Xenotransplantasyon

Dr. Kenan Keven,

Ankara Üniversitesi Tıp Fakültesi

Nefroloji B.D.

In a First, Surgeons Attached a Pig Kidney to a Human, and It Worked

21 Ekim 2021

A kidney grown in a genetically altered pig functions normally, scientists reported. The procedure may open the door to a renewable source of desperately needed organs.

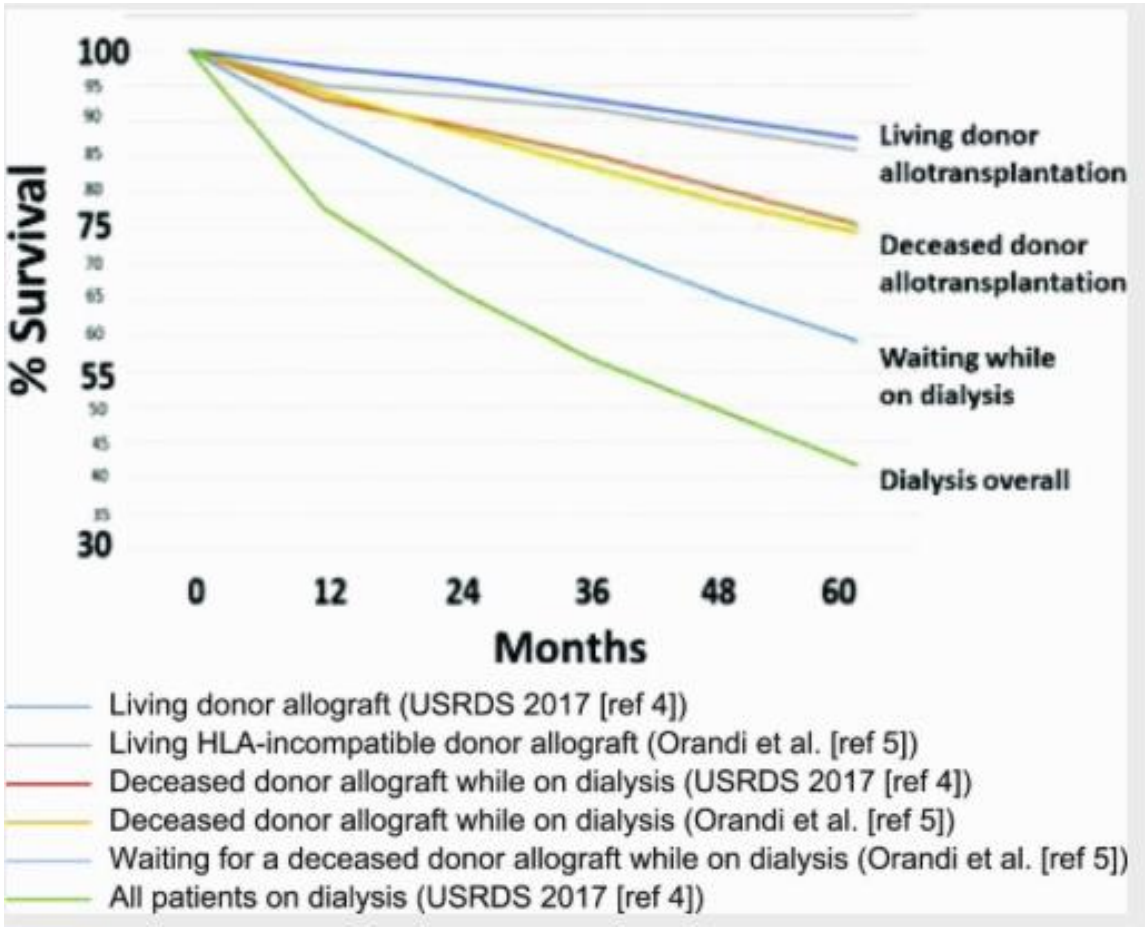
Dr. Robert Montgomery is director of the N.Y.U. Langone Transplant Institute in Manhattan. Genetically engineered pigs "could potentially be a sustainable, renewable source of organs," he said. Amir Hamja for The New York Times

NYT

A surgical team at the hospital in New York examined a pig kidney attached to the body of a brain-dead recipient for any signs of rejection. Joe Carrotta/N.Y.U. Langone Health, via Associated Press

Böbrek Nakli

- Kontrendikasyon olmadığı takdirde
  - Tüm SDBH olan hastalar böbrek nakline adaydır



USRDS,2020

Bekleme listesine (2011-2014) alınan hastaların sonraki 2 yılda nakil olma oranları
ABD

Kalp Akciğer	O	%36
	A	%7
	B	%10
	AB	%10
Kalp	O	%54
	A	%59
	B	%68
	AB	%75
Karaciğer	O	%46
	A	%47
	B	%55
	AB	%68
Böbrek	O	%12
	A	%17
	B	%11
	AB	%29

optn.transplant.hrsa.gov

BEKLEME LİSTESİ ABD

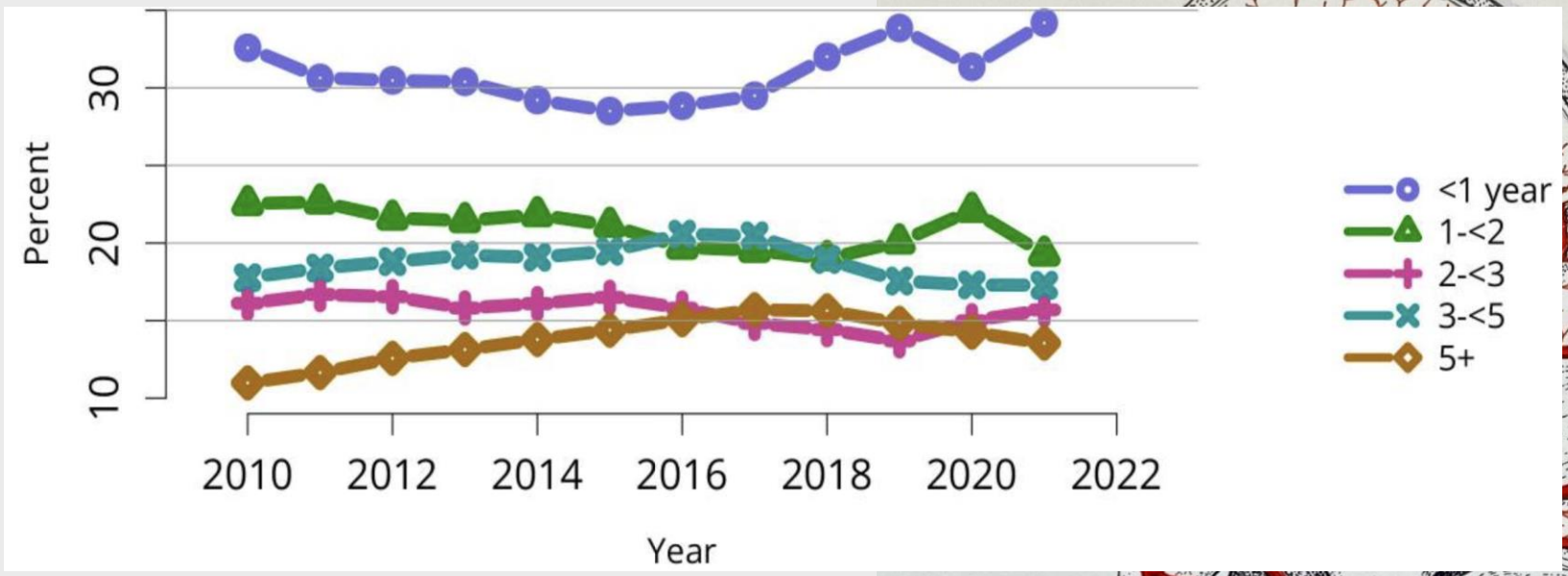
Böbrek	98.000
Karaciğer	12.000
Kalp	3500
Akciğer	1038

Yılda 35.000 hasta ortalama böbrek bekleme listesinden çıkıyor
Yılda eklenen hasta sayısı (böbrek) ortalama 38.000

20.000 transplant
4000 ex
4000 diğer nedenler ?
3400 nakil için çok hasta
3000 başka ülke-merkez nakil

NAKİL SAYILARI

Böbrek	20.736 (4900 canlı, 15.700 Kadavra)

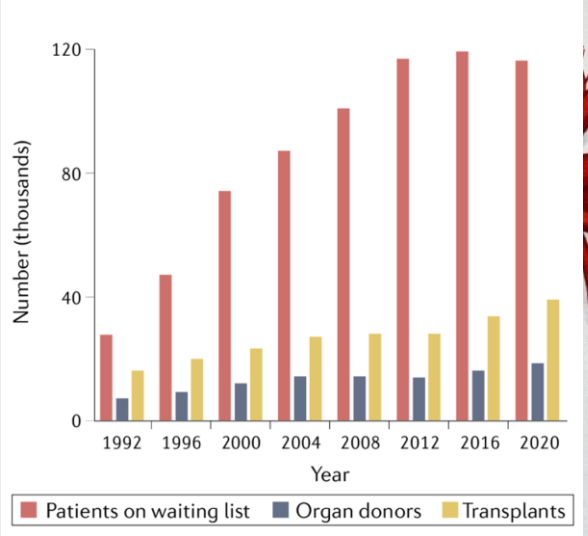


%34,2 <1yıldır bekleme listesinde
%13,6 ≥5 yıldır bekleme listesinde
%16,7 ≥6 yıldır diyalizde

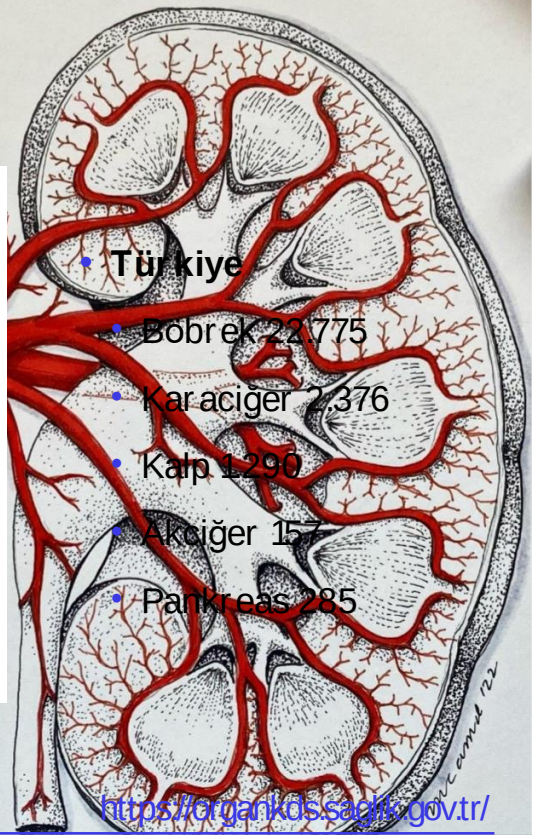
OPTN/SRTR 2021 Annual Data Report: Kidney.

Organ Nakli Bek

- **ABD**
  - Böbrek 90.223
  - Karaciğer 11338
  - Kalp 3.522
  - Akciğer 1053
  - Pankreas 2.581

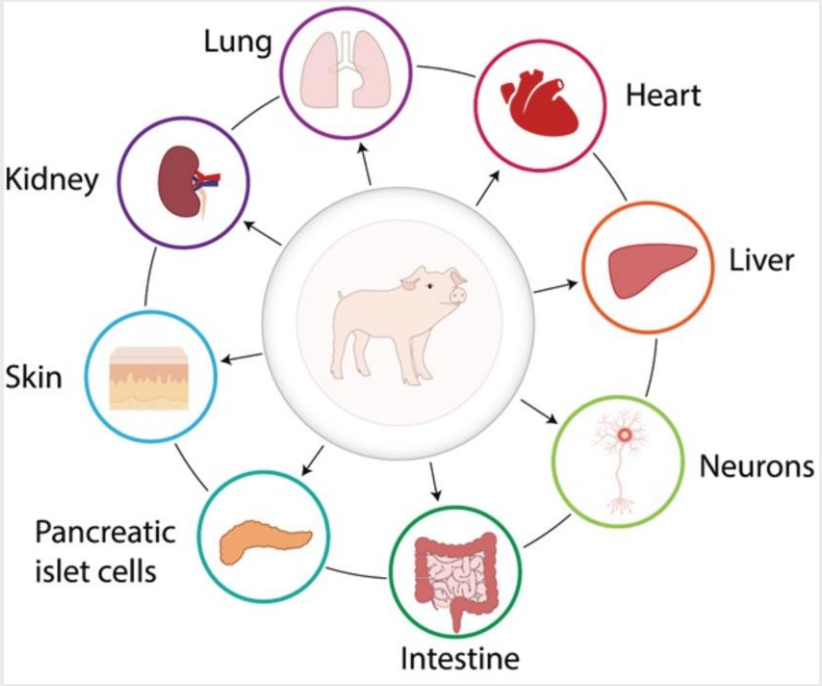


- **Türkiye**
  - Böbrek 22775
  - Karaciğer 2.376
  - Kalp 1.290
  - Akciğer 157
  - Pankreas 285



OPTN/SRTR 2021 Annual Data Report.

https://organkds.saglik.gov.tr/

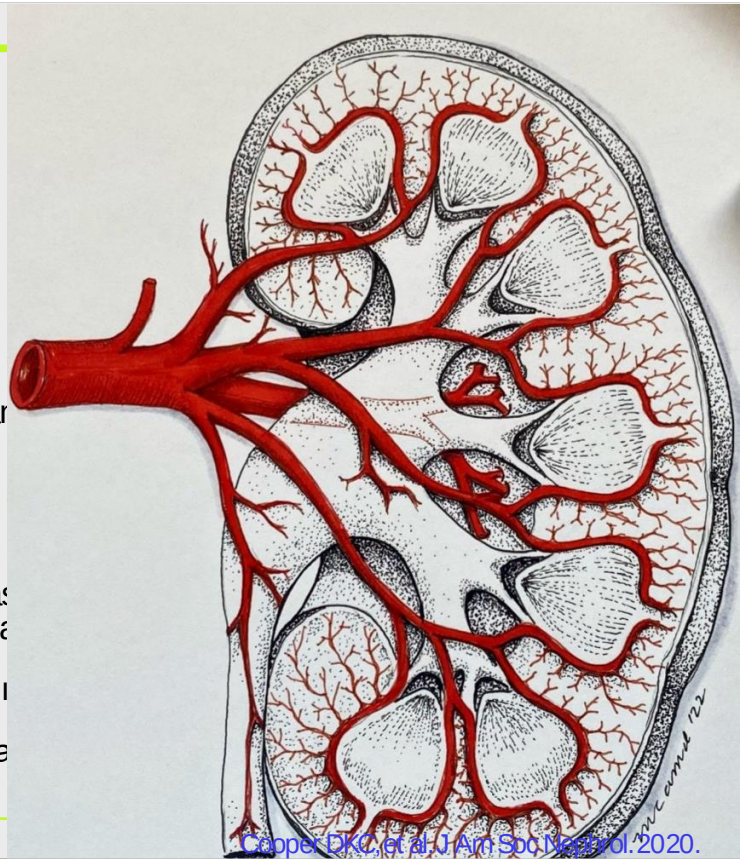


Xenotransplantasyon

Türler arası hücre, doku veya organ nakli

Xenotransplantasyon Avantajları

- Sınırsız donor organ kaynağı sağlaması
- Acil veya elektif ihtiyacın karşılanması
- Beyin ölümünün donor organlara olumsuz etkileri ortadan
- "Borderline" nakil adayları için de organ sağlanabilmesi
  - Kontrolsüz DM, ASKH, SVH, Periferik arter hastalığı gibi
- Nakil öncesi donorün ayrıntılı mikrobiyoloji taranması xenogeneik dokunun bazı insan patojenlerine dirençli olma
- Canlı donor ihtiyacının ve canlı donor riskini ortadan kaldı
- Kadavra donor bağışı için kültürel bariyerlerin ortadan ka



Cooper DKC, et al. J Am Soc Nephrol. 2020.

