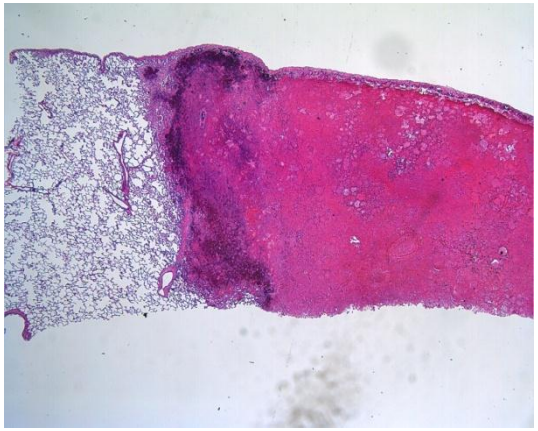


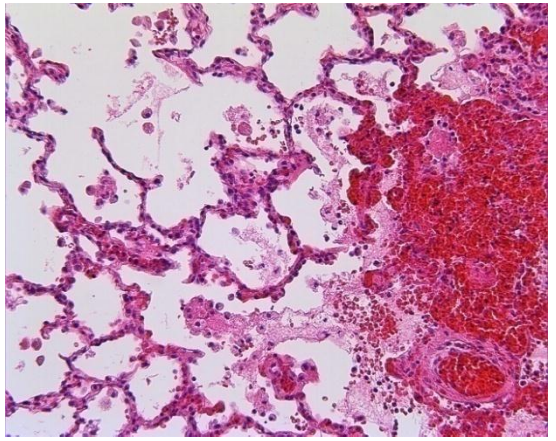
Kidney

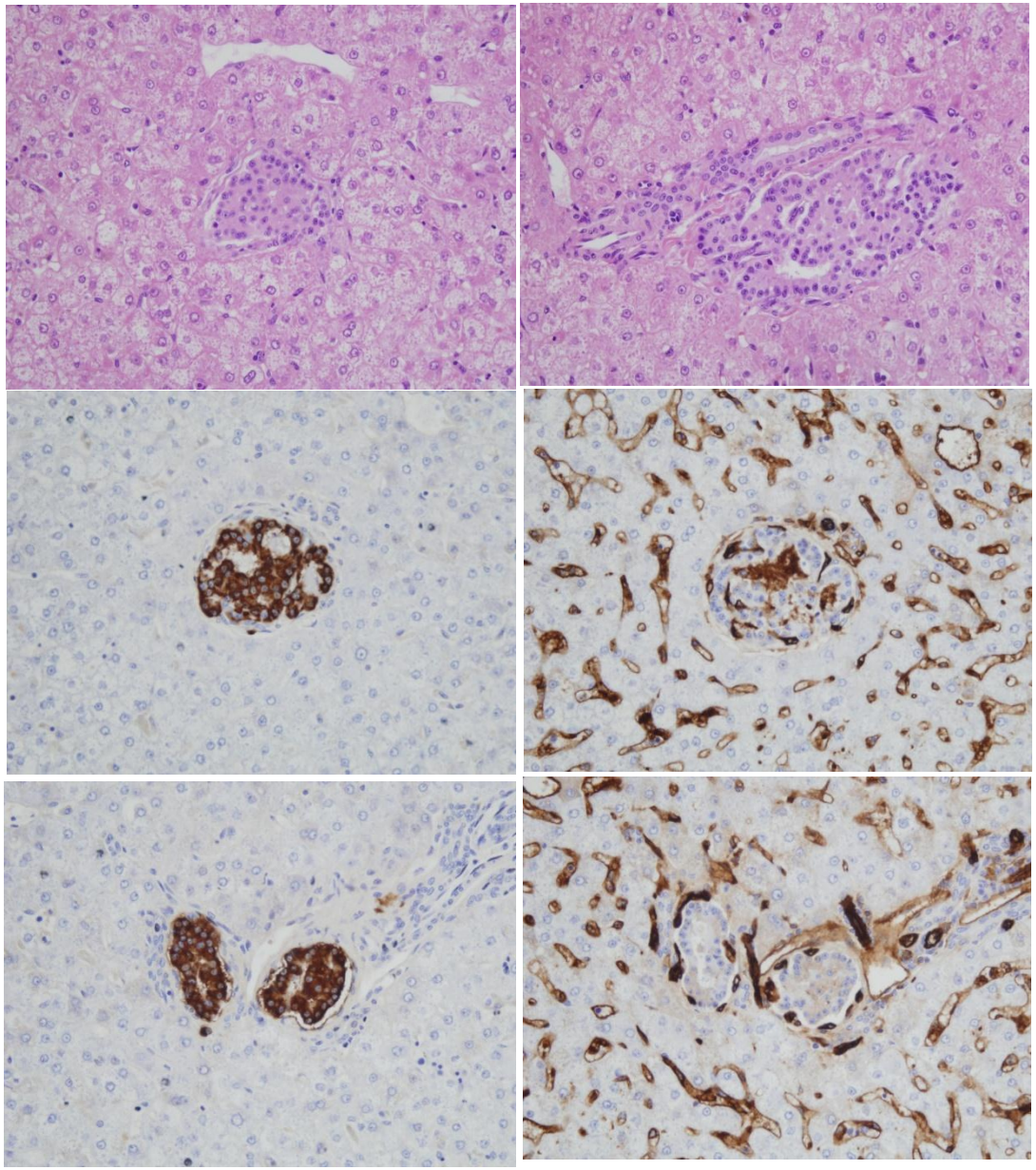




Lung







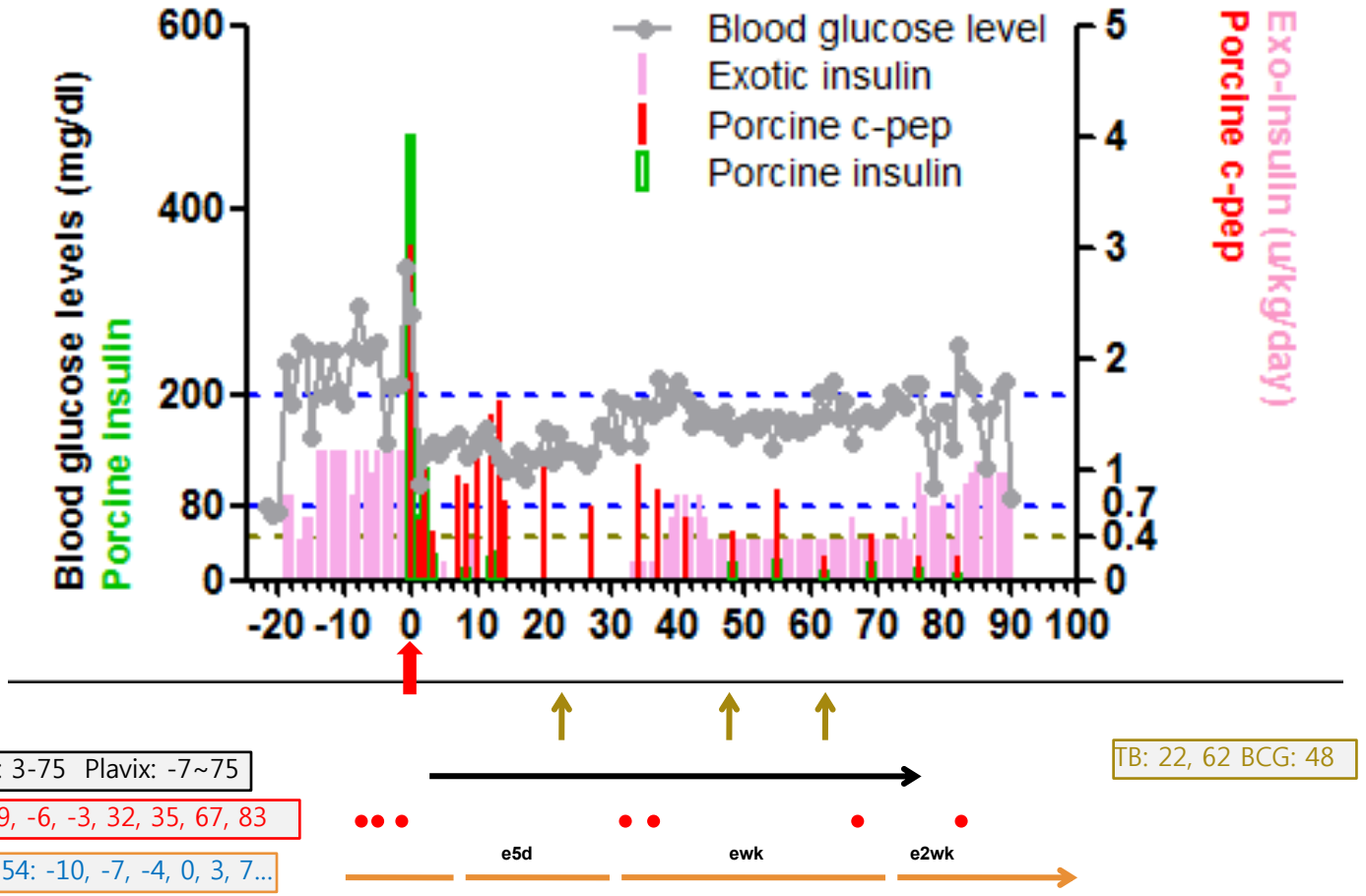
Insulin staining CD31 staining

이식된 돼지 췌도 간 조직에 생착

- Species : Rhesus macaque
- Origin: China
- Imported D: 2009. 05. 06
- Sex: Female
- BOD: 2006. 03. 08 (4y 3m)
- BW: 5.5 kg

Glucose monitoring

Pre-PIT	231 mg/dl, 5.1 u
Post-PIT	165 mg/dl, 1.5 u
Today	88 mg/dl, 5 u



The International Xenotransplantation Association Consensus Statement

