# Xenotransplantasyon : Güncel durum ve gelecek perspektifi

**Dr. Oğuz Söylemezoğlu**

# XENOTRANSPLANTASYON NEDİR ? Neden tartışılıyor?

**XENOTRANSPLANTASYON**

- Genetik olarak modifiye edilen hayvan organlarının insanlara transplantasyonu
- Böbrekler için genetik olarak modifiye edilmiş domuz organları kullanılıyor

- Bu gelişme organ bekleyen binlerce kişi için yüksek sayıda bir organ sağlanması için büyük umut oldu .

# İHTİYAÇ

Dünya genelinde 5-10 milyon Renal Replasman tedavisi gerekiyor

2.5 milyon Böbrek Nakli alıcısı var

İdame hemodiyaliz tedavisindeki hasta survisi 5 yıl için % 42

Bekleme listesinde böbrek nakli bekleyen hastaların %40 ı 5 yıl içinde ex oluyor

SDBY hastalarının 1/7 si bekleme listesinde

| Condition | 5 year survival |
|---|---|
| Prostate Cancer | 99% |
| Breast Cancer | 91% |
| Bladder Cancer | 79% |
| Colon Cancer | 66% |
| ESKD Peritoneal Dialysis | 51.7% |
| ESKD Hemodialysis | 41.8% |
| Lung Cancer | 19% |

## 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) |

| TÜRKİYE ORGAN VE DOKU BEKLEME LİSTESİ (Eki.2024) | |
|---|---|
| BÖBREK | 25.245 |
| KARACİĞER | 2.650 |
| KALP | 1.477 |
| AKCİĞER | 203 |
| PANKREAS | 275 |
| İNCE BARSAK | 1 |
| KORNEA | 3.647 |
| TOPLAM | 33.498 |

# Hayvan ve İnsan vücut parçalarının birleştirilmesinin mitolojik kökleri var

Minotaur

Gorgon

Ganesha

# Mathieu Jaboulay

. 1906- ilk solid organ böbrek xenotransplantasyon prosedürü
. 48 yaş kadın: oligüri, hipertansiyon başağrısı, görme işitme kaybı
. Organ kaynağı: domuz

https://commons.wikimedia.org/wiki/File:Professeur_Jaboulay_CIPH0224.jpg

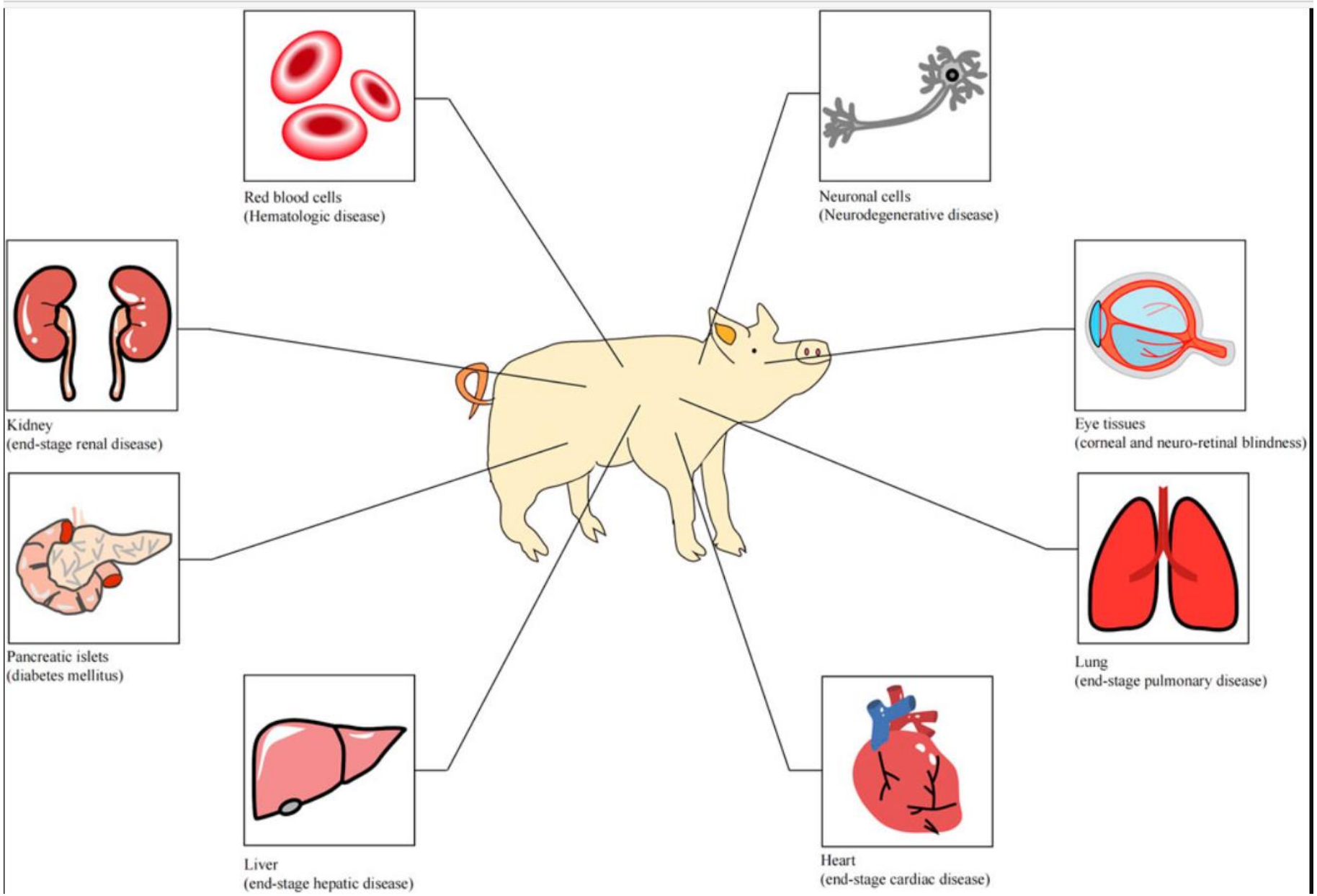
**1906:** Pig and goat kidneys transplanted onto arm vessels survive for 3 days