Xenotransplantasyon Tarihçe

Year	Surgeon (Location)	Donor Organ and Source	Survival
1906	Jaboulay (Lyon, France)	Pig kidney	3 days
1906	Jaboulay (Lyon, France)	Goat kidney	3 days
1910	Unger (Berlin, Germany)	Macaque monkey kidney	32 hours
1913	Schonstadt (?)	Monkey kidney	60 hours
1923	Neuhof (New York, NY)	Lamb kidney	9 days
1963	Hitchcock (Minneapolis, MN)	Baboon kidney	4 days
1963	Reemtsma (New Orleans, LA)	Rhesus monkey kidney	63 days
1964	Reemtsma (New Orleans, LA)	Chimpanzee kidneys (13 patients)	11 days to 9 months
1964	Starzl (Denver, CO)	Baboon kidneys (6 patients)	19 days to 3 months
1964	Hume (Richmond, VA)	Chimpanzee kidney	1 day
1964	Trager (Lyon, France)	Chimpanzee kidneys (3 patients)	< 49 days
1964	Hardy (Jackson, MS)	Chimpanzee heart	90 minutes
1966	Cortesini (Rome, Italy)	Chimpanzee kidney	31 days
1968	Ross (London, UK)	Pig heart	4 minutes
1968	Cooley (Austin, TX)	Sheep heart	10 minutes
1969	Marion (Lyon, France)	Chimpanzee heart	"quickly"
1969	Starzl (Denver, CO)	Chimpanzee liver	9 days
1970	Bertoye (Lyon, France)	Baboon liver (2 patients)	39 hours to 4 months
1970	Leger (Paris, France)	Baboon liver	72 hours
1971	Pouyet & Bérard (Lyon, France)	Baboon liver (2 patients)	< 2 days
1974	Starzl (Denver, CO)	Chimpanzee liver	14 days
1977	Barnard (Cape Town, South Africa)	Baboon heart	5.5 hours
1977	Barnard (Cape Town, South Africa)	Chimpanzee heart	4 days
1984	Bailey (Loma Linda, CA)	Baboon heart	20 days
1992	Religa & Czaplicki (Sosnowiec, Poland)	Pig heart	23 hours
1992	Makowka (Los Angeles, CA)	Pig liver	34 hours
1992	Starzl (Pittsburgh, PA)	Baboon liver	70 days
1993	Starzl (Pittsburgh, PA)	Baboon liver	Coma 26 days
1996	Baruah (Sonapur, India)	Pig heart	7 days

Deschamps J-Y, et al. Xenotransplantation. 2005.

Tromboza bağlı graft kaybı

İmmünosupresyon

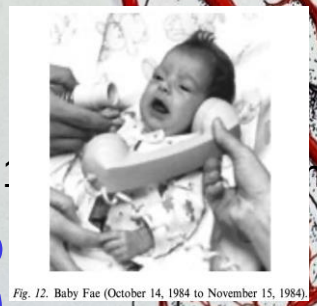


Fig. 12. Baby Fae (October 14, 1984 to November 15, 1984).

Takrolimus

Siems C, Huddleston S, John R. Ann Thorac Surg. 2022.

Xenotransplantasyon Sorunlar
Mevzuat Sınırlar Çalışmalar

Zaten öleceklerdi ….. Bu kavram dışlanıyor

1998 US Public Health Policy on Xenotransplantation FDA'e çalışmaları durdurma önerisi
(Etik sorunlar çözülene kadar ve iyi planlanmış araştırmalar yapılana kadar)

1998 UK ve İspanya: İnsan (Xeno) nakil öncesinde 6 aylık yaşam süresi ve güvenlik kanıtı

1999 US Department of Health and Human Services Secretary Advisory Comittee on Xenotransplantation

1999 Avrupa Birliği Komisyonu Xenotransplantasyonu yeterli kanıtlar elde edinceye kadar erteliyor

Organ Sağlayıcı Tür?

Genetik daha yakın
Rejeksiyonlar daha az gürültülü

Boyut yeterli
Çoğalma hızlı 3 ayda 10-12 domuz yavrusu
Gelişim hızlı
Tür tehdidi yok
Yetiştirmek nispeten kolay

Boyut sorunu : organlar küçük
Gelişim yavaş
Viral geçiş ihtimali daha yüksek
Yetiştirmek güç
Tür tükenme tehlikesi
Çoğalma yavaş

Genetik ve yapısal daha uzak
İmmunolojik reaksiyonlar daha sert
İnflamasyon ve koagülasyon aktivasyonu

Siems C, Huddleston S, John R. Ann Thorac Surg. 2022.

İnsandan Önce İdeal Başarılı Xenotransplantasyon Modeli

Donör: Domuz

Alıcı: NHP

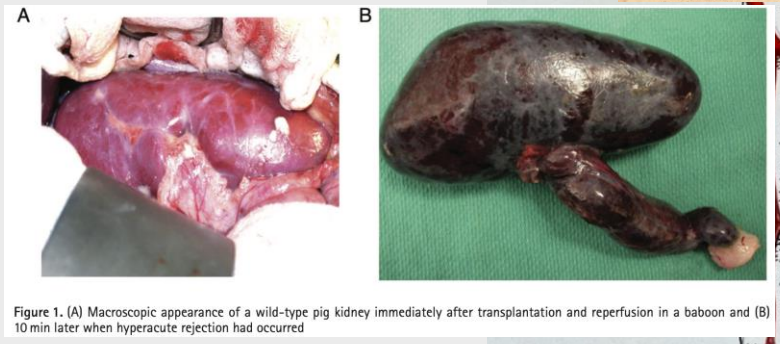
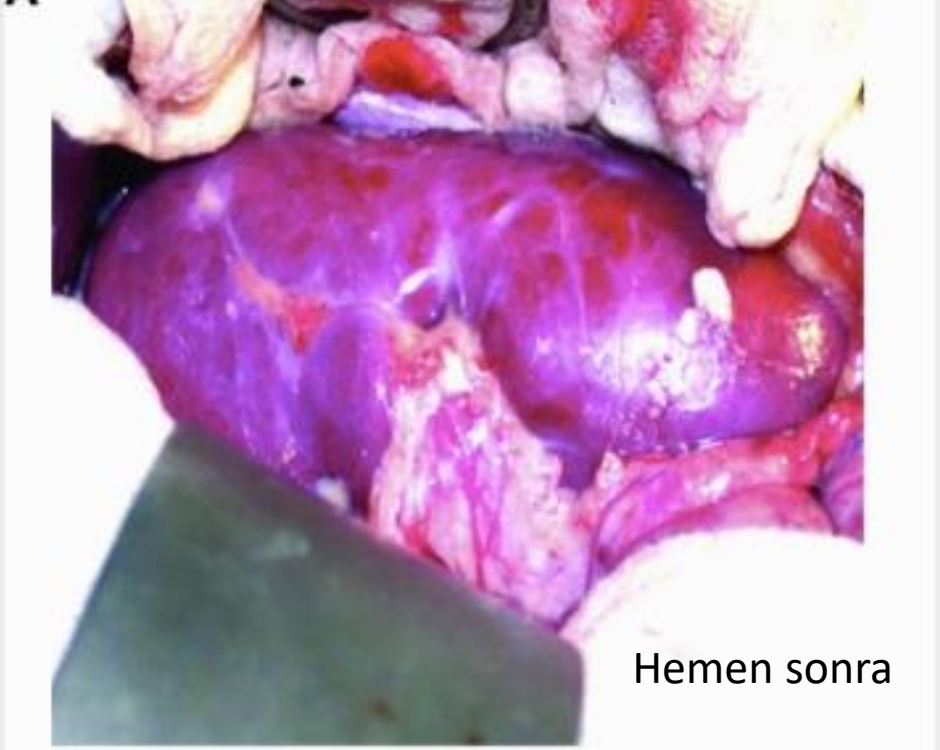


Figure 1. (A) Macroscopic appearance of a wild-type pig kidney immediately after transplantation and reperfusion in a baboon and (B) 10 min later when hyperacute rejection had occurred

Cooper DKC, et al. J Pathol. 2016.



Wild Type domuzdan

Baboon a nakil

Hiperakut Rejeksiyon

Xenotransplantasyon Bariyer

1. Hiperakut rejeksiyon	Dakikalar - Saatler
2. Akut humoral rejeksiyon	Saatler-Günler
3. T hücreli rejeksiyon	Günler Haftalar
4. Kronik rejeksiyon	Haftalar -Aylar

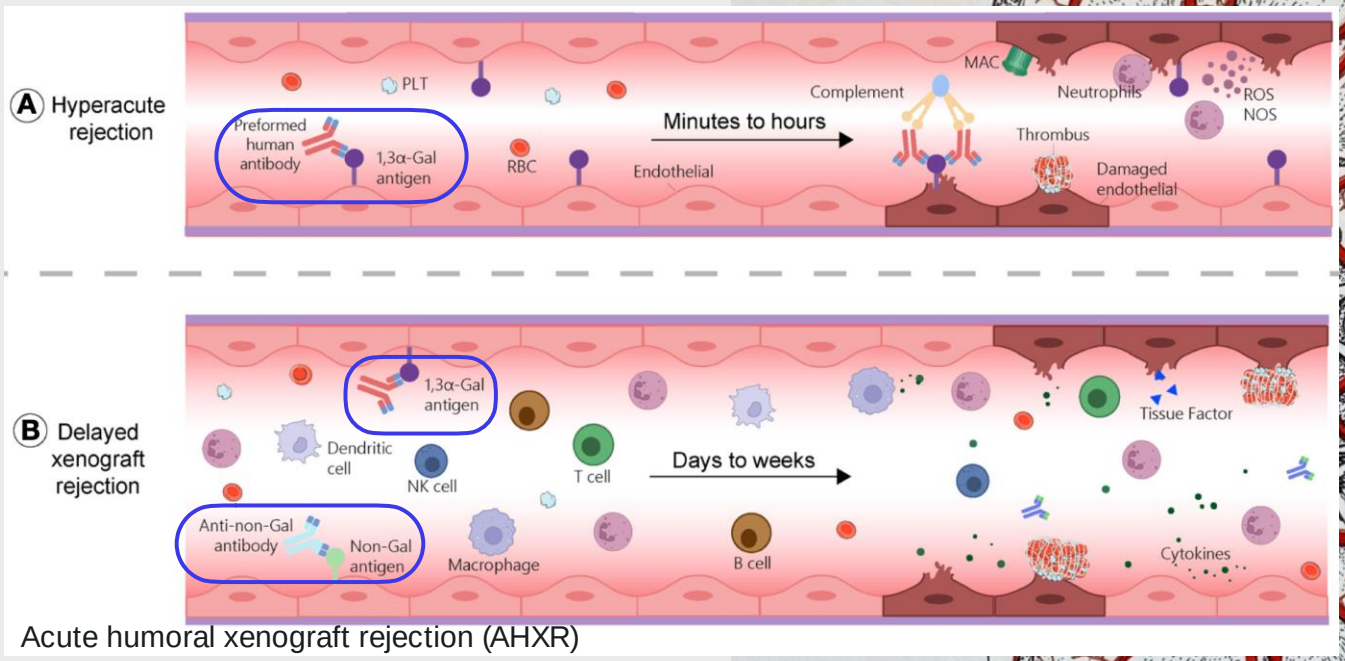
Koagülasyon Disregülasyonu

Trombotik mikroanjiopati
Sistemik consumptive koagülopati (sistemik reaksiyon) nadir
İskemi ve hasar ile sonuçlanır

Compleman Regülatör proteinler

Lu T, Front in Imm, 2020

İmmünolojik Bariyer



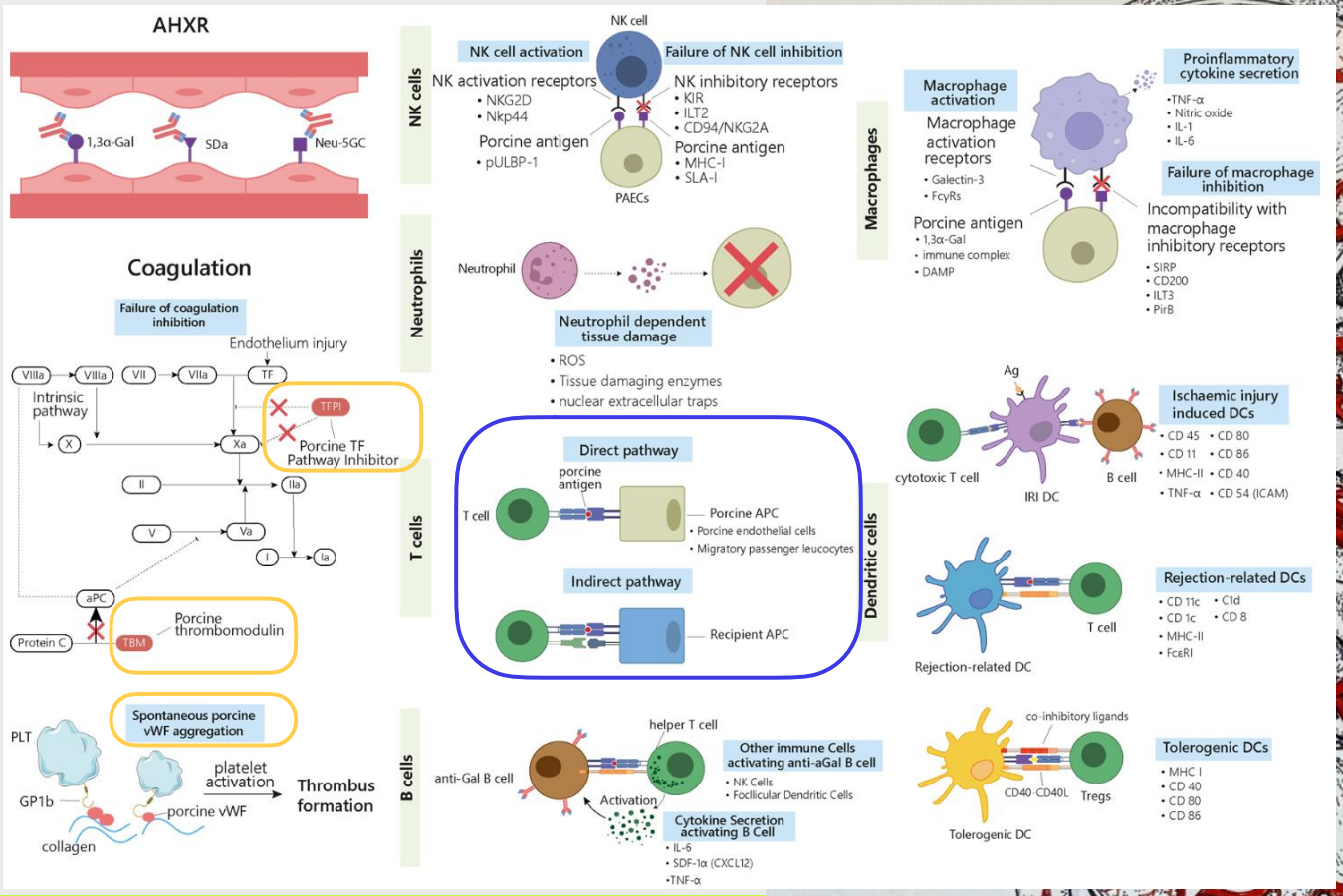
Acute humoral xenograft rejection (AHXR)

Cellular xenograft rejection

Coagulation dysregulation

Zhou Q, et al. Front Immunol. 2022.