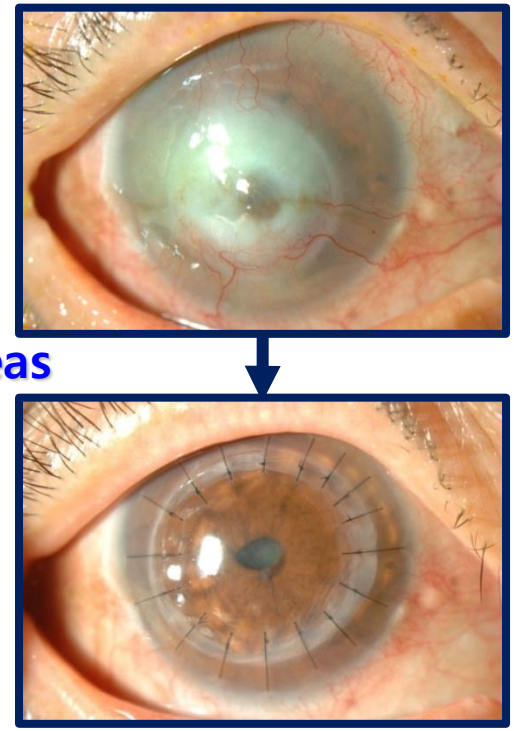
on Conditions for Undertaking Clinical Trials of Porcine Islet Products in Type 1 Diabetes

July 27, 2009

Development of Porcine Corneal Xenograft for Biomedical Application

Introduction

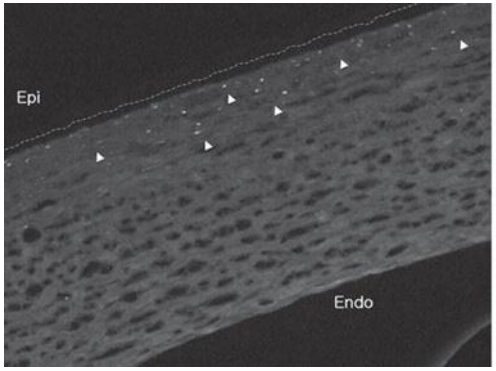
- Shortage of donor cornea
  - Eye donation is rare in Asian culture
  - **Emerging as a worldwide problem**
    - ⬆ *aging population*
    - ⬆ *refractive and cataract surgery*
    - ⬆ *infectious disease like HIV, CJD, hepatitis, etc.*
  - ➢ **Require other sources to substitute human corneas**