**1910:** A macaque kidney transplanted into a human survives for 32 hours

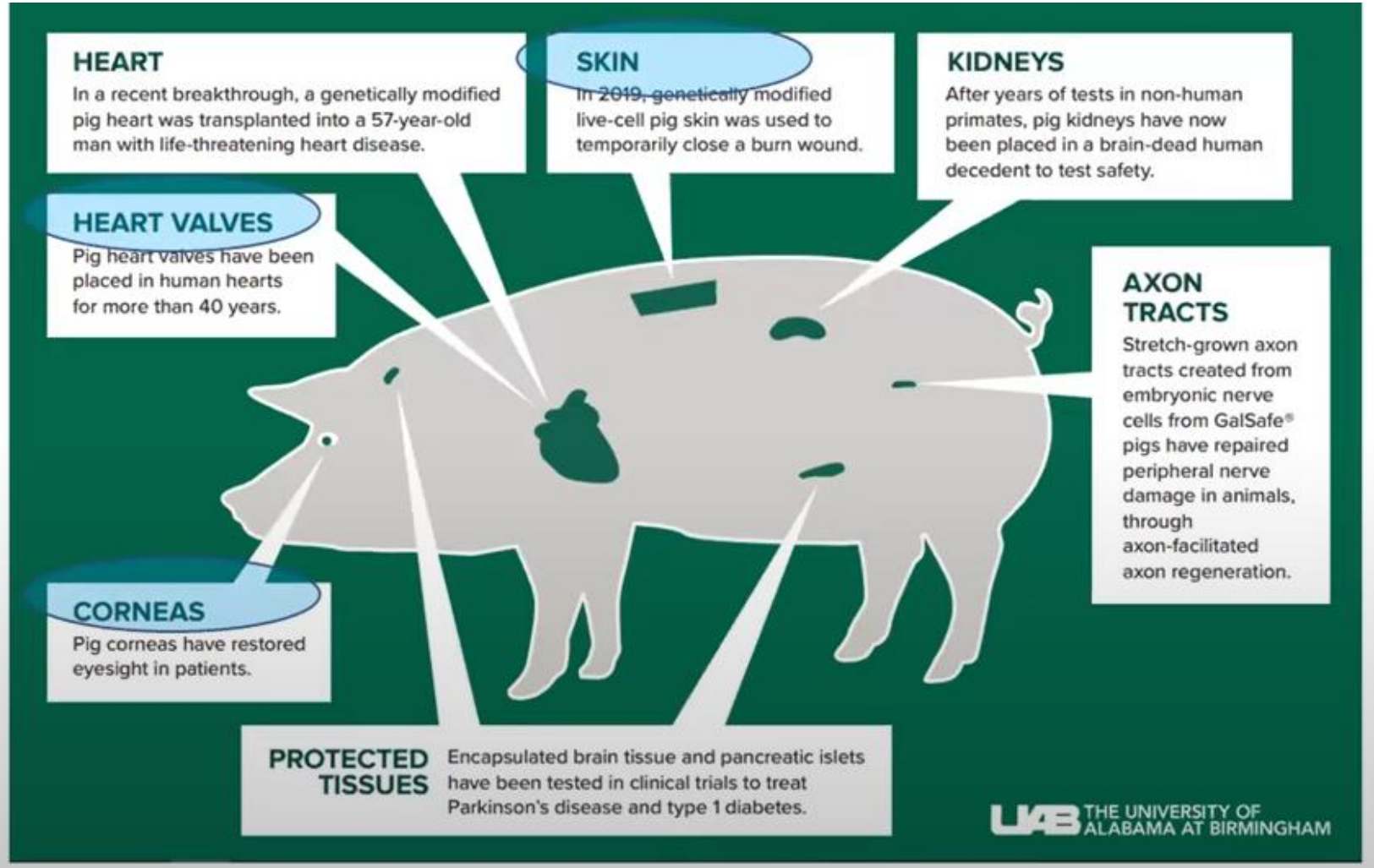
**1963:** A 23-year-old woman survives for 9 months with functioning chimpanzee kidneys