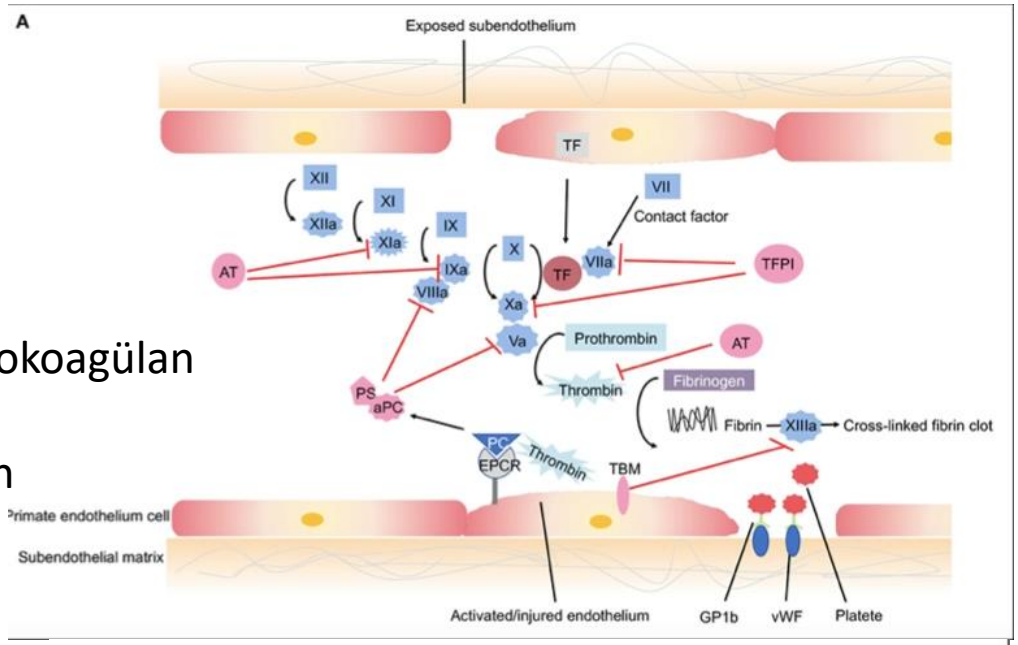
Hajibabaei Z, et al. Xenotransplantation. 2023.



Zhou Q et al. Front Immunol. 2022.

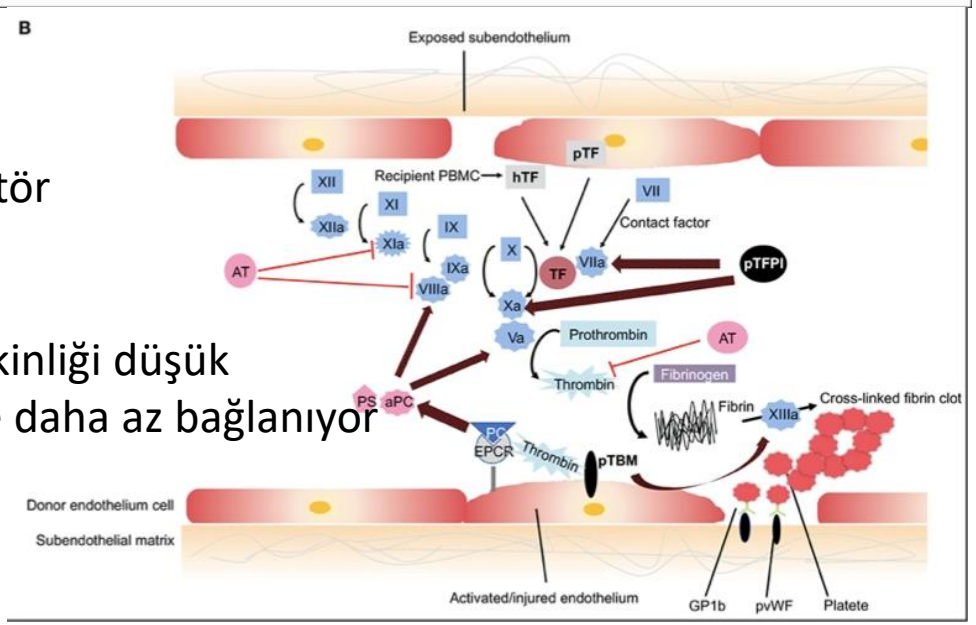
Koagülasyon Disregülasyonu

Domuz endoteli
- antikoagülan değil prokoagülan
- Tissue faktör
- Extrinsek koagülasyon
- …
- trombin



Tissue faktör pathway inhibitör
Domuz da daha az etkin

Domuz Thrombomodulin etkinliği düşük
Domuz TBM-İnsan Trombine daha az bağlanıyor
Protein C daha az aktive
Domuz vWB platelet aggre..



Genetik Mühendislik

- Xenoantijenlerin uzaklaştırılması
- Koagülasyonu aktive eden mekanizmaların kaldırılması
- Koagülasyonu regüle eden proteinlerin expre sağlanması
- İnsan kompleman regülatör proteinlerin expre sağlanması
- İnflamasyon aktivasyon genlerin inhibisyonu
- İnflamasyon regülatör genlerin aktivasyonu

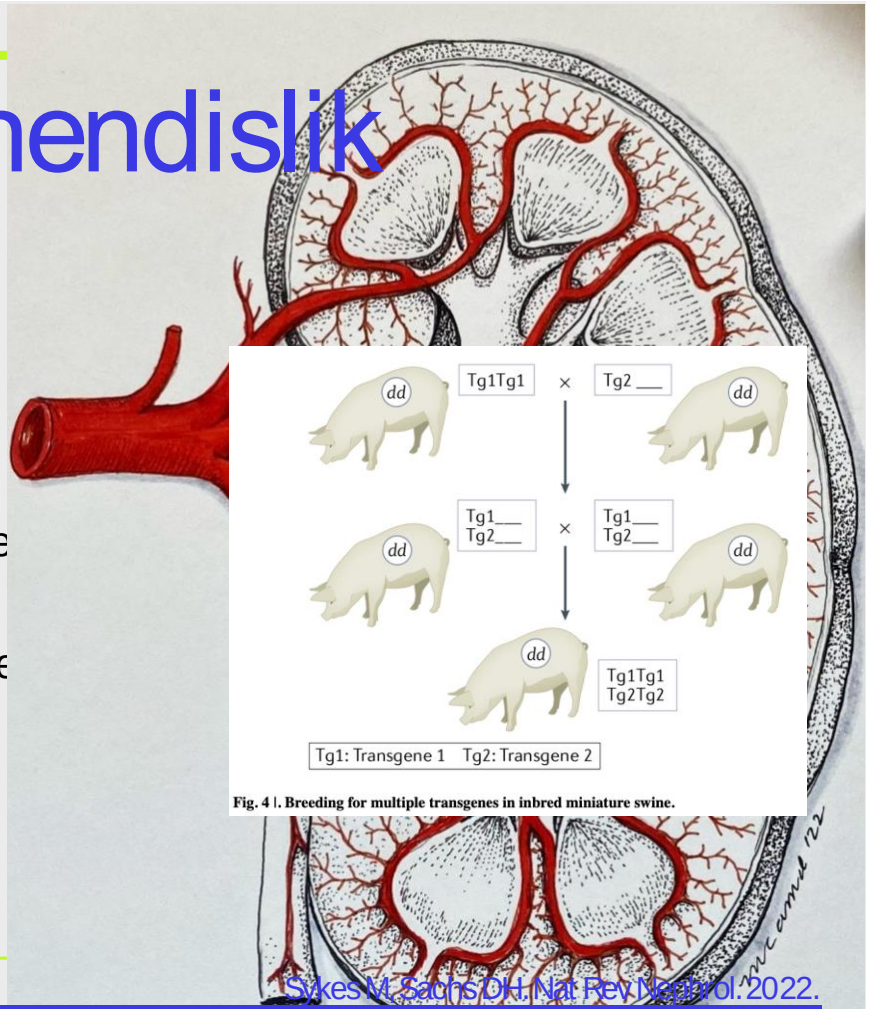


Fig. 4 |. Breeding for multiple transgenes in inbred miniature swine.

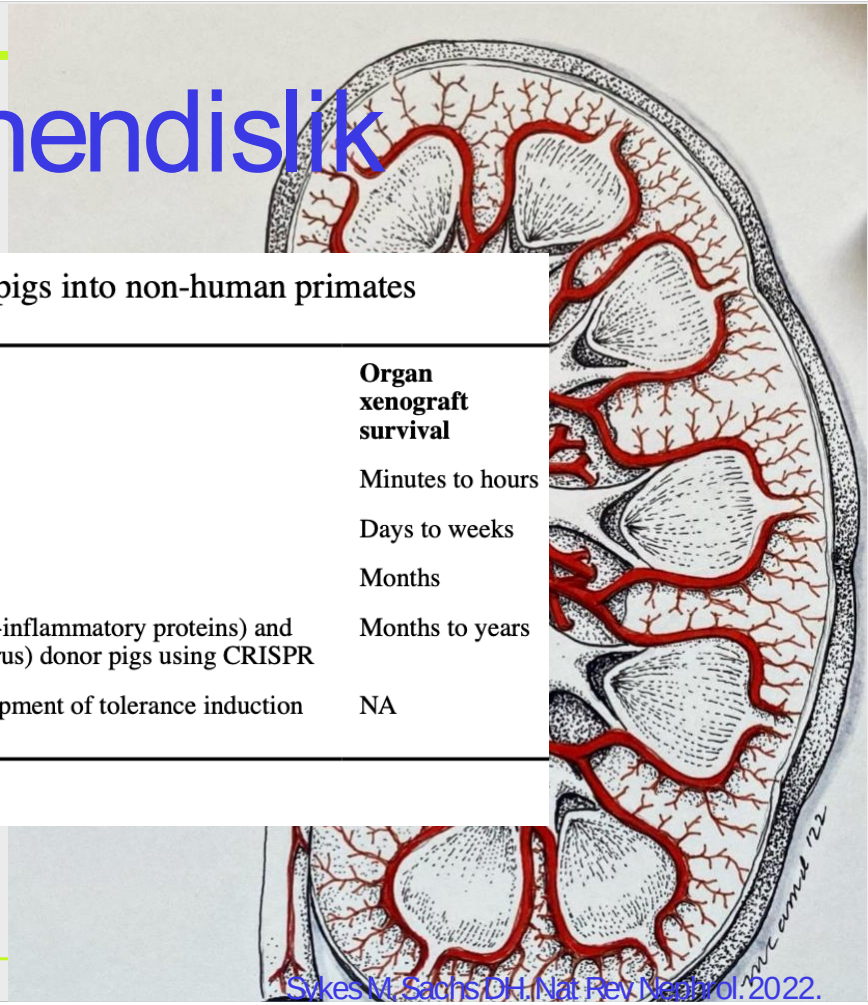
Sykes M, Sachs DH. Nat Rev Nephrol. 2022.

Genetik Mühendislik

Chronology of progress in xenotransplantation from pigs into non-human primates

Date	Innovation	Organ xenograft survival
1980s	Natural antibody absorption	Minutes to hours
1990s	Human CRP transgenic donor pigs	Days to weeks
2000s	GGTA1-knockout donor pigs	Months
2010s	New transgenic (CRPs, hCD47, coagulation inhibitors, anti-inflammatory proteins) and knockout (B4GalNT2, CMAH, porcine endogenous retrovirus) donor pigs using CRISPR	Months to years
2020s	First human xenotransplants, potential clinical trials, development of tolerance induction approaches	NA

CRP, complement regulatory protein; NA, not available.



Sykes M, Sachs DH. Nat Rev Nephrol. 2022.

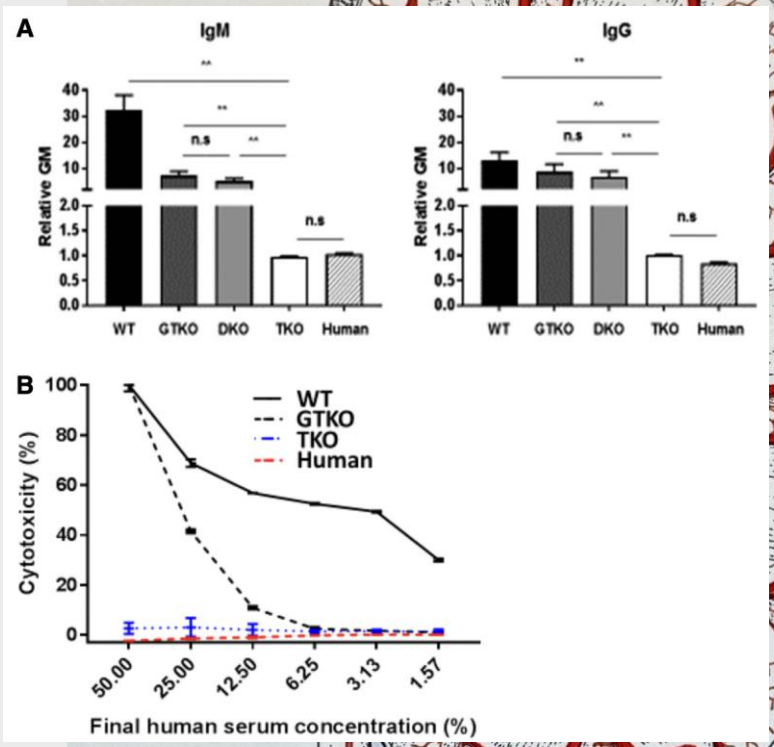
Genetik Mühendislik

Known carbohydrate xenoantigens expressed on pig cells

Carbohydrate (Abbreviation)	Responsible Enzyme	Gene-Knockout Pig
Galactose-α1,3-galactose (Gal)	α1,3-galactosyltransferase	GTKO
N-glycolylneuraminic acid (Neu5Gc)	CMAH[a]	CMAHKO
Sd[a]	β-1,4N-acetylgalactosaminyltransferase	β4GalNT2KO

Triple-knockout (TKO) domuz- İnsan

Antikor bağlama ve serum sitotoksisite görülmemektedir.



Li Q, et al. Xenotransplantation. 2019.