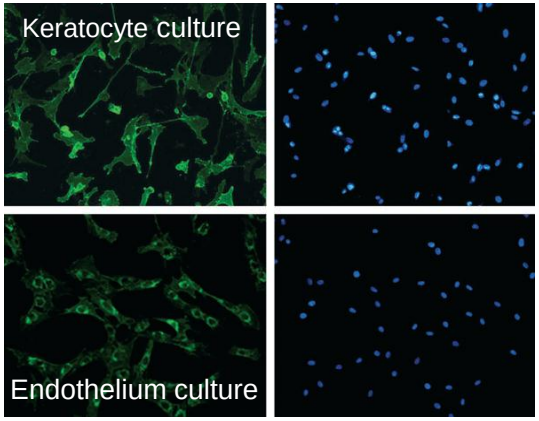


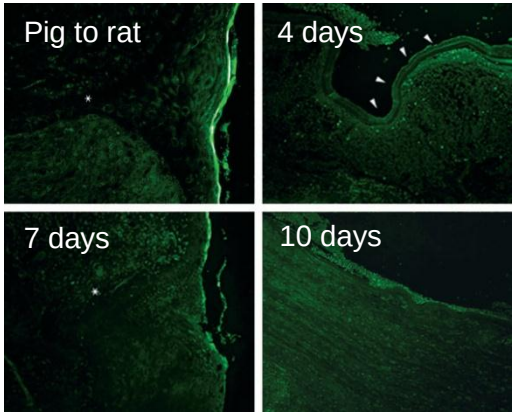
- Pig as the most suitable xenograft donor
  - Similar physical and optical properties to the human cornea
  - Genetically manipulated to improve suitability
    - α1,3-galactosyltransferase knockout (GTKO) pigs
    - Complement regulatory protein transgenic pigs

Alpha-Gal in xenocorneal transplantation

- Alpha-Gal expression in porcine cornea
  - **Mostly confined the anterior stromal keratocytes**
  - **↑ during in vitro culture or after xenotransplantation**

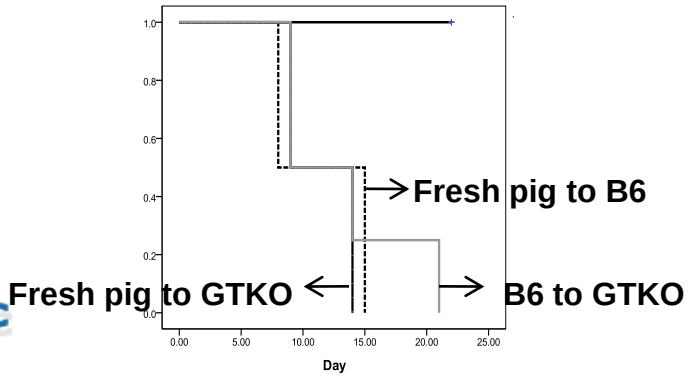


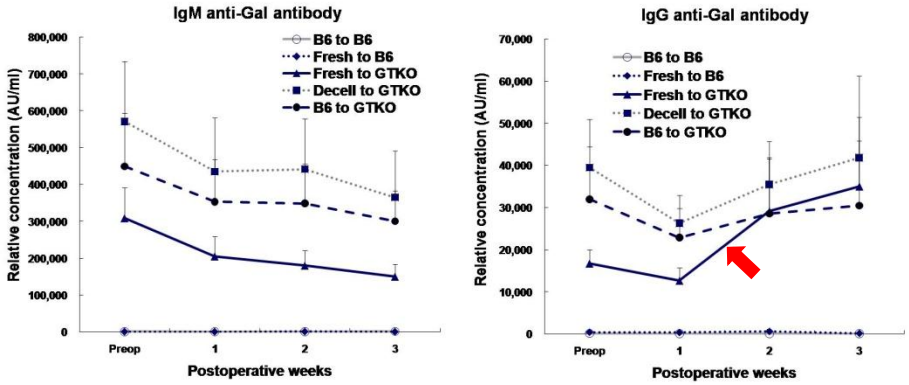




Lee HI, et al. Xenotransplantation 2007;14:612-8.