**1984:** A newborn, Baby Fae, survives for 20 days with a baboon heart

# Domuz dokularının kullanımı

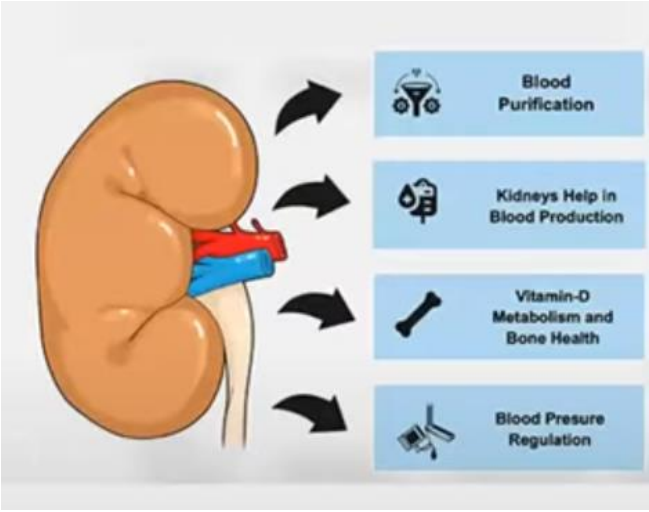


# Domuz dokularının Medikal kullanımı umut verici



# Neden Domuzlar ?

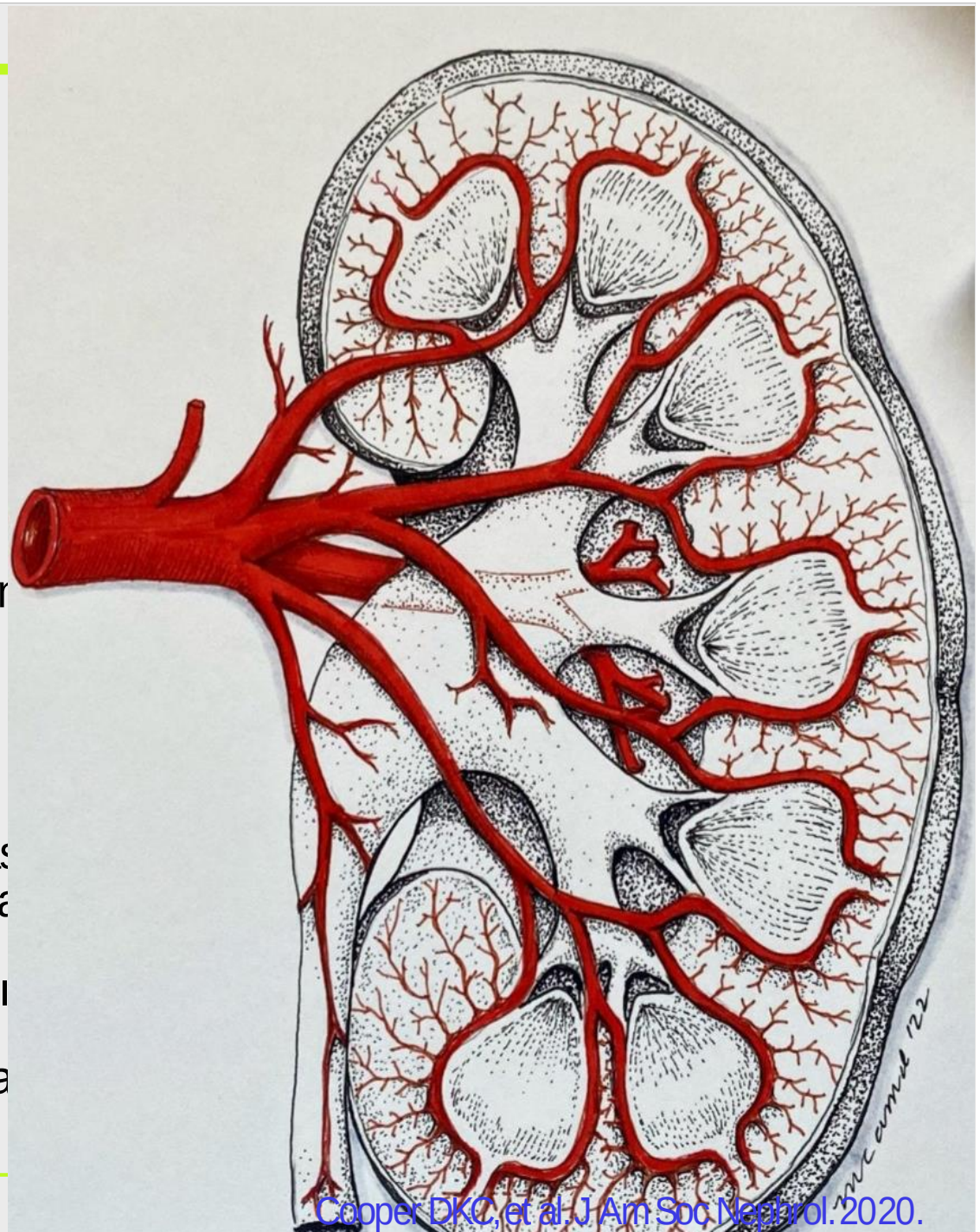

- Domuz böbrekleri insan böbrek büyüklük ve fonksiyonuna benzer özellikler taşıyor
  - Sıvı ve atık atımı
  - Kan basıncı kontrolü
  - Vitamin ve mineral dengesi
  - Boyut insan böbreği ile benzer
  - Kısa sürede Çok sayıda elde ediliyor
  - Rejeksiyonu önlemek için Genetik olarak modifiye edilebiliyor
  - İnfeksiyon geçiş riski az




- Domuz böbrek yaşam süresinin 30 yıl civarında olması allotransplant (insan donor böbreği ) ile benzer.

# Xenotransplantasyon Avantajları

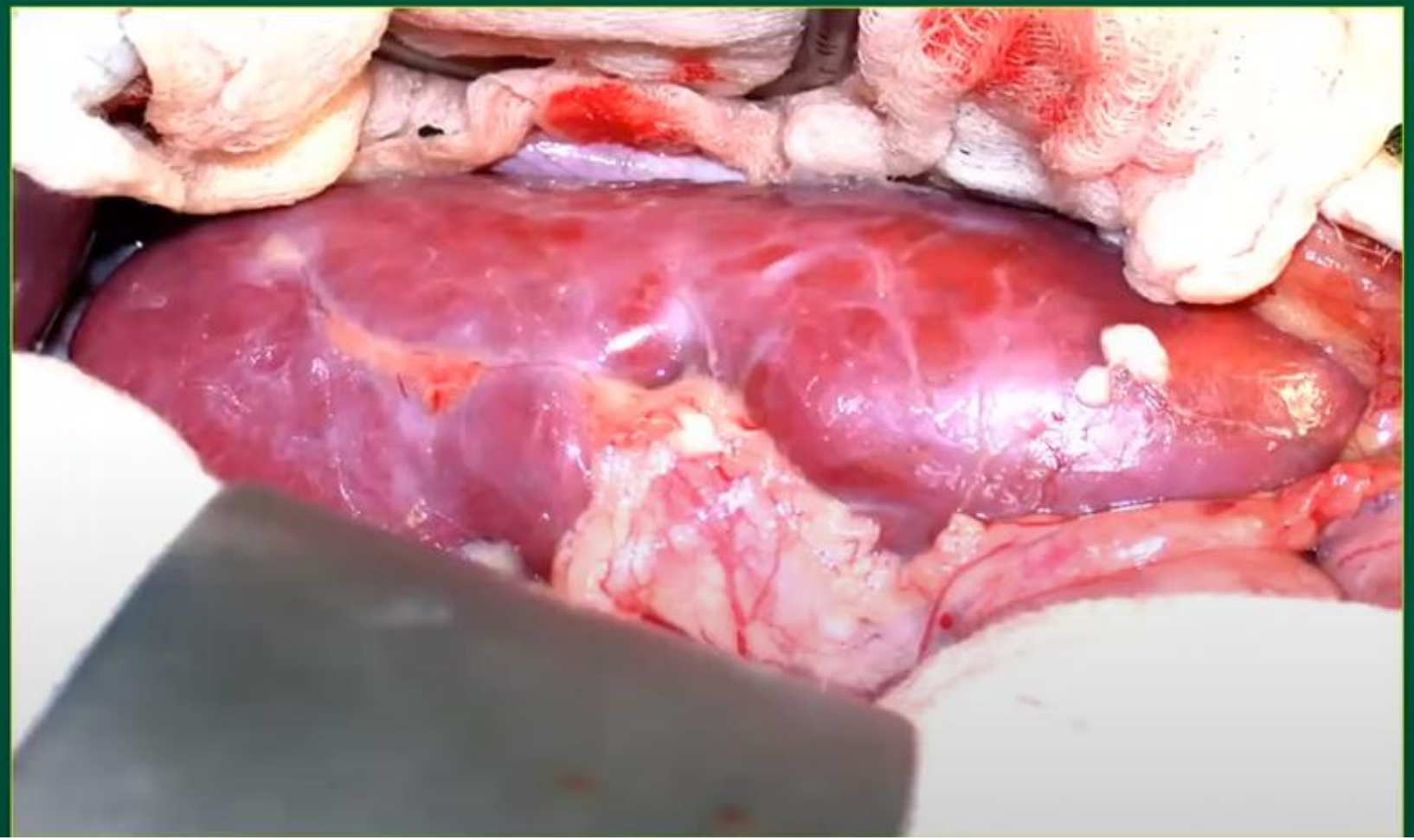
- Sınırsız donor organ kaynağı sağlaması
- Acil veya elektif ihtiyacın karşılanması
- Beyin ölümünün donor organlara olumsuz etkileri ortadan
- "Borderline" nakil adayları için de organ sağlanabilmesi
  - Kontrolsüz DM, ASKH, SVH, Periferik arter hastalığı gibi
- Nakil öncesi donorün ayrıntılı mikrobiyoloji 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



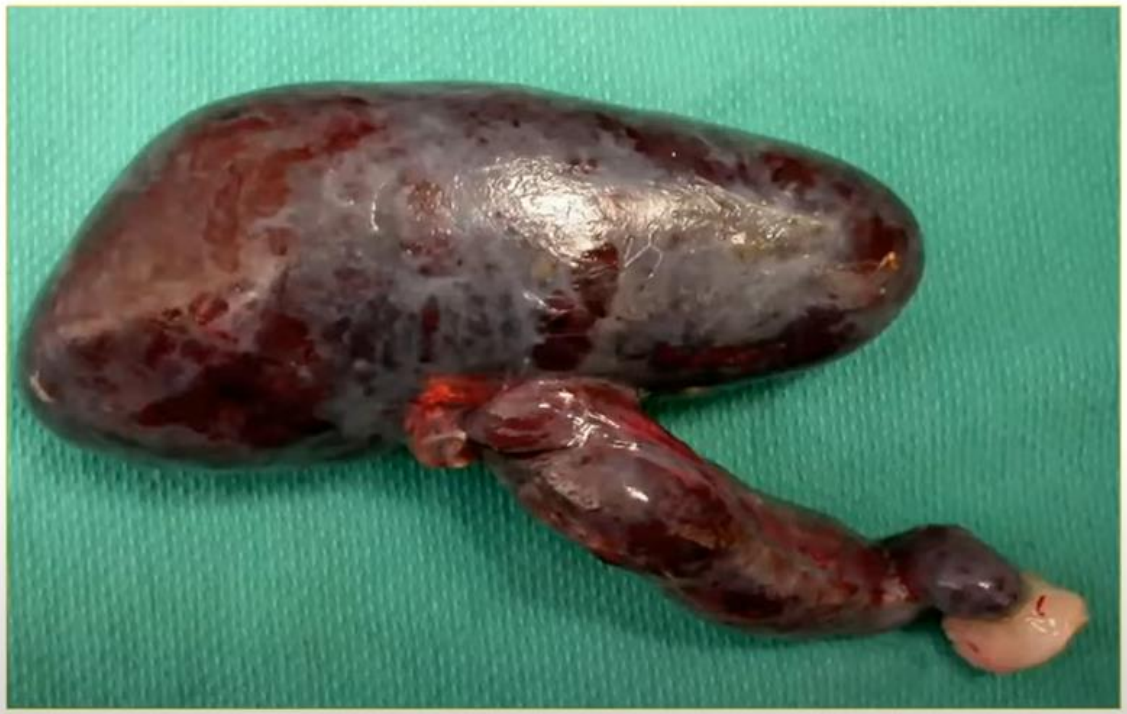
Cooper DKC, et al. J Am Soc Nephrol. 2020.