Genetik Mühendislik

Knockouts

Prevention of natural antibody-mediated rejection

- α1,3-galactosyl transferase
- CMAH
- B4GalNT2

Prevention of infection

- Porcine endogenous retroviruses

Limitation of organ size

- GHR

Knock-ins

Prevention of graft thrombosis

- Human vWF

Transgenes

Complement inhibition

- Human DAF
- Human CD46
- Human CD59

Coagulation inhibition

- Human CD39
- Human thrombomodulin
- Human endothelial protein C receptor

Anti-inflammatory genes

- HO-1
- A20

Immunosuppressive molecules

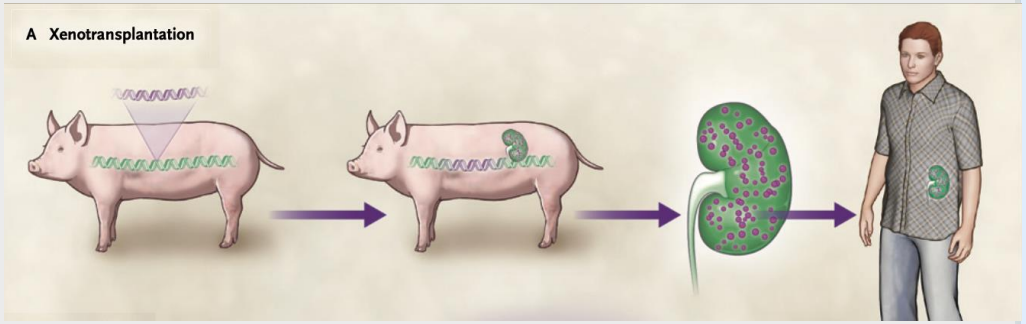
- Anti-CD2
- CTLA4Ig
- Human CD47
- PD-L1
- FasL

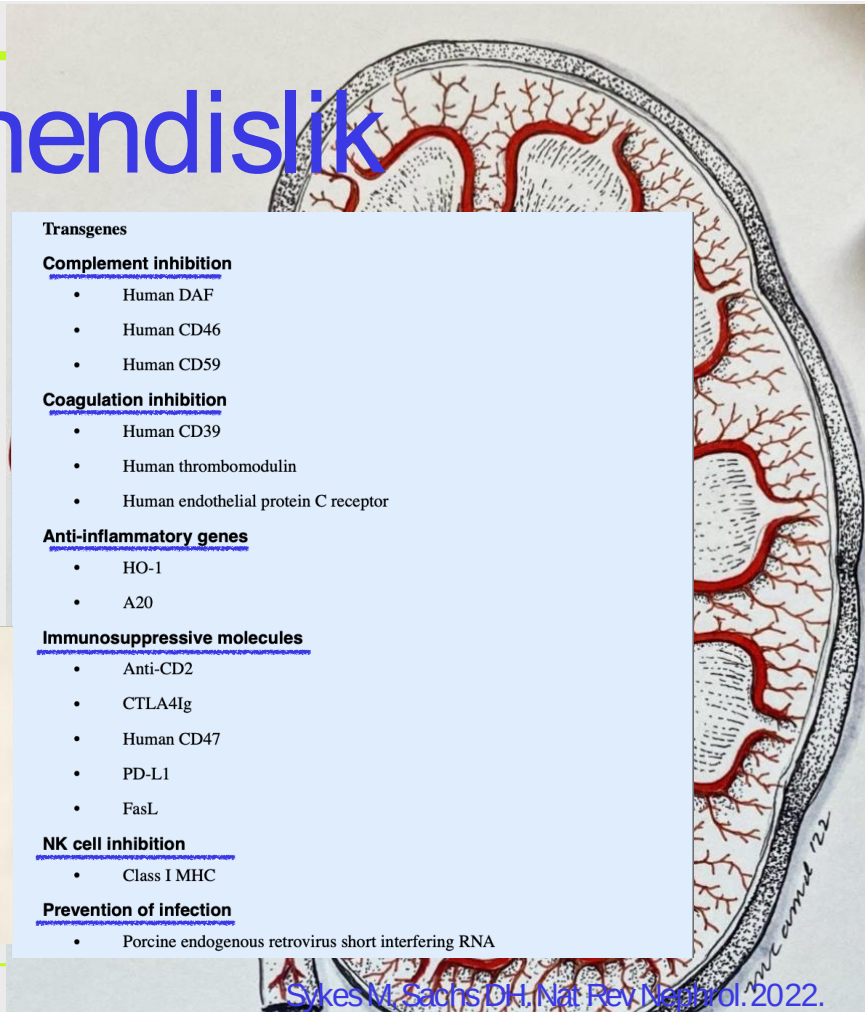
NK cell inhibition

- Class I MHC

Prevention of infection

- Porcine endogenous retrovirus short interfering RNA





Sykes M, Sachs DH. Nat Rev Nephrol. 2022.

TABLE 1 | Genetically modified pigs currently available for xenotransplantation research.

Abbreviation	Gene name	Function
GTKO	1,3-galactosyltransferase KO (GGTA1 KO)	Deletion of αGal epitope
CMAH KO	CMP-*N*-acetylneuraminic acid hydroxylase KO	Deletion of Neu5Gc epitope
β4GalNT2 KO	β-1,4*N*-acetylgalactosaminyltransferase KO	Deletion of SDa epitope
hCD46 (MCP)	Human membrane cofactor protein transgene	Inactivation complement factors C3b and C4b
hCD55 (DAF)	Human decay accelerating factor transgene	Acceleration of complement decay
hCD59 (MAC-IP)	Human membrane attack complex C5b-9 inhibitory protein transgene	Inhibition of the complement membrane attack complex C5b-9
hTBM	Human thrombomodulin	Anticoagulation (activates protein C)
hTFPI	Human tissue factor pathway inhibitor	Antagonize the function of tissue factor
hCD39 (hENTPD1)	Human ectonucleoside triphosphate diphosphohydrolase-1 transgene	Anticoagulation and anti-inflammatory
hA20	Human tumor necrosis factor alpha-induced protein-3 transgene	Inhibition of NF-kappa B activation and TNF-mediated apoptosis
hCD47	Human integrin associated protein transgene	Regulation of macrophage activation and phagocytosis
CTLA4-Ig	Cytotoxic T-lymphocyte-associated protein 4-immunoglobulin transgene	Cellular immune response: Inhibition of T-cell costimulation via CD86/CD80
CIITA-DN	MHC class II transactivator dominant negative	Suppression of T-cell activation
hHO1	Human heme oxygenase 1 transgene	Antiapoptosis; cytoprotection; anti-inflammatory
ASGR1 KO	Asialoglycoprotein receptor 1	Decreases human platelet phagocytosis by pig sinusoidal endothelial cells
PERV inactivation	Porcine endogenous retroviral virus inactivation	Xenozoonosis

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

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 termination. No features of a consumptive coagulopathy (e.g., thrombocytopenia, decreased fibrinogen) or of a protein-losing nephropathy were observed. There was no evidence of an elicited anti-pig antibody response. Death was from septic shock (*Myroides* spp). Histology of a biopsy on day 103 was normal, but by day 136, the kidney showed features of glomerular enlargement, thrombi, and mesangial expansion. The combination of (i) a graft from a specific genetically engineered pig, (ii) an effective immunosuppressive regimen, and (iii) anti-inflammatory agents prevented immune injury and a protein-losing nephropathy, and delayed coagulation dysfunction. This outcome encourages us that clinical renal xenotransplantation may become a reality.

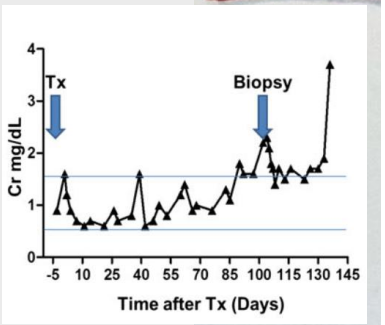


Table 1. Immunosuppressive, anti-inflammatory, and adjunctive therapy

Agent	Dose (Duration)
Immunosuppressive	
Induction	
Thymoglobulin (ATG) (Genzyme, Cambridge, MA)	10 mg/kg (day-3)
Anti-CD20mAb (Rituximab) (Genentech, South San Francisco, CA)	10 mg/kg (day-2)
Cobra venom factor (Complement Technology, Tyler, Texas)	100 IU (days-1 and 0)
Maintenance	
Anti-CD40mAb (2C10R4) (NIH NHP Resource Center, Boston, MA)	50 mg/kg (days-1, 0, 4, 7, 14, and weekly)
Rapamycin (LC Laboratories, Woburn, MA)	0.01 mg/kgx2/day (target 8-12 ng/ml) (from day-3)
Methylprednisolone (MP) (Astellas, Deerfield, IL)	5 mg/kg/day tapering to 0.25 mg/kg/day
Anti-inflammatory	
Tocilizumab (IL-6R blockade) (Genentech, South San Francisco, CA)	10 mg/kg (days-1, 7, 14 and every 2 weeks)
Etanercept (TNF-α antagonist) (Amgen, Thousand Oaks, CA)	0.5 mg/kg (days 0, 3, 7, 28, 40)
Adjunctive	
Aspirin (Bayer, Deland, FL)	40 mg p.o. (alternate days)
Low-molecular weight heparin (LMWH) (Eisai, Woodcliff Lake, NJ)	700 IU/day s.c

Iwase H, et al. Xenotransplantation. 2015.

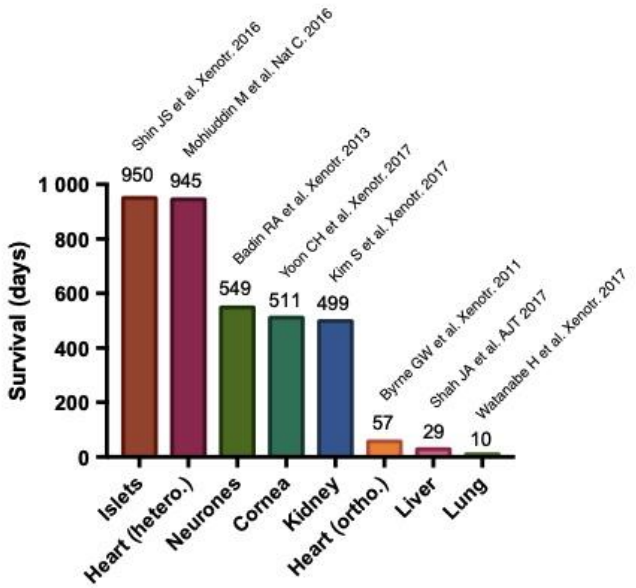


Figure 1 Survival of xenogeneic organs/tissue transplanted in nonhuman primates.

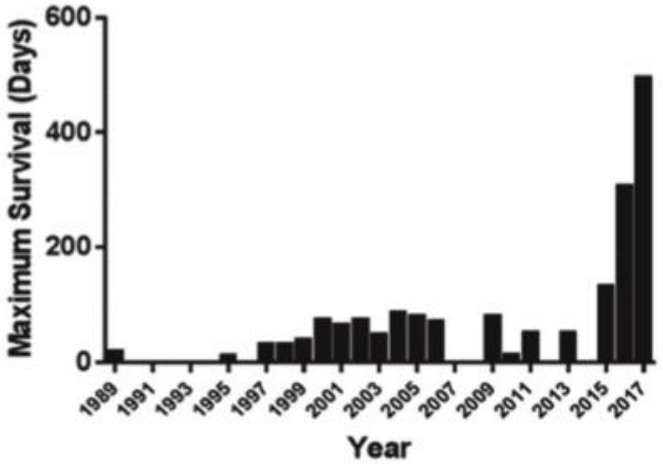


Figure 2. Reported maximum survivals of nonhuman primates with life-supporting pig kidney grafts, 1989–2017. Details can be found in Lambrigts *et al.*,[19] and Cooper *et al.*[73] In some years, no results were reported.

Cooper DKC et al. J Am Soc Nephrol. 2020.

FDA approves genetically engineered pigs

The first approval for both food and medical use

By Kait Sanchez | @crisp_red | Dec 14, 2020, 7:03pm EST

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Curing Human Disease Through Regenerative Medicine

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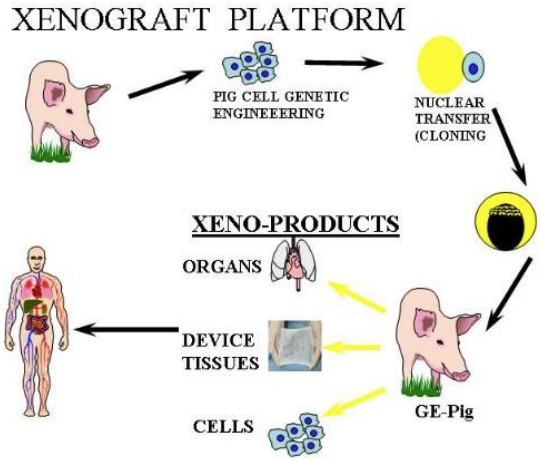
Xenotransplantation Technology

Islet Transplantation Technology

Infectious Disease Technology

Xenotransplantation Technology

Revivicors goal is to produce **genetically engineered** (GE) pigs to provide human compatible cells (i.e., islets) for treatment of diabetes and organs and tissues for use in transplant surgery (xenografts).



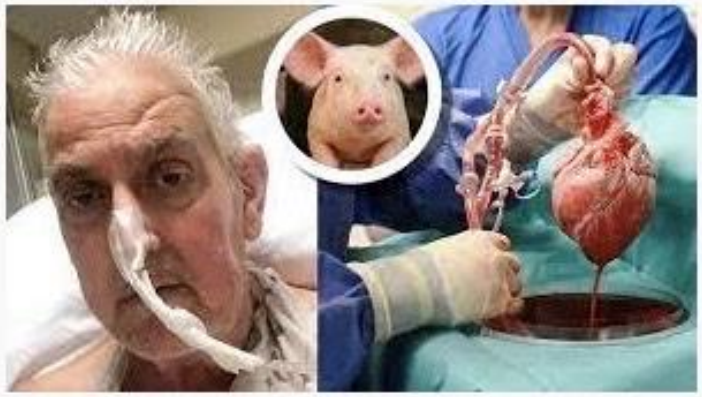
Xenotransplantasyon Klinik Çalışma

- FDA: 2 yıldan düşük yaşam beklentisi olan hastalar alınmalı
- 6 hasta 1 yıl takip ile başlayabilir
- İlk hasta 3 ay sorun yok
  - Diğer hastalar alınır
- Alıcı adayları
- Kronik diyaliz tedavisi uygulanmaya başlanmış olmalı
- Vasküler giriş yolu sorunu olanlardan
- Yüksek PRA hasta ?
- Tek değil 2 böbrek nakil edilebilir ?

Genetiği değiştirilmiş Domuz
4 knock-out gen
6 eklenmiş genetik

David Bennett world first human receives a Pig Heart Transplant

Sumit Arora Published On January 13th, 2022



"It was either die or do this transplant. I want to live. I know it's a shot in the dark, but it's my last choice," said Mr. Bennett, the patient, a day before the surgery was conducted.

FDA ilk defa acil kullanım onayı verdi

The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

Genetically Modified Porcine-to-Human Cardiac Xenotransplantation

Bartley P. Griffith, M.D., Corbin E. Goerlich, M.D., Ph.D., Avneesh K. Singh, Ph.D., Martine Rothblatt, Ph.D., Christine L. Lau, M.D., Aakash Shah, M.D., Marc Lorber, M.D., Alison Grazioli, M.D., Kapil K. Saharia, M.D., Susie N. Hong, M.D., Susan M. Joseph, M.D., David Ayares, Ph.D., and Muhammad M. Mohiuddin, M.D.

SUMMARY

A 57-year-old man with nonischemic cardiomyopathy who was dependent on venoarterial extracorporeal membrane oxygenation (ECMO) and was not a candidate for standard therapeutics, including a traditional allograft, received a heart from a genetically modified pig source animal that had 10 individual gene edits. Immunosuppression was based on CD40 blockade. The patient was weaned from ECMO, and the xenograft functioned normally without apparent rejection. Sudden diastolic thickening and failure of the xenograft occurred on day 49 after transplantation, and life support was withdrawn on day 60. On autopsy, the xenograft was found to be edematous, having nearly doubled in weight. Histologic examination revealed scattered myocyte necrosis, interstitial edema, and red-cell extravasation, without evidence of microvascular thrombosis — findings that were not consistent with typical rejection. Studies are under way to identify the mechanisms responsible for these changes. (Funded by the University of Maryland Medical Center and School of Medicine.)