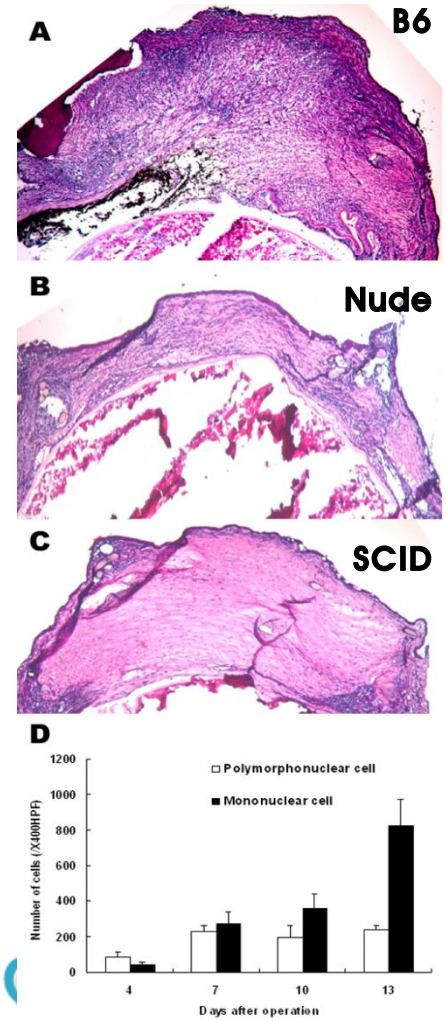
- Effect of alpha-Gal on pig-to-mouse corneal transplantation
  - **No hyperacute rejection**
  - **↑ IgG anti-Gal antibody in GTKO mice recipients**