# Domuz Xenotransplantasyon modeli: Wild type pigs

# wild type PIG-TO-BABOON KIDNEY TX (DAY 0)



# Majör Xenoantijenin tanımlanması



**Baboons and Human have naturally occurring antibodies to pig organs that attack the organs**

**Solution:**
**Treat the recipient with Immunosuppression (over treated)**

# Xenotransplantasyonun 100 yılı


**Box 1 | 100 years of xenotransplantation**

**1906:** Pig and goat kidneys transplanted onto arm vessels survive for 3 days

**1910:** A macaque kidney transplanted into a human survives for 32 hours

**1963:** A 23-year-old woman survives for 9 months with functioning chimpanzee kidneys

**1984:** A newborn, Baby Fae, survives for 20 days with a baboon heart

**1994:** Discovery of natural antibodies against alpha-Gal, which causes the rejection of pig organs

**2004:** Genetic editing of pigs to modify alpha-Gal, a discovery followed by additional genetic modifications to prevent rejection

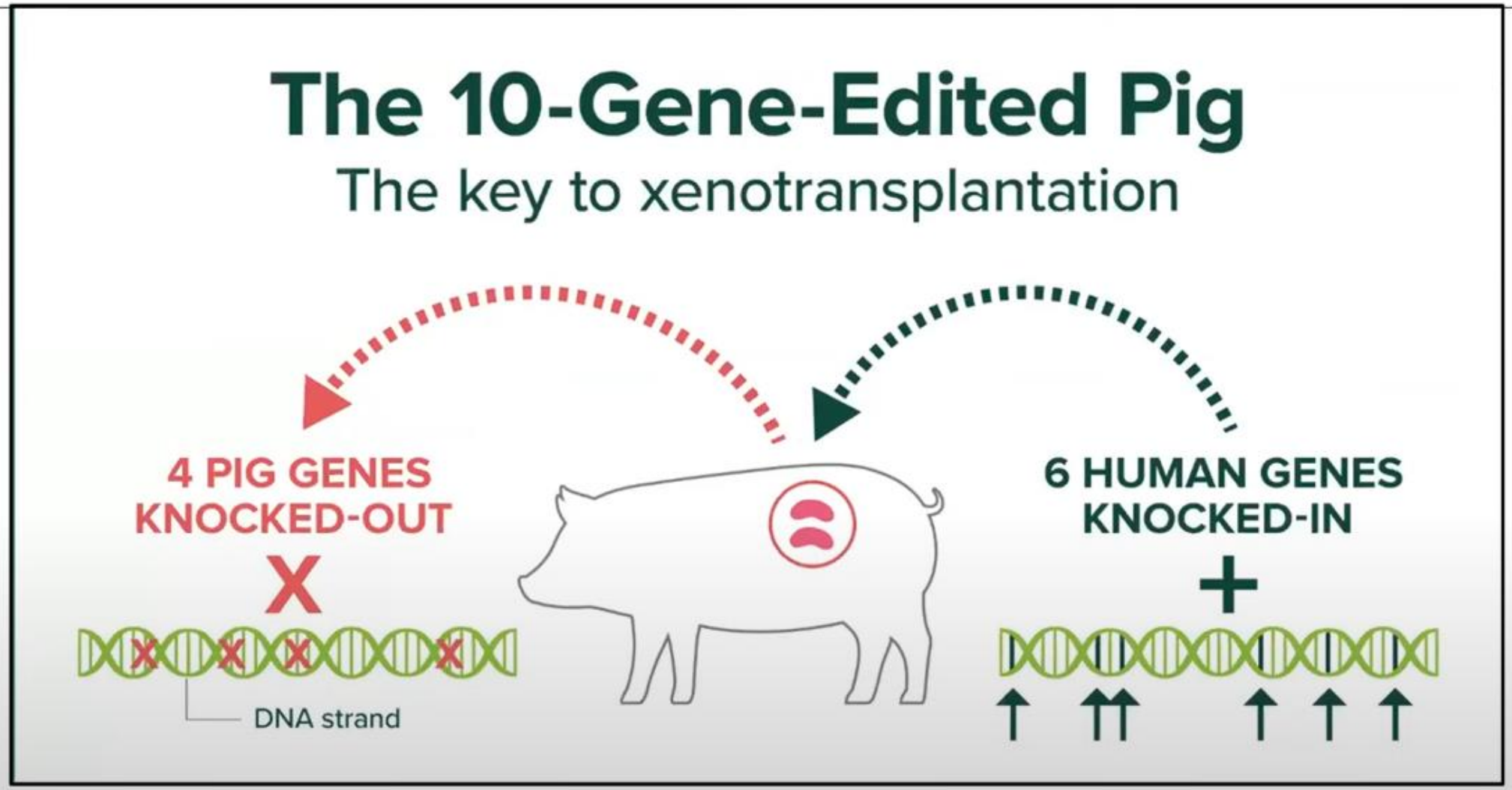
**2022:** Transplantation of genetically modified pig kidneys into two deceased humans