Surgeon Bartley P Griffith pictured with David Bennett earlier this month

The first person in the world to get a heart transplant from a genetically-modified pig has died.

nature medicine

Article

https://doi.org/10.1038/s41591-023-02471-9

Pig-to-human heart xenotransplantation in two recently deceased human recipients

Received: 20 January 2023

Accepted: 26 June 2023

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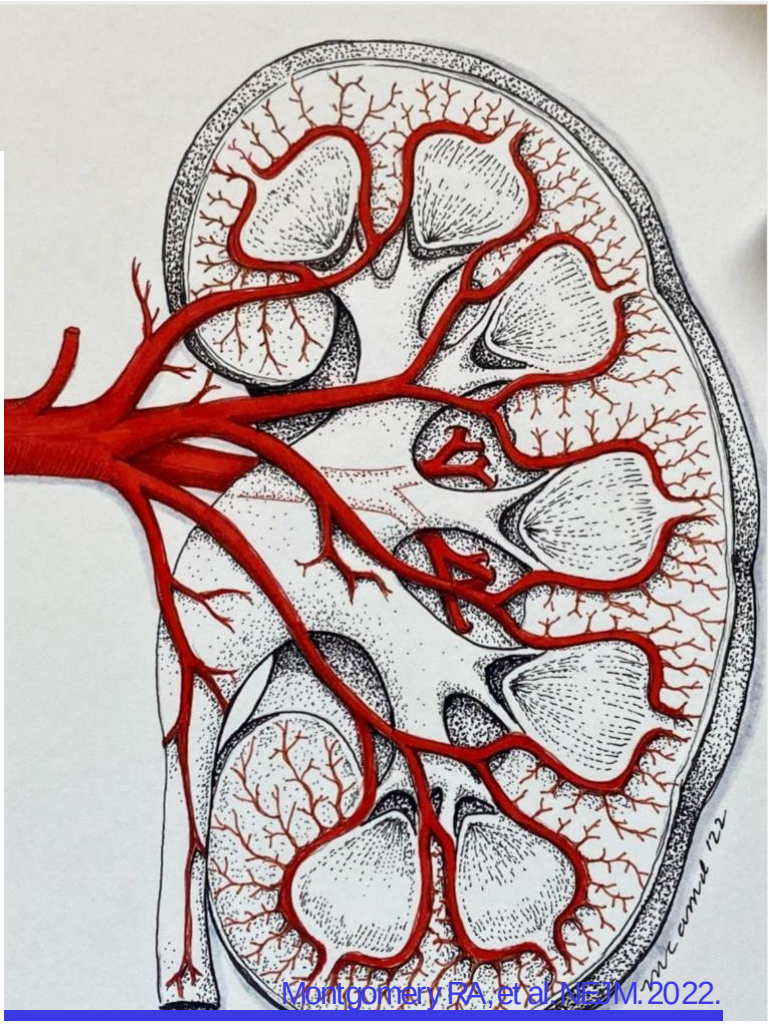
Check for updates

Nader Moazami[1], Jeffrey M. Stern[2], Karen Khalil[2], Jacqueline I. Kim[2], Navneet Narula[3], Massimo Mangiola[2], Elaina P. Weldon[2], Larisa Kagermazova[4], Les James[1], Nikki Lawson[2], Greta L. Piper[5], Philip M. Sommer[6], Alex Reyentovich[7], Daniel Bamira[7], Tajinderpal Saraon[7], Bernard S. Kadosh[7], Michael DiVita[7], Randal I. Goldberg[7], Syed T. Hussain[1], Justin Chan[1], Jennie Ngai[6], Thomas Jan[6], Nicole M. Ali[2], Vasishta S. Tatapudi[2], Dorry L. Segev[5,8], Shivani Bisen[9], Ian S. Jaffe[9], Benjamin Piegari[10], Haley Kowalski[9], Maria Kokkinaki[11], Jeffrey Monahan[11], Lori Sorrells[11], Lars Burdorf[11], Jef D. Boeke[4,12], Harvey Pass[1], Chandra Goparaju[1], Brendan Keating[13], David Ayares[11], Marc Lorber[14], Adam Griesemer[2], Sapna A. Mehta[2], Deane E. Smith[1] & Robert A. Montgomery[2]

Genetically modified xenografts are one of the most promising solutions to the discrepancy between the numbers of available human organs for transplantation and potential recipients. To date, a porcine heart has been implanted into only one human recipient. Here, using 10-gene-edited pigs, we transplanted porcine hearts into two brain-dead human recipients and monitored xenograft function, hemodynamics and systemic responses over the course of 66 hours. Although both xenografts demonstrated excellent cardiac function immediately after transplantation and continued to function for the duration of the study, cardiac function declined postoperatively in one case, attributed to a size mismatch between the donor pig and the recipient. For both hearts, we confirmed transgene expression and found no evidence of cellular or antibody-mediated rejection, as assessed using histology, flow cytometry and a cytotoxic crossmatch assay. Moreover, we found no evidence of zoonotic transmission from the donor pigs to the human recipients. While substantial additional work will be needed to advance this technology to human trials, these results indicate that pig-to-human heart xenotransplantation can be performed successfully without hyperacute rejection or zoonosis.



Montgomery RA, et al. NEJM. 2022.

2. Genetiği Değiştirilmiş Domuzdan İnsana kalp nakli

Second recipient of genetically modified pig heart dies, six weeks after surgery

By Deborah Balthazar Oct. 31, 2023

Reprints

Kliniğe geçmeden önceki insan çalışması

Received: 7 December 2021 | Revised: 15 December 2021 | Accepted: 16 December 2021

DOI: 10.1111/ajt.16930

ORIGINAL ARTICLE

AJT

First clinical-grade porcine kidney xenotransplant using a human decedent model

Paige M. Porrett[1] | Babak J. Orandi[1] | Vineeta Kumar[1] | Julie Houp[1] | Douglas Anderson[1] | A. Cozette Killian[1] | Vera Hauptfeld-Dolejsek[1] | Dominque E. Martin[2] | Sara Macedon[1] | Natalie Budd[1] | Katherine L. Stegner[1] | Amy Dandro[3] | Maria Kokkinaki[3] | Kasinath V. Kuravi[3] | Rhiannon D. Reed[1] | Huma Fatima[1] | John T. Killian Jr.[1] | Gavin Baker[1] | Jackson Perry[1] | Emma D. Wright[1] | Matthew D. Cheung[1] | Elise N. Erman[1] | Karl Kraebber[1] | Tracy Gamblin[1] | Linda Guy[1] | James F. George[1] | David Ayares[3] | Jayme E. Locke[1]

Xenograft alıcı adayı

- 18 yaş üzeri beyin ölümü gerçekleşmiş ancak organ vericisi olmaları reddedilmiş ve genetiği değiştirilmiş böbrek nakli için onam alınmış hemodinamik stabil alıcı adayları çalışmaya alınmış

Domuz Kaynağı

- Porcine renal xenografts were procured from genetically engineered (GE) pigs provided by Revivicor, Inc.
- The GE pigs harbor ten genetic modifications (10-GE pigs),
  - including targeted insertion of two human complement inhibitor genes (hDAF, hCD46),
  - two human anti- coagulant genes (hTBM, hEPCR), and two immunomodulatory genes (hCD47, hHO1),
  - as well as deletion (knockout) of 3 pig carbohydrate antigens and the pig growth hormone receptor gene.
  - Importantly, 10-GE pigs do not express red blood cell antigens and are therefore universal donors with respect to blood type.

FCXM test nakil öncesi yapılıyor

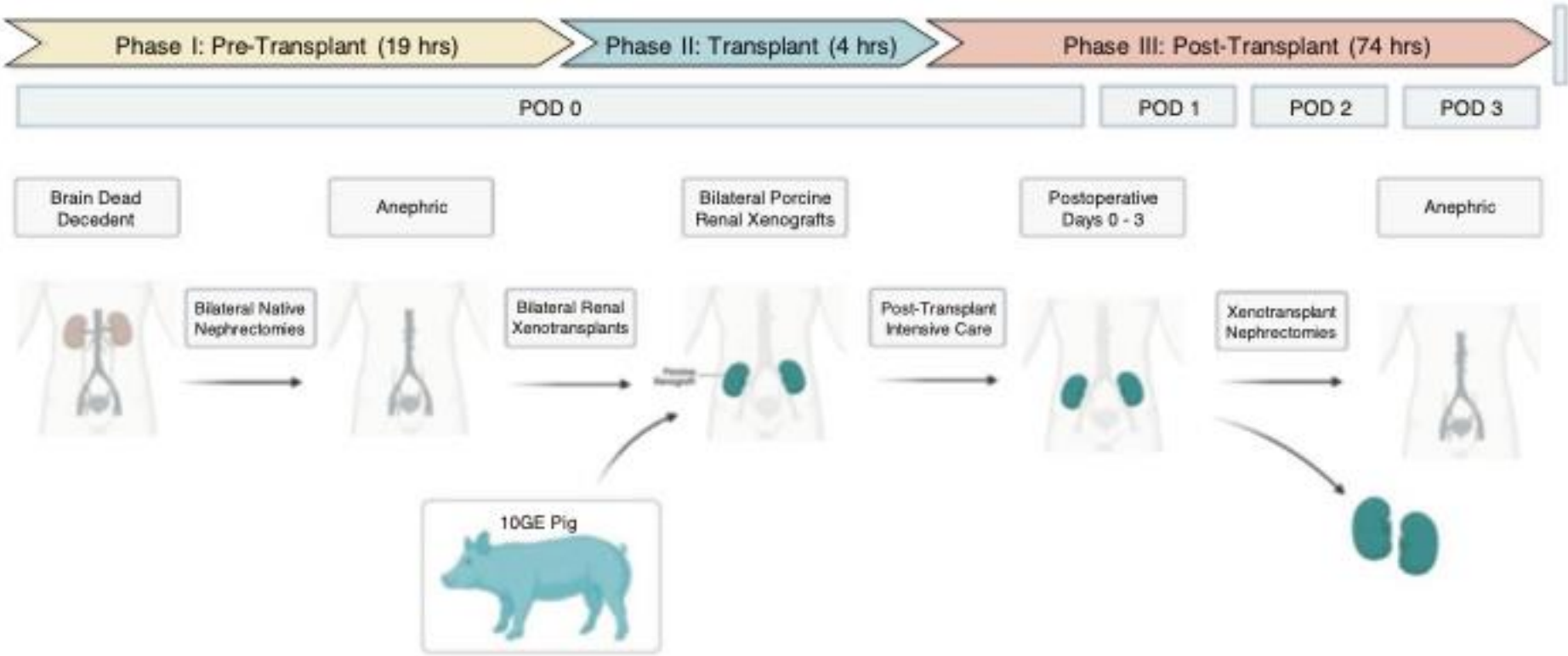
İmmunsupresyon

İndüksiyon

- Metil prednizolon (MP)
- ATG (6 mg/kg)
- Anti-CD20

İdame

- MP
- MMF
- Tacrolimus



• Family authorization • Prospective crossmatch • Xenograft procurement • Pre-transplant xenograft histology • Removal of bilateral decedent native kidneys	• Induction immunosuppression • Transplantation of right and left kidney xenografts • Visual assessment for hyperacute rejection • Visual assessment of xenograft perfusion • Post-implantation xenograft biopsies	• Induction and maintenance immunosuppression • Assessment of xenograft function • Visual inspection of xenografts, including vascular and ureteral anastomoses • Assessment of xenograft perfusion • Assessment of sensitization (anti-HLA antibodies) • Assessment for transmission of porcine endogenous retroviruses • Serial xenograft biopsies • Bilateral xenograft nephrectomies

FIGURE 1 Study timeline and event summary. Created with BioRender.com

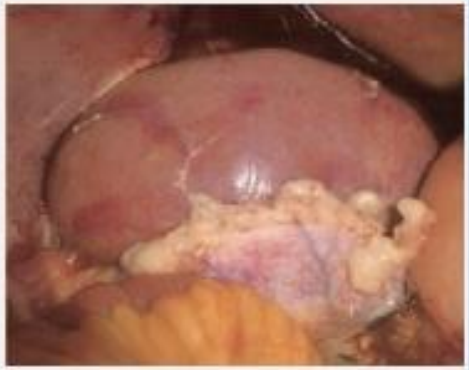
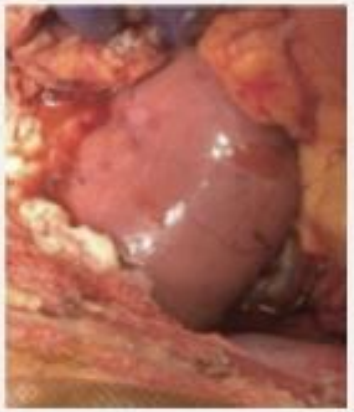
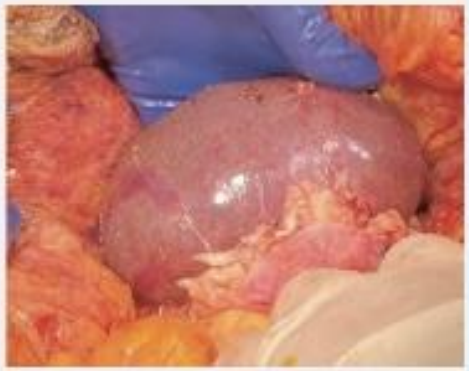
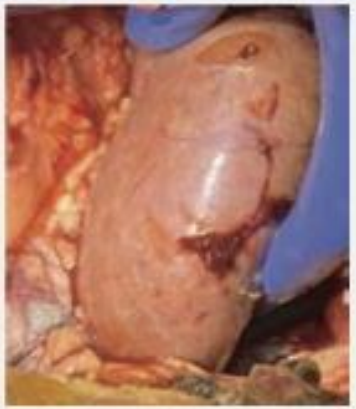
(A)

Right Porcine Renal Xenograft Reperfusion

(B)

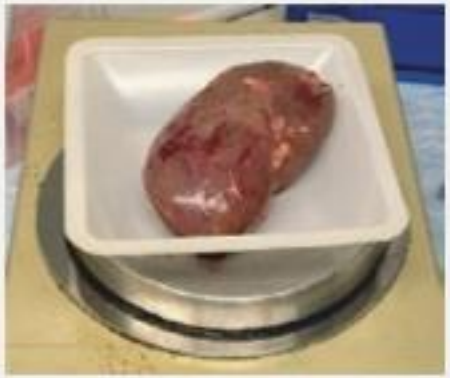
Right Porcine Renal Xenograft Urine Output

0 h 23 min (Day 0: 16:01)	0 h 40 min (Day 0: 16:18)	4 h 15 min (Day 0: 19:53)
i	ii	iii

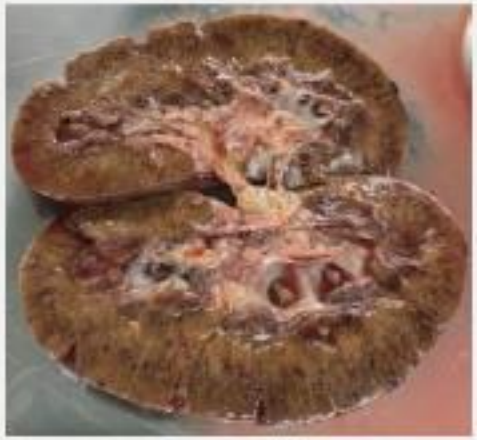
	Right	Left
POD 1	**19 h 32 min (Day 1: 10:06)** 	**16 h 41 min (Day 1: 09:56)** 
POD 3	**72 h 54 min (Day 3: 16:32)** 	**71 h 56 min (Day 3: 16:19)** 

Explant

Ex vivo (Day 3: 23:25)



Ex vivo (Day 3: 23:22)