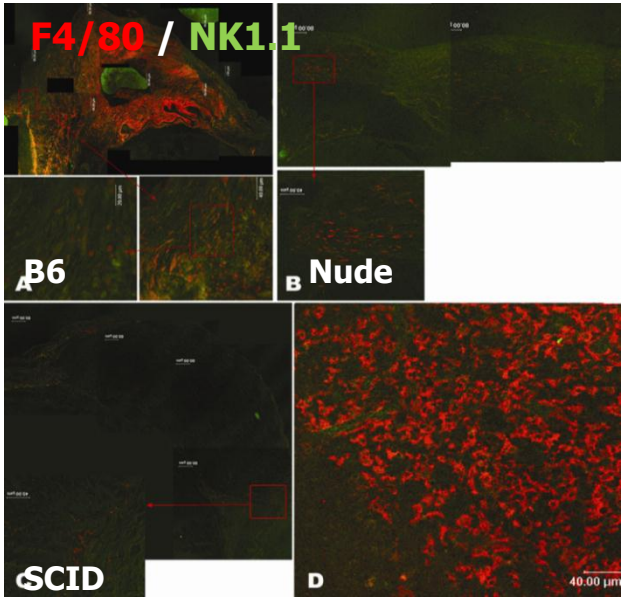


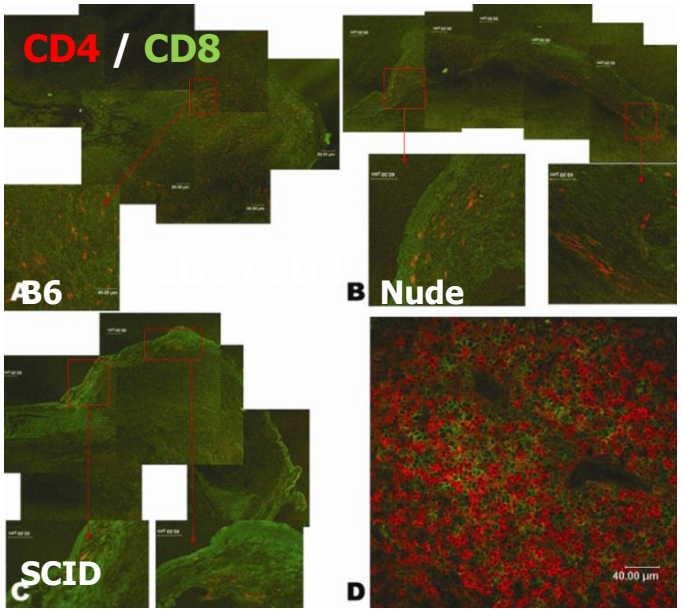


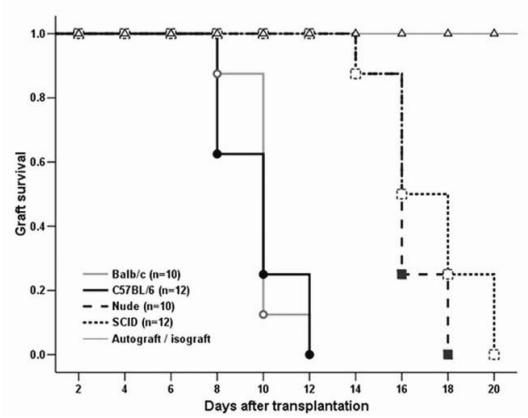
Mechanism of xenocorneal graft rejection

- Pig-to-mouse full thickness corneal transplantation
  - **Importance of CD4 T cell, Macrophage, and Neutrophil**