**2022:** Transplantation of a genetically modified pig heart into a human, who lived for a further 2 months


# Modifiye Donör

# Knock out Domuzlar

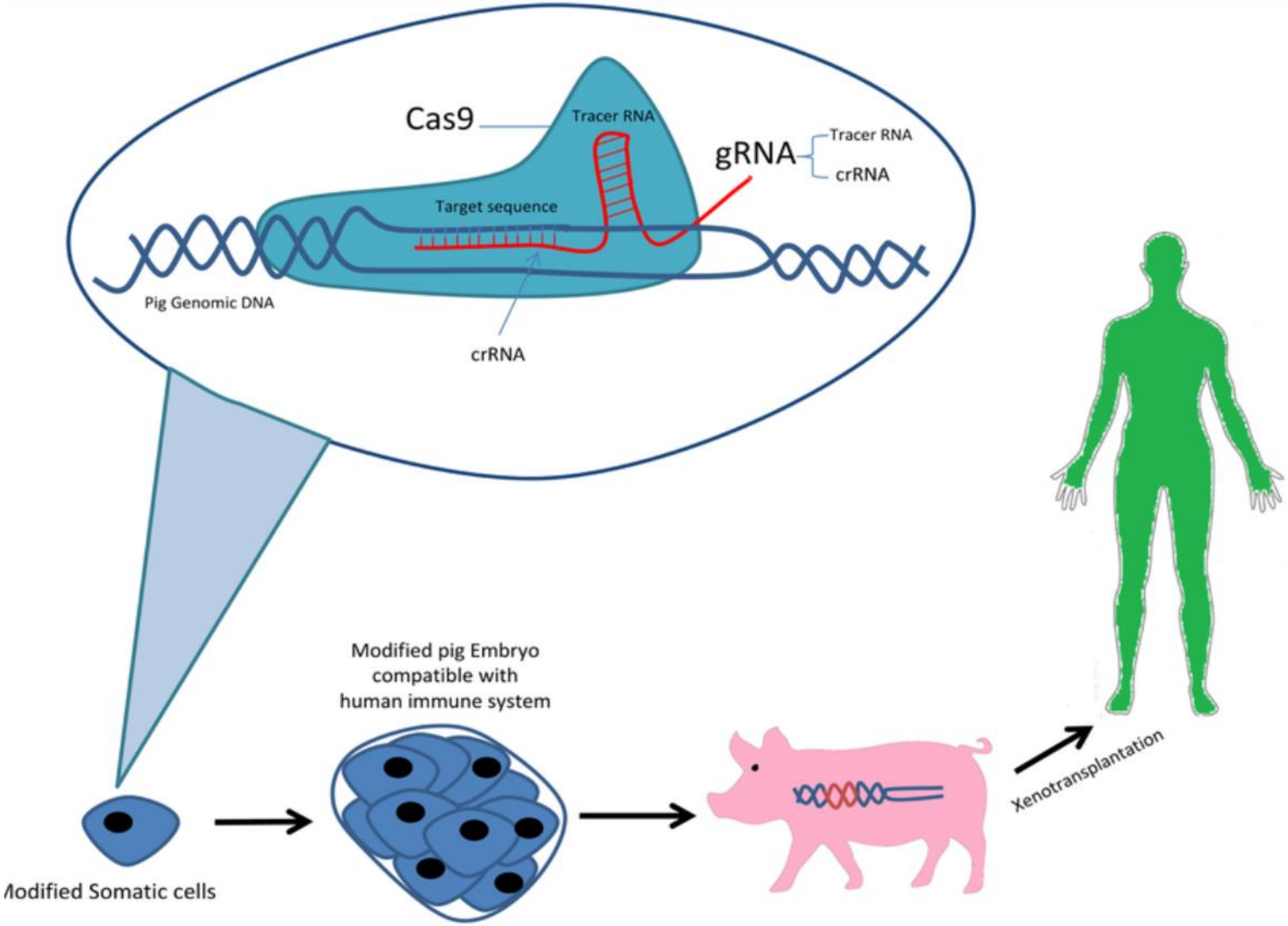
# Donorü insana benzer hale getirmek



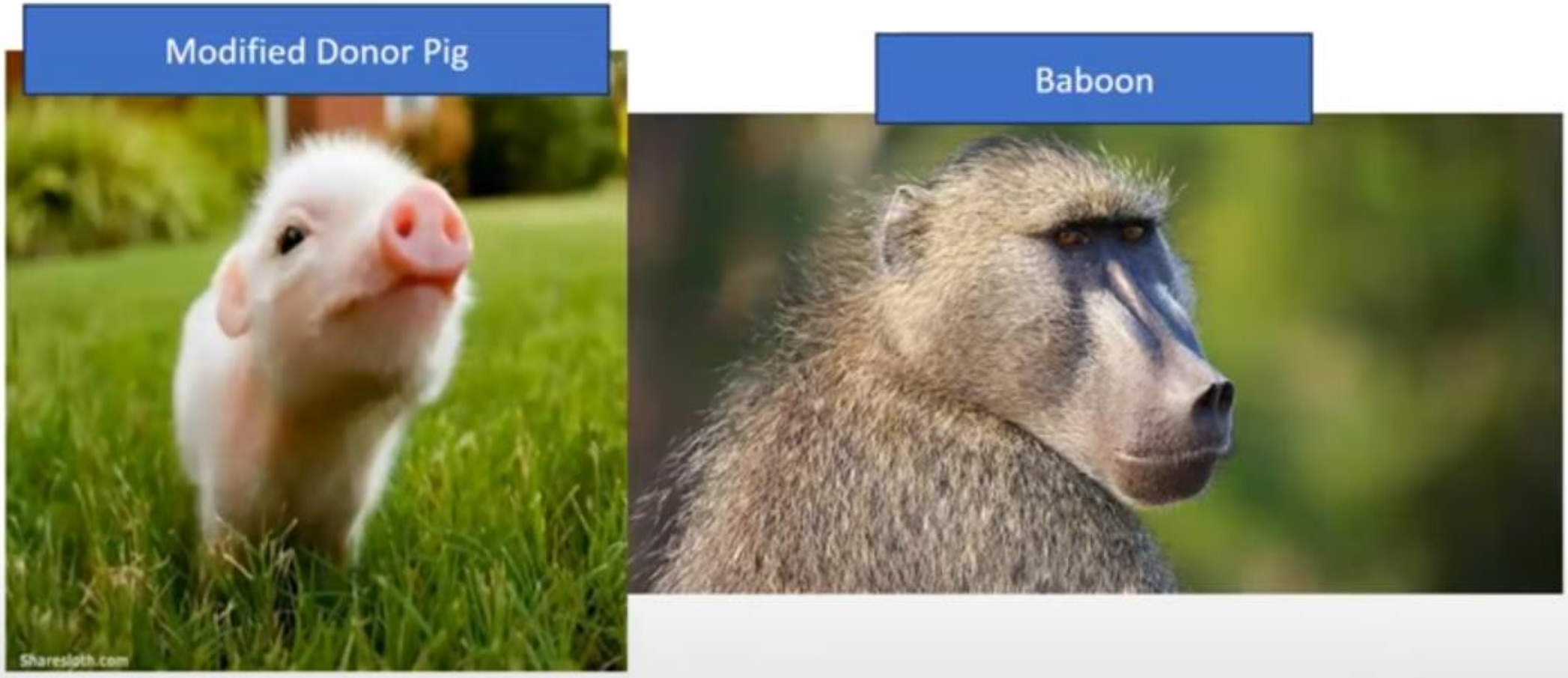

**Knock –out: GGTA1,CMAH, B4GALLNT2, GHR**

**Knock-in: CD46,CD55,CD47, THBD, PROCR, HMOX1**

# CRISPR-Cas9 in Xenotransplantation

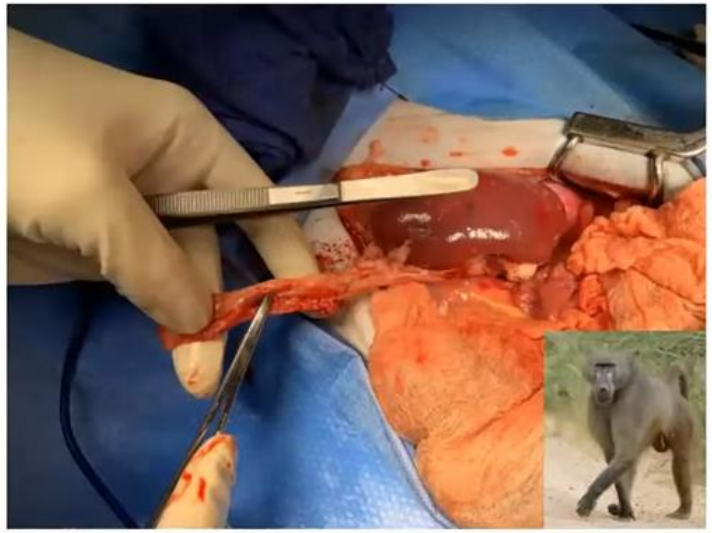


# Knockout domuz organ modeli :
# Neler öğrendik



# insan böbreğine benzeyen domuz önce Baboon larda denendi

Modified Pig → Baboon
Modified Pig X Human



Tested in Baboons (NHP)