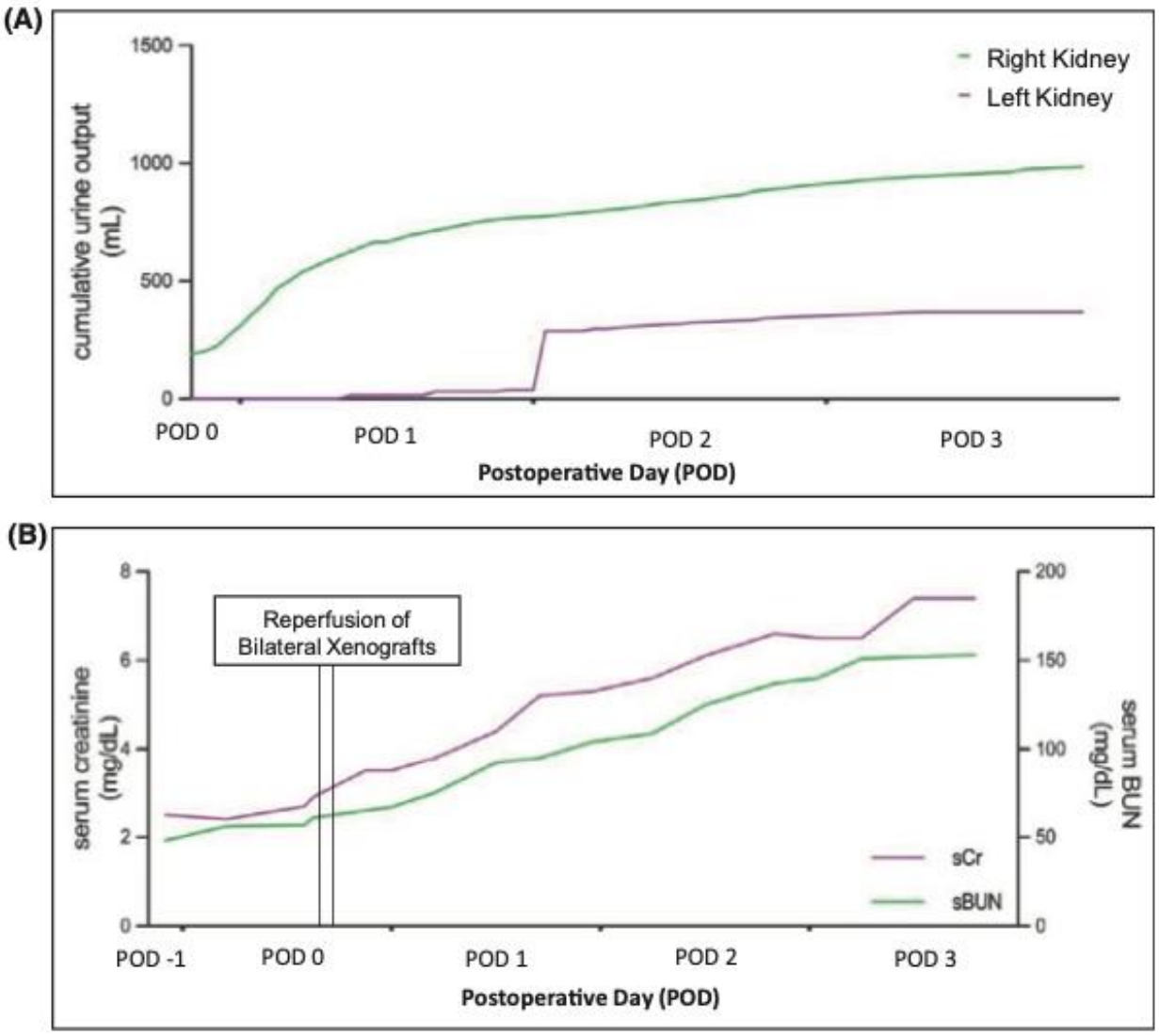


FIGURE 6 Porcine renal xenotransplant function in the human decedent. (A) Cumulative posttransplant urine output from transplantation to study end from right and left xenografts. (B) BUN and creatinine in the decedent's serum. Results prior to POD 0 reflect function of decedent's native kidneys prior to native nephrectomies

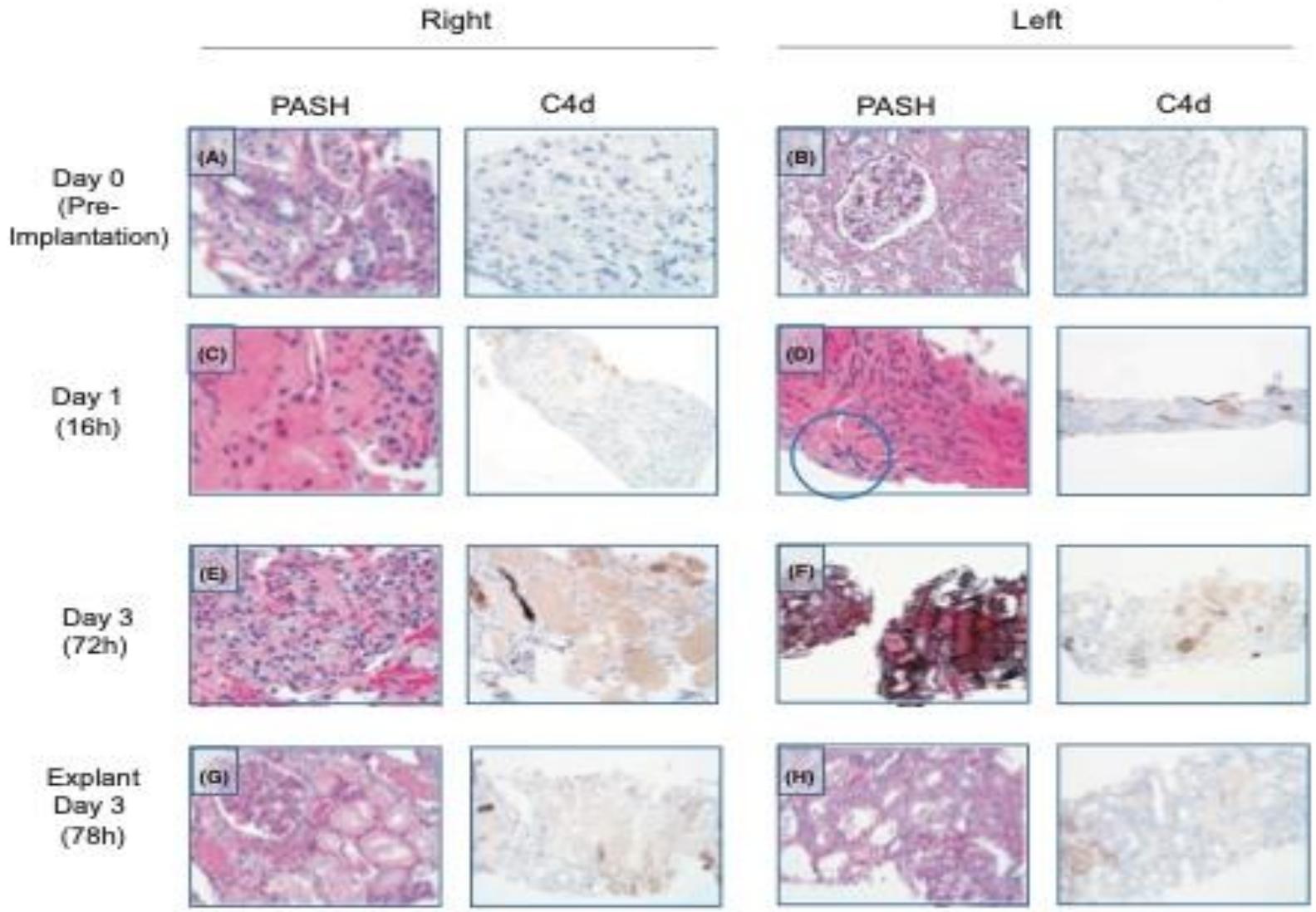


FIGURE 7 Serial histologic examination of the porcine kidney xenografts. All biopsies represent core biopsies. (A, B, G, and H) Were obtained *ex vivo*. (C, D, E and F) Were obtained *in vivo*. Sections are stained with PASH and are 10X, except for (C and D) (40X) and (F) (silver stain). C4d negative throughout. (A and B) Mild to moderate acute tubular injury from cold ischemia. Normal appearance of the capillary network, the mesangium, and the podocytes. (C and D) Glomerulus with multiple fibrin thrombi (blue circle). There is diffuse glomerular capillary congestion with swollen endothelial cells and near complete obliteration of the peripheral capillary lumina. There is presence of fibrin thrombi and fragmented red blood cells consistent with thrombotic microangiopathy (TMA). There is evidence of progressive tubular injury with extensive acute tubular necrosis (ATN). No mesangiolysis is appreciated. (E and F) Glomerular congestion and acute tubular necrosis. Endothelial cells remain segmentally swollen with partially obliterated lumina and rare fibrin thrombi with improvement of glomerular injury. (G and H) Acute tubular injury persists. Glomeruli with segmental endothelial swelling. No fibrin thrombi

Histologic findings on post-operative day 1

- were consistent with thrombotic microangiopathy, TMA
- with diffuse glomerular capillary congestion, KONJESYON
- swollen endothelial cells,
- and near complete obliteration of the peripheral capillary lumina along with the presence of fibrin thrombi. (FİBRİN TROMBUS)

On post-operative day 3

- there was evidence of progressive tubular injury with extensive acute tubular necrosis, (ATN)
- but additional features of TMA including mesangiolysis were not observed.
- C4d was negative at both time points as well as IgM, IgG, IgA, C1q, and C3.
- Wedge biopsies from study termination demonstrated no evidence of cortical necrosis or interstitial hemorrhage
- and glomerular capillary congestion was no longer diffuse (data not shown).
- Post-termination analysis of renal tissue confirmed expression of the human transgenes within the porcine kidney parenchyma (Figure S11).

ORIGINAL ARTICLE

Results of Two Cases of Pig-to-Human Kidney Xenotransplantation

Robert A. Montgomery, M.D., D.Phil., Jeffrey M. Stern, M.D., Bonnie E. Lonze, M.D., Ph.D., Vasishta S. Tatapudi, M.D., Massimo Mangiola, Ph.D., Ming Wu, M.D., Elaina Weldon, M.S.N., A.C.N.P.-B.C., Nikki Lawson, R.N., Cecilia Deterville, M.S., Rebecca A. Dieter, Pharm.D., B.C.P.S., Brigitte Sullivan, M.B.A., Gabriella Boulton, B.A., Brendan Parent, J.D., Greta Piper, M.D., Philip Sommer, M.D., Samantha Cawthon, B.S., Erin Duggan, M.D., David Ayares, Ph.D., Amy Dandro, M.S., Ana Fazio-Kroll, Ph.D., Maria Kokkinaki, Ph.D., Lars Burdorf, M.D., Ph.D., Marc Lorber, M.D., Jef D. Boeke, Ph.D., Harvey Pass, M.D., Brendan Keating, Ph.D., Adam Griesemer, M.D., Nicole M. Ali, M.D., Sapna A. Mehta, M.D., and Zoe A. Stewart, M.D., Ph.D.

ABSTRACT

BACKGROUND

Xenografts from genetically modified pigs have become one of the most promising solutions to the dearth of human organs available for transplantation. The challenge in this model has been hyperacute rejection. To avoid this, pigs have been bred with a knockout of the alpha-1,3-galactosyltransferase gene and with subcapsular autologous thymic tissue.

METHODS

We transplanted kidneys from these genetically modified pigs into two brain-dead human recipients whose circulatory and respiratory activity was maintained on ventilators for the duration of the study. We performed serial biopsies and monitored the urine output and kinetic estimated glomerular filtration rate (eGFR) to assess renal function and xenograft rejection.

RESULTS

The xenograft in both recipients began to make urine within moments after reperfusion. Over the 54-hour study, the kinetic eGFR increased from 23 ml per minute per 1.73 m^2 of body-surface area before transplantation to 62 ml per minute per 1.73 m^2 after transplantation in Recipient 1 and from 55 to 109 ml per minute per 1.73 m^2 in Recipient 2. In both recipients, the creatinine level, which had been at a steady state, decreased after implantation of the xenograft, from 1.97 to 0.82 mg per deciliter in Recipient 1 and from 1.10 to 0.57 mg per deciliter in Recipient 2. The transplanted kidneys remained pink and well-perfused, continuing to make urine throughout the study. Biopsies that were performed at 6, 24, 48, and 54 hours revealed no signs of hyperacute or antibody-mediated rejection. Hourly urine output with the xenograft was more than double the output with the native kidneys.

CONCLUSIONS

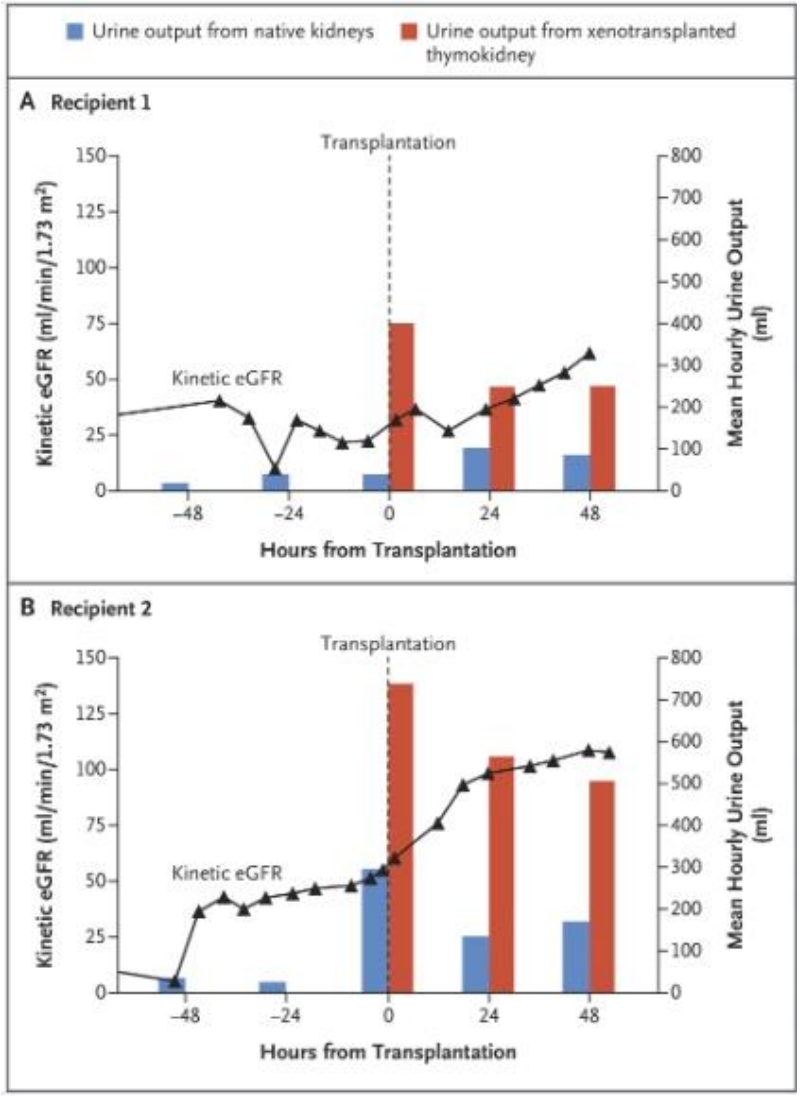
Genetically modified kidney xenografts from pigs remained viable and functioning in brain-dead human recipients for 54 hours, without signs of hyperacute rejection. (Funded by Lung Biotechnology.)

From the New York University (NYU) Langone Transplant Institute (R.A.M., J.M.S., B.E.L., V.S.T., M.M., E.W., N.L., C.D., R.A.D., B.S., G.B., G.P., N.M.A., S.A.M., Z.A.S.), the Departments of Pathology (M.W.), Anesthesia (P.S.), Biochemistry and Molecular Pharmacology (J.D.B.), and Cardiothoracic Surgery (H.P.), and the Institute for Systems Genetics (J.D.B.), NYU Langone Health, the Department of Population Health, Division of Medical Ethics (B.P.), NYU Grossman School of Medicine (S.C.), and the Columbia Center for Translational Immunology and the Department of Surgery, Columbia University (E.D., A.G.) — all in New York; Revivicor, Blacksburg, VA (D.A., A.D., A.F.-K., M.K., L.B.); United Therapeutics, Silver Spring, MD (M.L.); and the Department of Surgery, University of Pennsylvania, Philadelphia (B.K.). Dr. Montgomery can be contacted at robert.montgomery@nyulangone.org or at NYU Langone Health, 550 First Ave., New York, NY 10016.