Median survival time

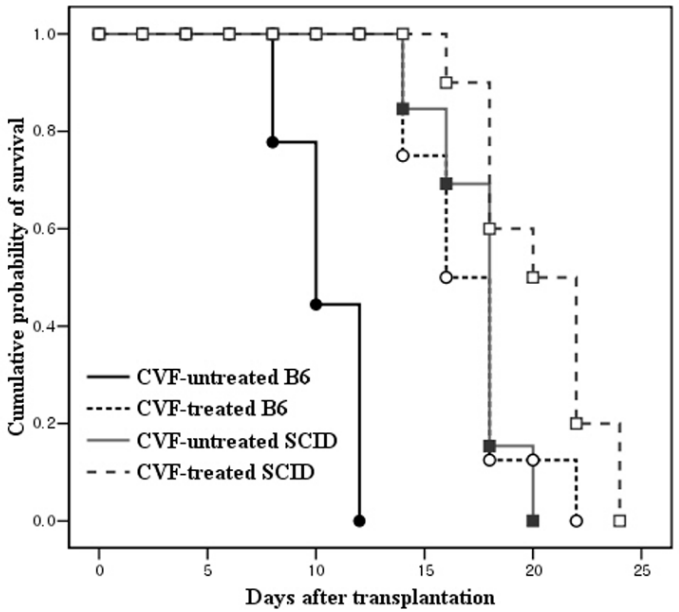
- Balb/C : 9.0 days
- B6 : 9.4 days
- **Nude : 14.8 days**
- **SCID : 16.4 days**

($p < 0.05$)

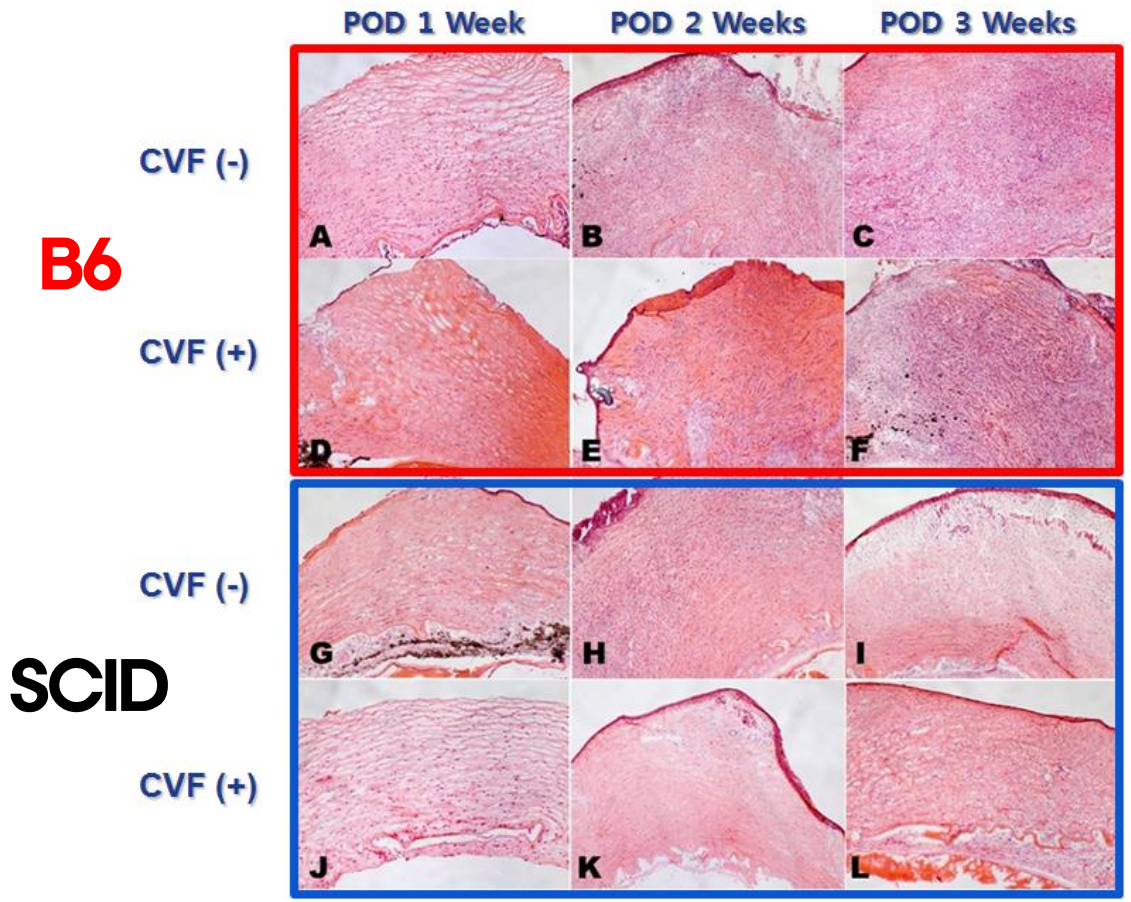
Oh JY, et al. Xenotransplantation 2009;16:74-82.