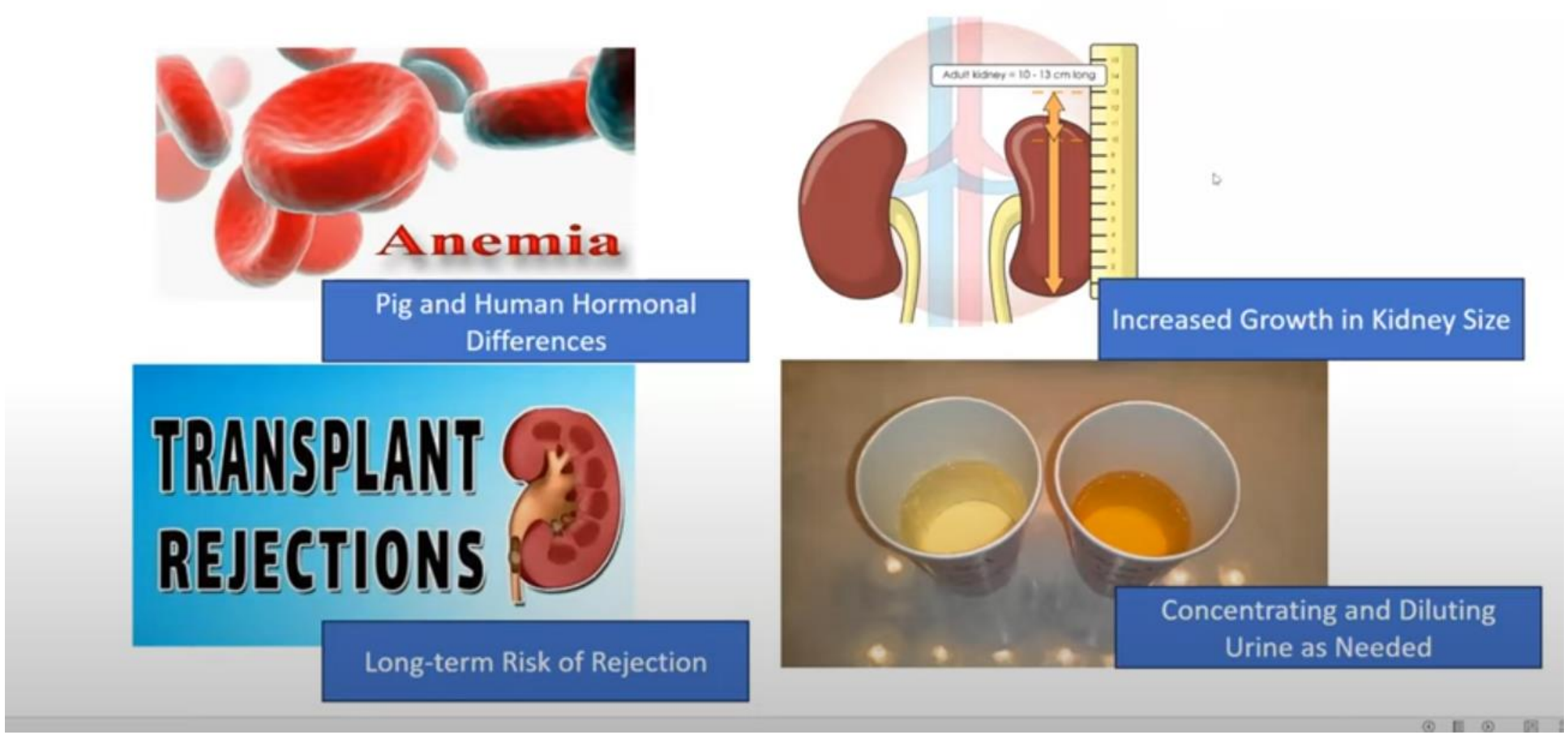
Genetically engineered to look less "foreign" to human immune system:

- TKO & GHRKO
- 6 human transgenes

BUT. . .

1. NHP immunologically different than human
2. Needs to be tested with conventional immunosuppression

# Modifiye domuz böbreği insan böbreği ile bazı farklılıklar taşıyor



*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.

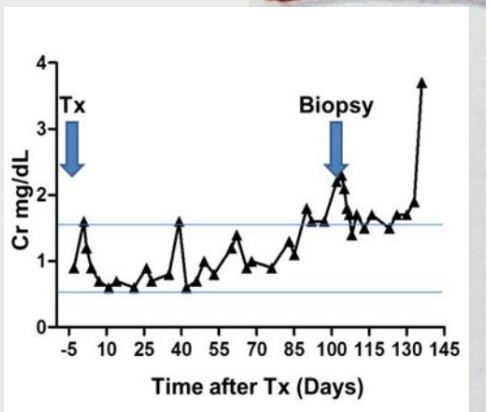



Table 1. Immunosuppressive, anti-inflammatory, and adjunctive therapy

| 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.