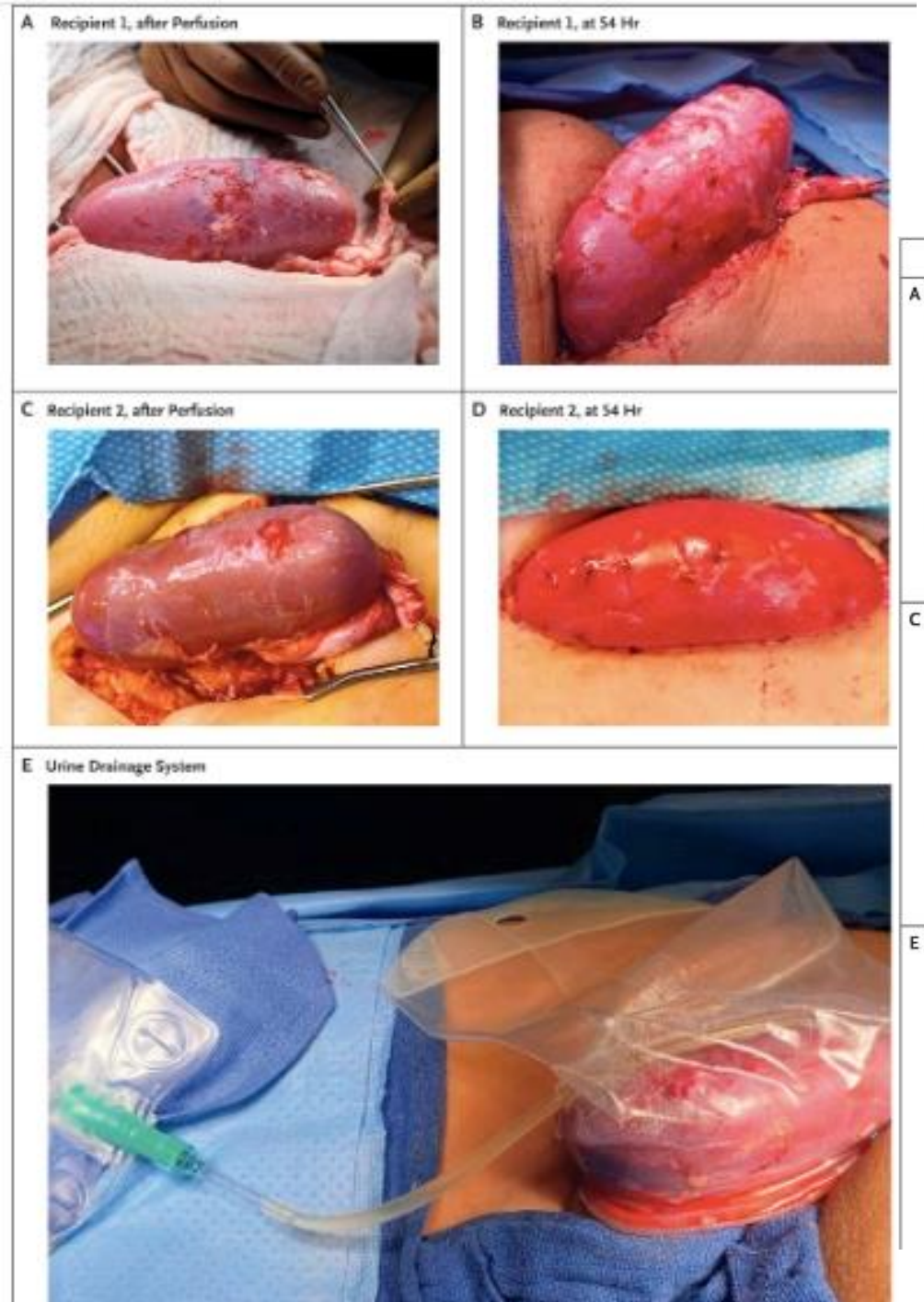
N Engl J Med 2022;386:1889-98.
DOI: 10.1056/NEJMoa2120238

Mayıs
2022

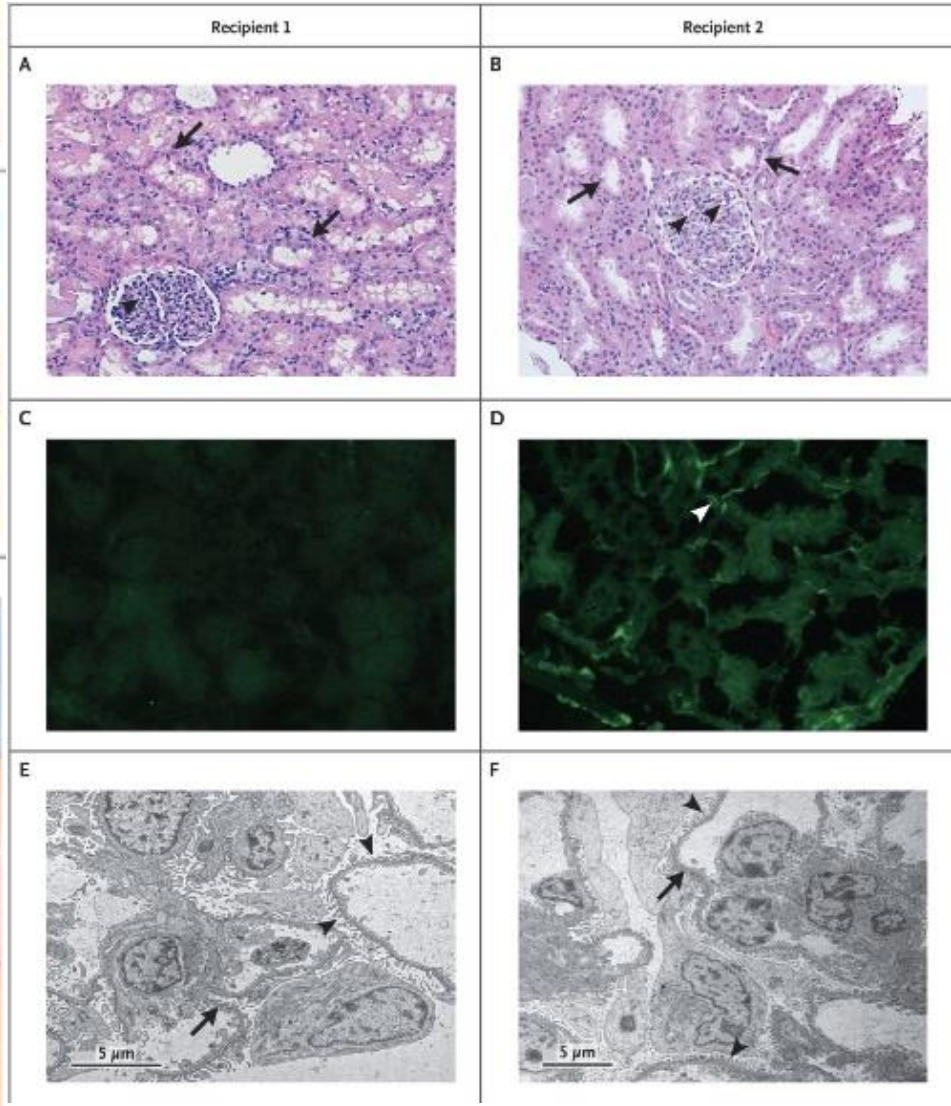
1000 mg/gün Prednisolon
2x1000 mg MMF IV



PRA iki alıcıda da negatif
CDC XM
1. Olguda düşük CDC pozitifliği
2. Olguda CDC XM daha güçlü pozitif



54 saatte
Hiperakut R. AMR veya T hücreli AR yok
TMA (-), C4d (-) Kapiller fibrin trombüs (-)
Trombositopeni yok



RESEARCH LETTER

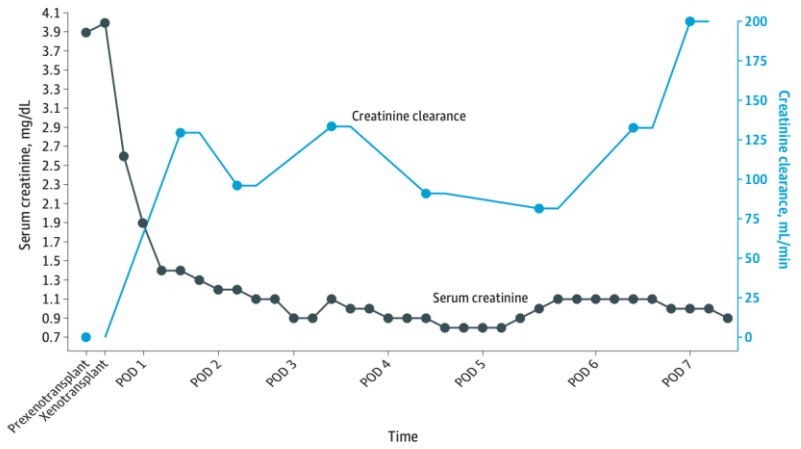
Normal Graft Function After Pig-to-Human Kidney Xenotransplant

JAMA Surgery Published online August 16, 2023

Jayme E. Locke, MD, MPH
Vineeta Kumar, MD
Douglas Anderson, MD
Paige M. Porrett, MD, PhD

Results | A male in his 50s who was declared brain dead and had acute kidney injury superimposed on a history of CKD (stage 2) and hypertension underwent bilateral native nephrectomy and cessation of dialysis followed by crossmatch-compatible xenotransplant with 10-gene–edited pig kidneys (UKidney). The decedent received a complement inhibitor (anti-C5; eculizumab) 24 hours before xenotransplant followed by standard induction therapy, including a solumedrol taper, antithymocyte globulin (6 mg/kg total), and rituximab. Maintenance immunosuppression included tacrolimus, mycophenolate mofetil, and prednisone. Goal tacrolimus levels (8-10 ng/dL) were reached by postoperative day (POD) 2 and maintained through study completion. Xenografts were transplanted en bloc with pig vasculature anastomosed to the decedent's right-side common iliac artery and distal inferior vena cava and the pig ureters anastomosed to the decedent's bladder. Within 4 minutes of reperfusion, the xenografts made urine, producing more than 37 L in the first 24 hours. Urine concentrated over time, with concurrent decreases in urine volume to a median of 14.1 L (IQR, 13.8-20 L) on PODs 1 to 3 and a median of 5.1 L (IQR, 5-6 L) on PODs 4 to 7. Before xenotransplant, serum creatinine was 3.9 mg/dL after cessation of dialysis and bilateral native nephrectomy. After xenotransplant, serum creatinine decreased to 1.9 mg/dL within the first 24 hours, normalized to 1.1 mg/dL at 48 hours, remained within normal limits through study duration, and was 0.9 mg/dL on POD 7 at study completion. Creatinine clearance also improved (POD 0, 0 mL/min; POD 7, 200 mL/min) (**Figure 1**).

Figure 1. Kidney Function Over Time After 10-Gene–Edited Pig-to-Human Xenotransplant



Xenograft-associated declining serum creatinine and increasing creatinine clearance in the absence of native kidneys and dialysis were consistent with life-sustaining kidney function after pig-to-human kidney xenotransplant. POD indicates postoperative day.

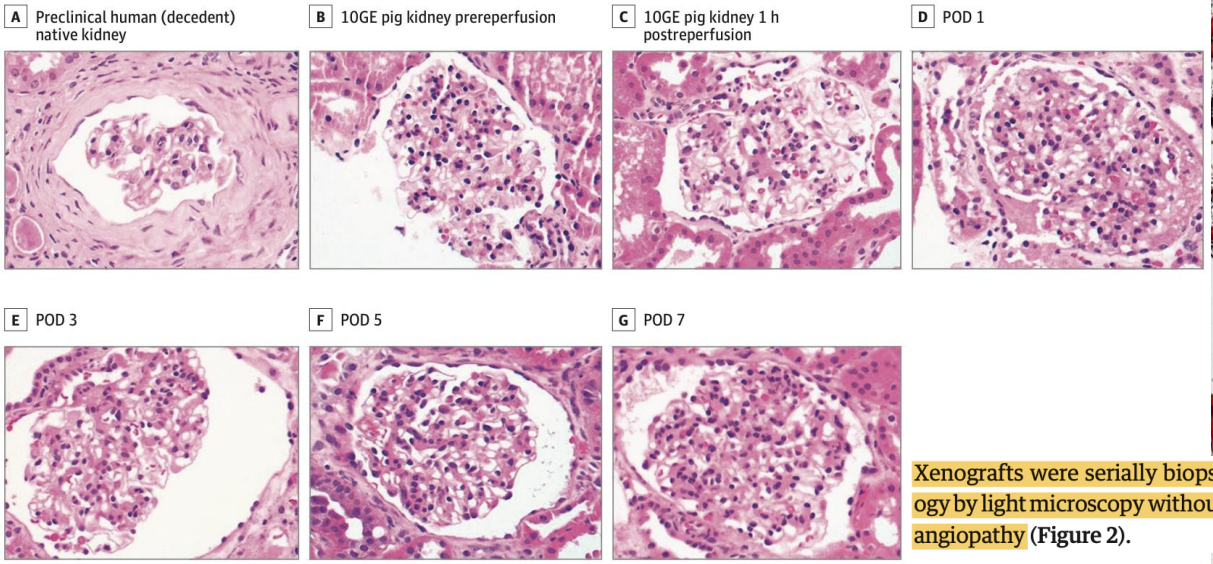
RESEARCH LETTER

Normal Graft Function After Pig-to-Human Kidney Xenotransplant

JAMA Surgery Published online August 16, 2023

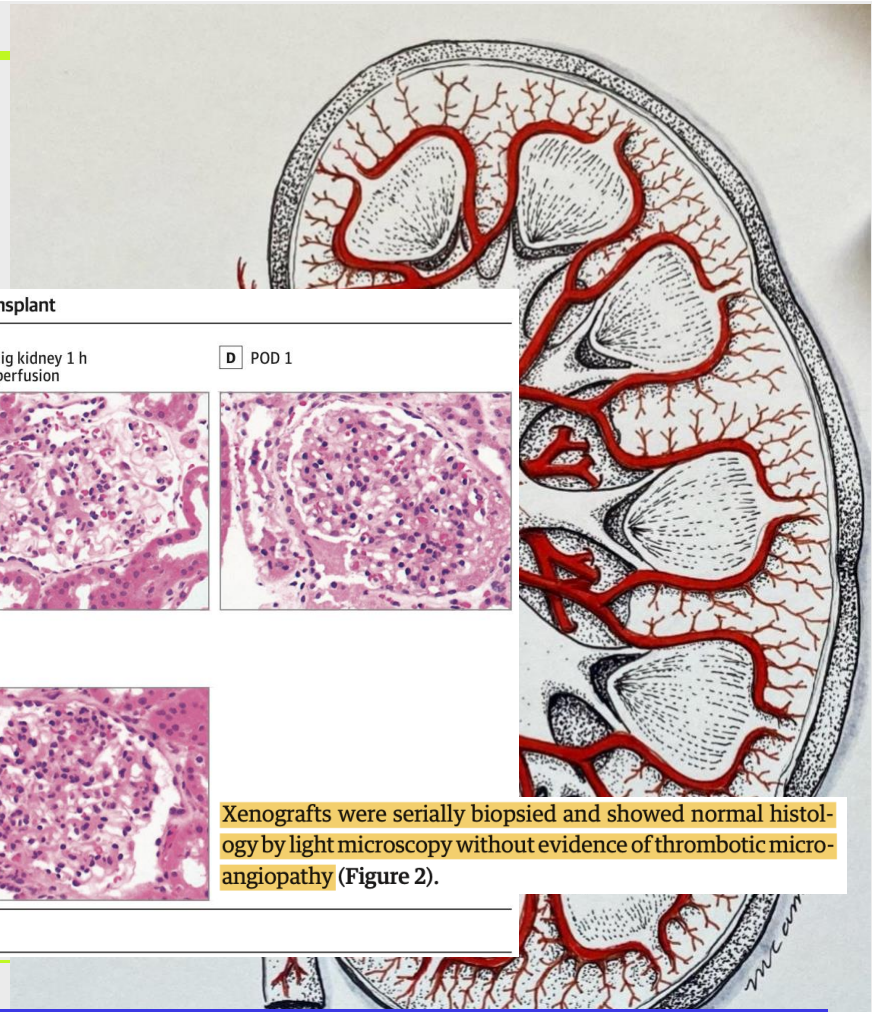
Jayme E. Locke, MD, MPH
Vineeta Kumar, MD
Douglas Anderson, MD
Paige M. Porrett, MD, PhD

Figure 2. Kidney Histopathology After 10-Gene–Edited (10GE) Pig-to-Human Xenotransplant



Original magnification ×40. POD indicates postoperative day.