– Role of complement in xenocorneal graft rejection

- ↑ graft survival by complement depletion
- Cellular infiltration : delayed and decreased by complement depletion



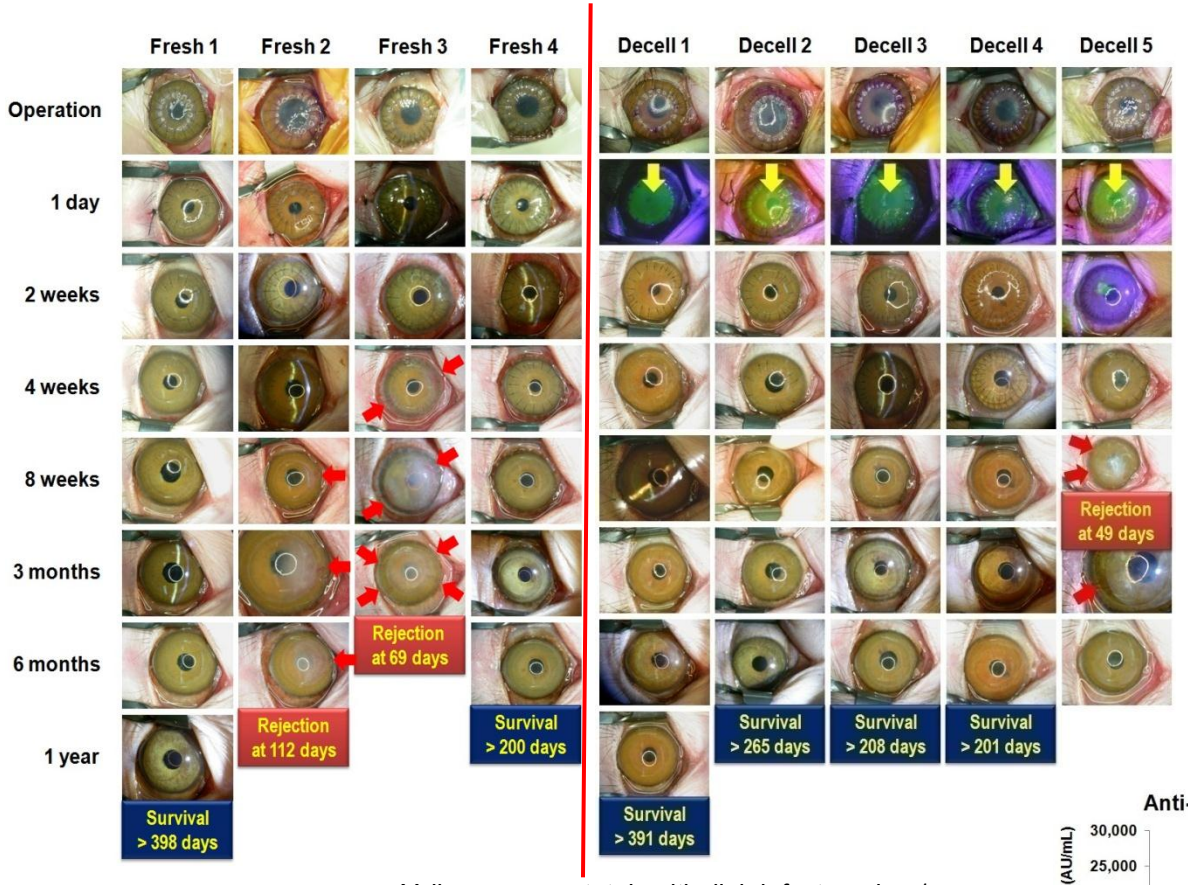
(p<0.05, log-rank test)