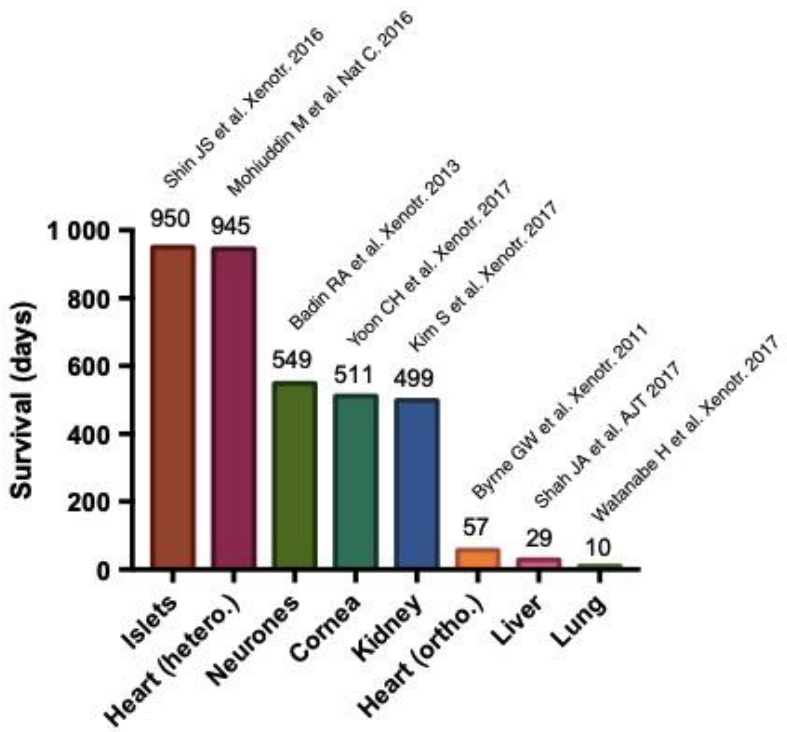



**Figure 1** Survival of xenogeneic organs/tissue transplanted in nonhuman primates.



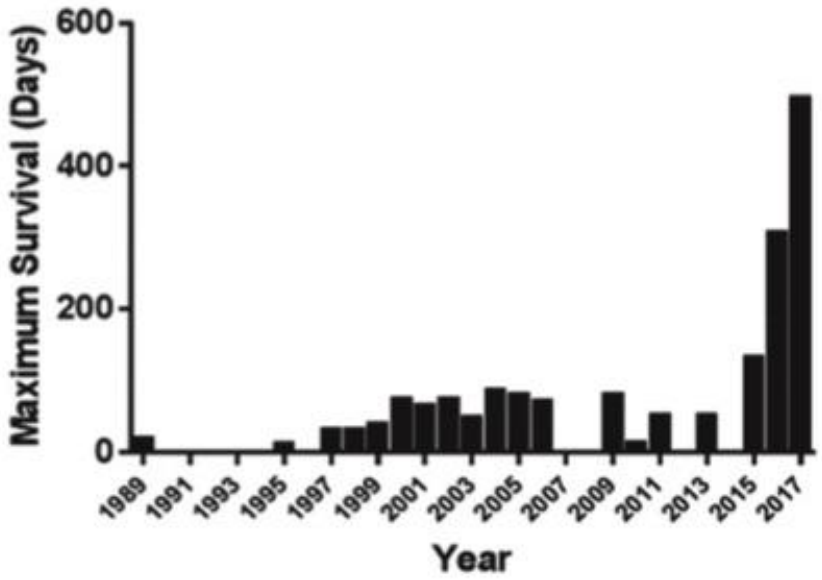
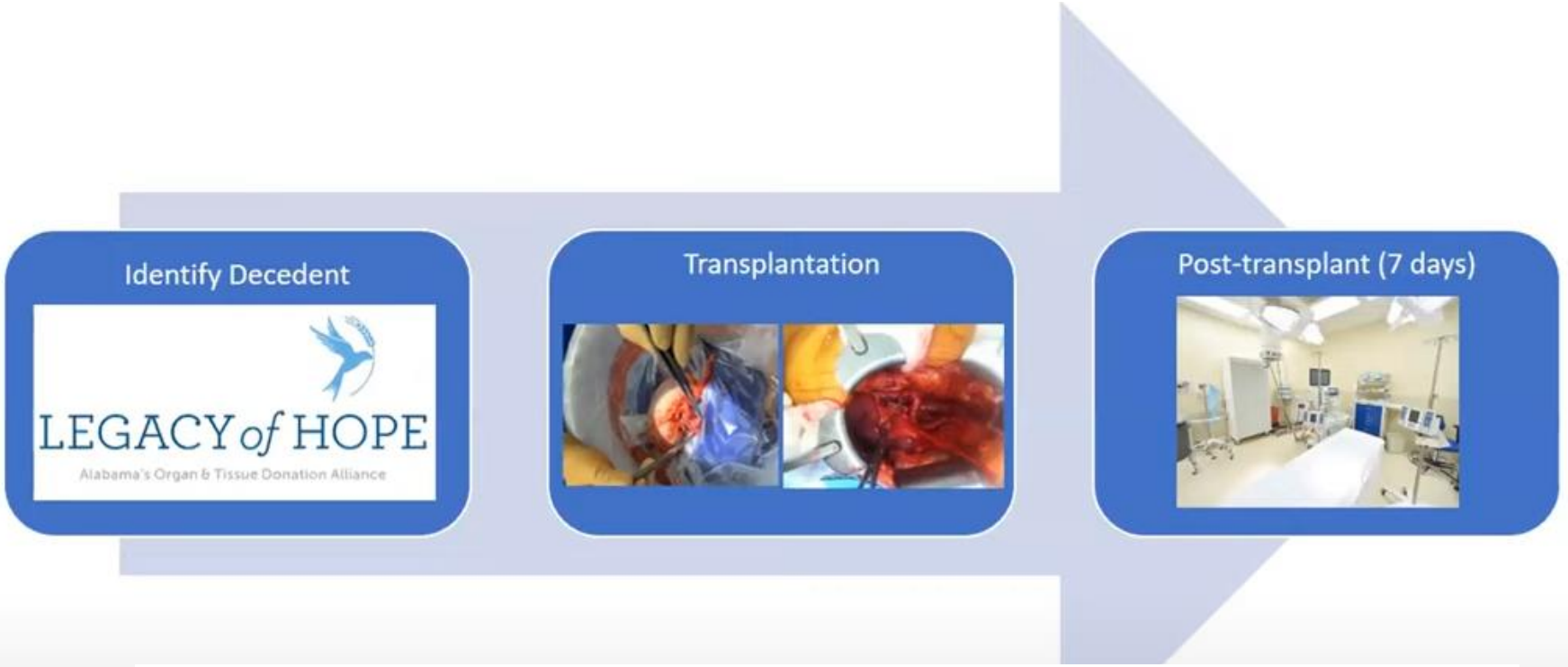



**Figure 2.** Reported maximum survivals of nonhuman primates with life-supporting pig kidney grafts,
some years, n

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

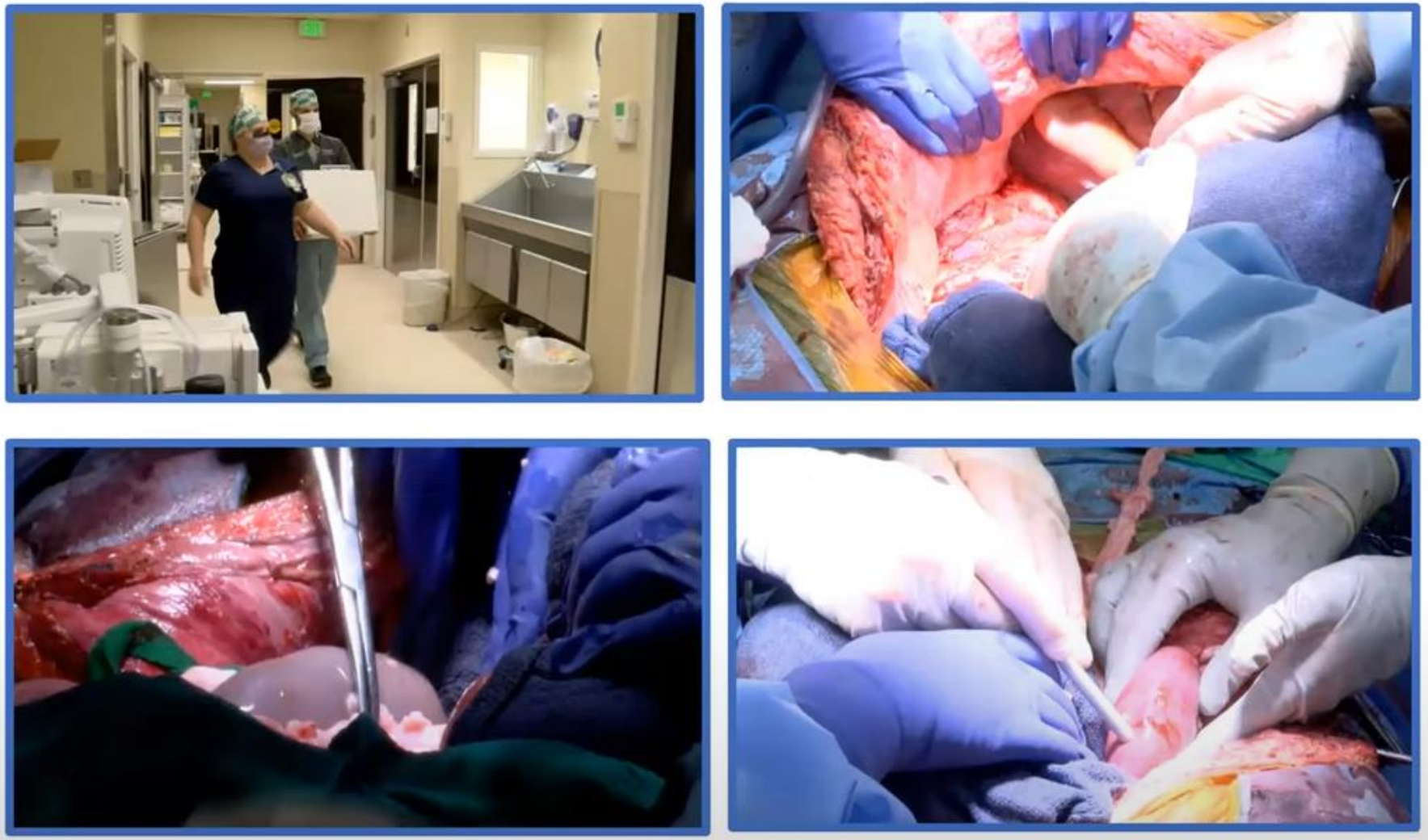
# insan böbreği modeli gerekiyor: Beyin ölümü modeli