Xenografts were serially biopsied and showed normal histology by light microscopy without evidence of thrombotic microangiopathy (**Figure 2**).

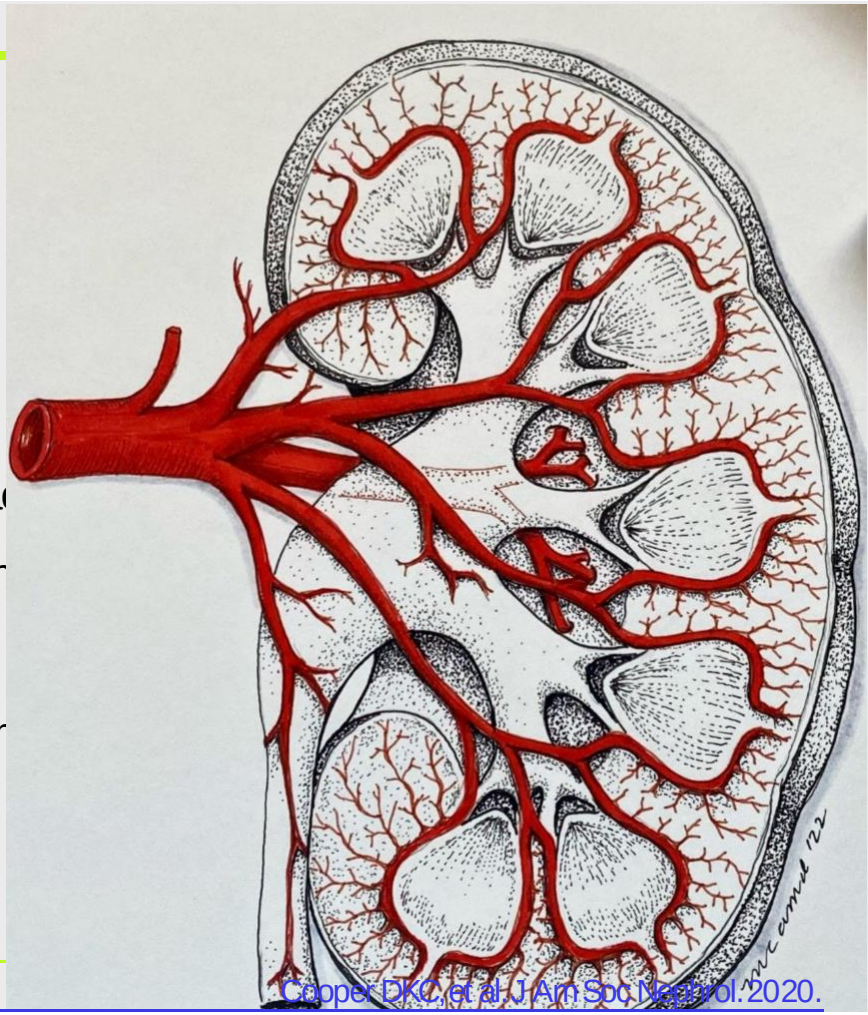


RESEARCH, INNOVATION, PRESS RELEASES | SEPTEMBER 14, 2023

Two-Month Study of Pig Kidney Xenotransplantation Gives New Hope to the Future of the Organ Supply

Xenotransplantasyon Başarılanlar

- Yapılan böbrek biyopsilerinde hiperakut, antikor-ar a
- Hemodinamik instabilite ve biyokimyasal inflamasyor
- İdrar çıkış ve GFR'de artış sağlandı.
- Domuz kaynaklı retrovirüs (PERV) aktivasyonu izlenr



Cooper DKC, et al. J Am Soc Nephrol. 2020.

Table 4. Potential conditions for which initial clinical trials of pig kidney xenotransplantation may be justified

Elderly patients without significant concomitant disease
Patients of blood group B or O often wait for >5 yr for a suitable donor. The mortality of waitlist patients is 40% at 5 yr, and so many of these patients, particularly in the age range 55–65 yr, will not survive until a deceased human kidney becomes available.
Recurrent kidney disease
Recurrent FSGS
Recurrence can be very rapid in some patients. If recurrence occurs rapidly in the pig kidney, this may not be a valid test of xenotransplantation.
Other potentially recurrent diseases
Recurrence is slower in several other disease states, *e.g.*, Ig A nephropathy, membranoproliferative GN type 2, and so these patients might possibly be candidates for a trial of pig kidney transplantation.
High sensitization to HLA
There is evidence for some cross-reactivity between anti-HLA antibodies and swine leukocyte antigens, suggesting that patients sensitized to HLA should be excluded from the first clinical trials.
Loss of vascular access for dialysis
These patients have often been on dialysis for some time (years rather than months) and may have diseased blood vessels making kidney transplantation technically difficult. They are frequently less than ideal candidates even for allotransplantation.

Cooper DKC, 2020, JASN

16 Mart 2024

In a First, Genetically Edited Pig Kidney Is Transplanted Into Human

Procedure marks milestone in quest to provide more organs to patients in need

By MASS GENERAL BRIGHAM COMMUNICATIONS | March 21, 2024 | Research, Care Delivery
7 min read

RS 62Y, Male
DM
HT ASKH
African American
Vascular Access problem
2. Nakil (İlk nakil 5 yıl)
69 gen edit
Tegoprubart Eledon Pharm
- Anti CD40L
Ravulizumab Alexion

The modified kidney was provided by eGenesis,
a xenotransplantation therapy company co-founded by HMS geneticist George Church and former HMS postdoctoral fellow Luhan Yang.
Over the past five years, Mass General and eGenesis have conducted extensive research, with findings published in *Nature* in Oct. 2023.

3 knockout
7 genetik ekleme
59 gen editing

MEDTECH

Patient discharged with eGenesis' genetically engineered pig kidney after successful xenotransplant procedure

By **Conor Hale** · Apr 4, 2024 12:27pm

eGenesis | xenotransplantation | CRISPR | Massachusetts General Hospital

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World's first pig kidney transplant recipient discharged from hospital

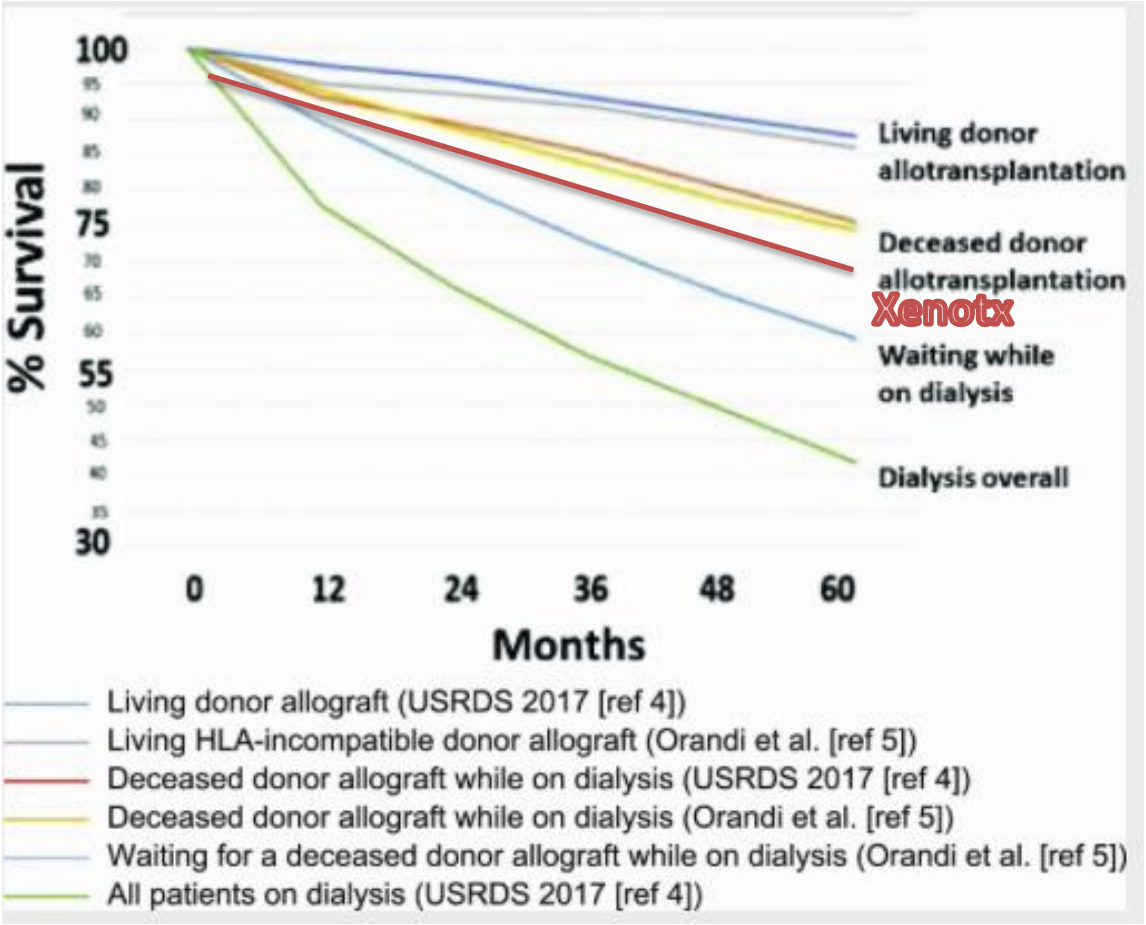
Sorunlar

- Etik şartlar
- Hangi genetiği değiştirilmiş domuz modeli daha uygun ?
  - Domuz sağlayıcı firmaların regülasyonu
- Xenoturizm-Donor Domuz ticareti-getirtilmesi ?
- Daha genişletilmiş nakil alıcı endikasyonları
- Hasta allogenetik nakil bekleme listesinde kalacak mı?
- 2 böbrek nakil vs 1 böbrek ?
- PERV takibi
- Dini görüş ?

İslam ve Yahudilikte Domuz makbul bir hayvan değil

Böbrek Nakli

- Xenotransplantasyon sonuçları : beklenti



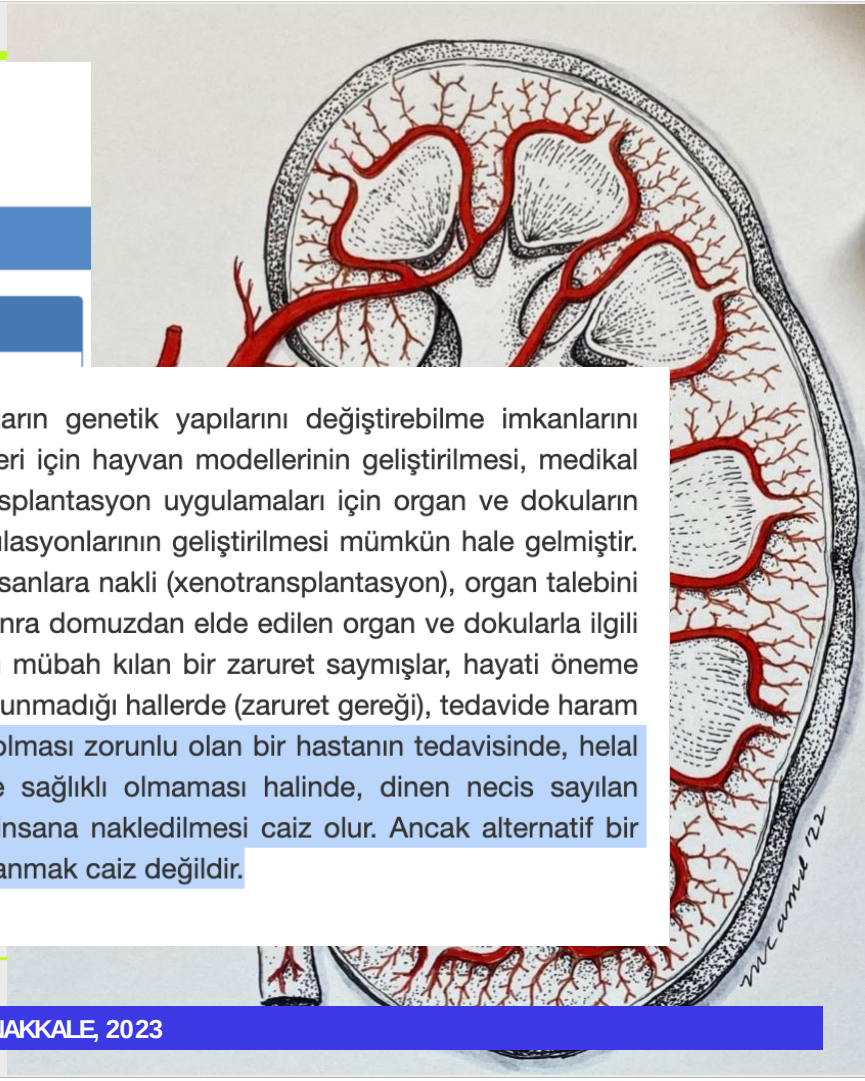
USRDS,2020

Din İşleri Yüksek Kurulu

Kurumsal | Kurul | Karar ve Mütalaalar | Yayınlar | Alo 190 / Fetva Hattı

Geçmiş Sorularım

Biyoteknoloji ve genetik alanındaki gelişmeler, bilim insanlarına hayvanların genetik yapılarını değiştirebilme imkanlarını sağlamıştır. Hayvanlarda biyomedikal araştırmalar ile hastalıklar ve tedavileri için hayvan modellerinin geliştirilmesi, medikal önemi olan hormon ve enzimlerin hayvanlarda üretilmesi, türler arası transplantasyon uygulamaları için organ ve dokuların geliştirilmesi ve kısa yoldan istenen verim özelliklerini taşıyan hayvan popülasyonlarının geliştirilmesi mümkün hale gelmiştir. Ayrıca türler arası nakil, özellikle çiftlik hayvanlarının organ ve dokularının insanlara nakli (xenotransplantasyon), organ talebini karşılamaya bir çözüm olarak gözükmektedir. Bu genel bilgilendirmeden sonra domuzdan elde edilen organ ve dokularla ilgili sorunuza gelince; İslam alimleri açlık ve susuzluk gibi, hastalığı da haramı mübah kılan bir zaruret saymışlar, hayati öneme sahip bir tedavinin helal olan nesneler ve yöntemlerle yapılabilme imkanı bulunmadığı hallerde (zaruret gereği), tedavide haram olan nesnelerden de yararlanılmasını caiz görmüşlerdir. Bu itibarla, tedavi olması zorunlu olan bir hastanın tedavisinde, helal yollarla bir alternatif bulunmaması ya da bulunan çözümlerin verimli ve sağlıklı olmaması halinde, dinen necis sayılan hayvanların dokularının ve bu hayvanlarda geliştirilen doku ve organların insana nakledilmesi caiz olur. Ancak alternatif bir tedavi yöntemi olduğu sürece dinen haram olan yol ve yöntemlerden faydalanmak caiz değildir.



SONUÇ

- Xenotransplantasyon önümüze gelecek
  - Hasta (kime ?)
  - Genetik değişim yapan domuz organ sağlayıcı
    - Revivicor, eGenesis
  - Cerrahi (Güvenli operasyon ve postop. Bakım)
  - Nefroloji – Tedavi ve Yönetimi
  - İmmünolog (Hangi testler, XM ?, PRA ??)
  - İnfeksiyon takibi (Anti viral tedaviler ?)

The future of xenotransplantation is brighter than at any previous time because what must be done to succeed has become remarkably clear.
Thomas Starzl -2007

"Xenotransplantation is ju
but it may be a very

Sir Roy Calne, 1995