Oh JY, et al. Xenotransplantation 2010;17:140-6.

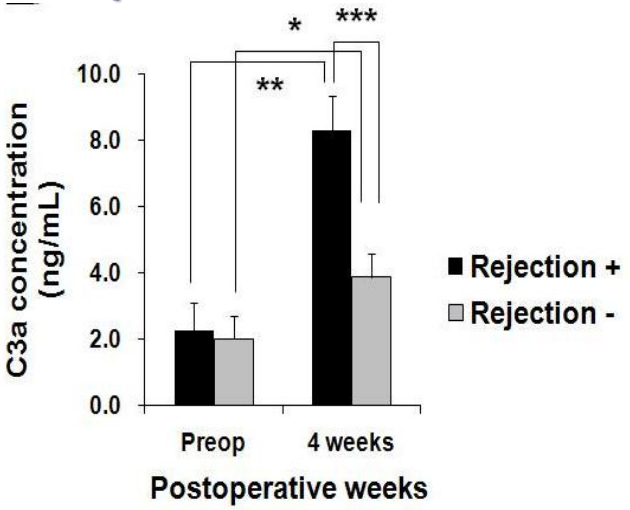
Pig-to-rhesus partial thickness corneal transplantation

- **Clinical data**



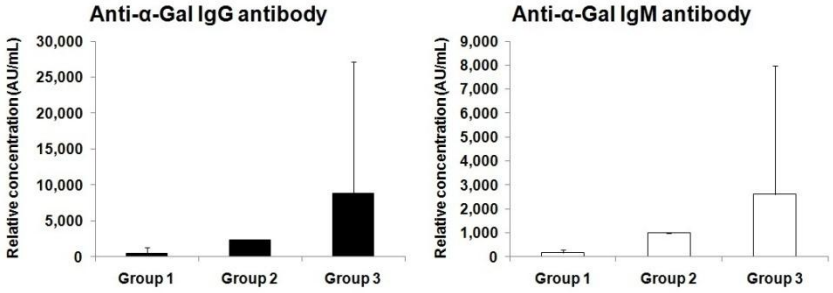
Yellow arrows : total epithelial defect on day 1
Red arrows : new vessels

Complement (C3a) in aqueous humor



Plasma alpha-Gal

Group 1 : partial thickness corneal transplantation
Group 2 : full thickness corneal transplantation
Group 3 : islet cell transplantation



Conclusion

- Porcine cornea as a substitute for a human cornea
  - Decellularized porcine cornea : promising substitute
  - Fresh porcine cornea : needs additional tools
    - More potent immunosuppression
    - Genetically engineered pig as a source

- Future directions
  : For full-thickness xenocorneal transplantation
  - Clinically applicable potent immunosuppression
  - Tissue engineered cornea using decellularized porcine corneal matrix
  - Genetically engineered pig as a source

바이오이종장기개발사업단

사업단장 김상준

Pig 관리
이왕재, 염수청

면역팀
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강희정,전태훈, 김정식

영장류팀
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박정규, 신준섭, 진상만, 민상일, 김강석

췌도 캡슐화 – 윤건호

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각막 이식
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심장 판막 도관 – 김용진

총 연구원 170명