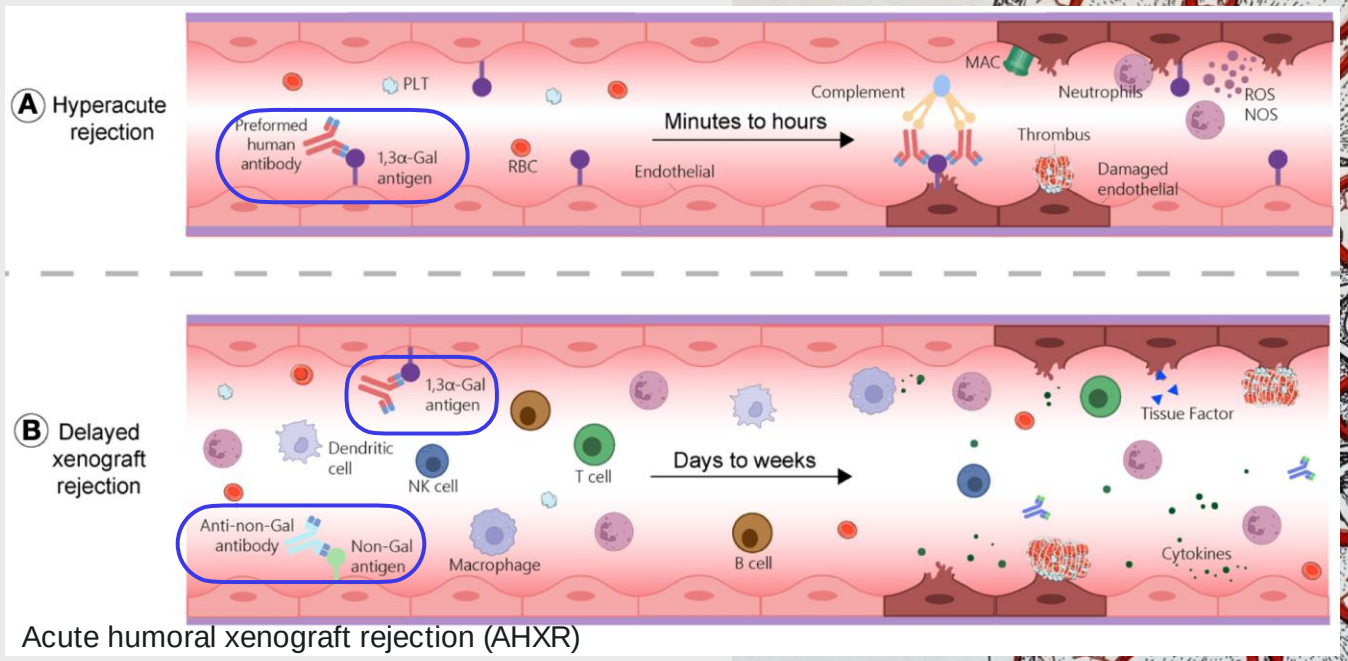

**Avantajları :**

1. **Yaşayan birisi için zararsız**
2. **Bir insanda genetik mühendisliği test etme şansı ?**
3. **Bir insanda immunsupresifleri test şansı ?**

# Xeno-böbrek transplantasyonu cerrahisi benzer bir cerrahi işlem



# İmmünolojik Bariyer




Acute humoral xenograft rejection (AHXR)

Cellular xenograft rejection

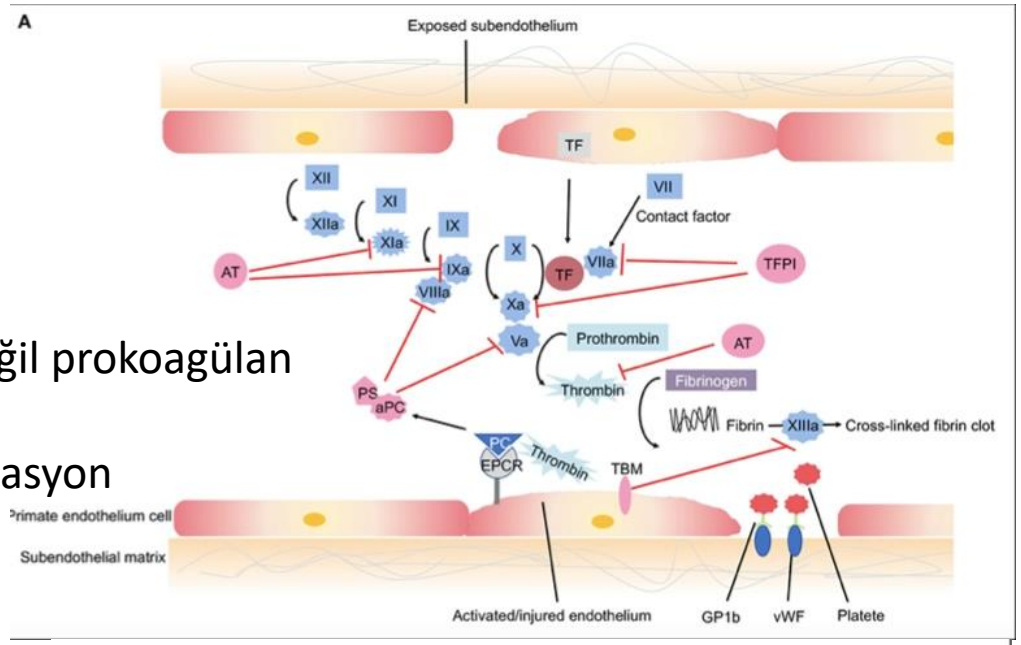
Coagulation dysregulation

Zhou Q, et al. Front Immunol. 2022.

Habibabadv Z, et al. Xenotransplantation. 2023.

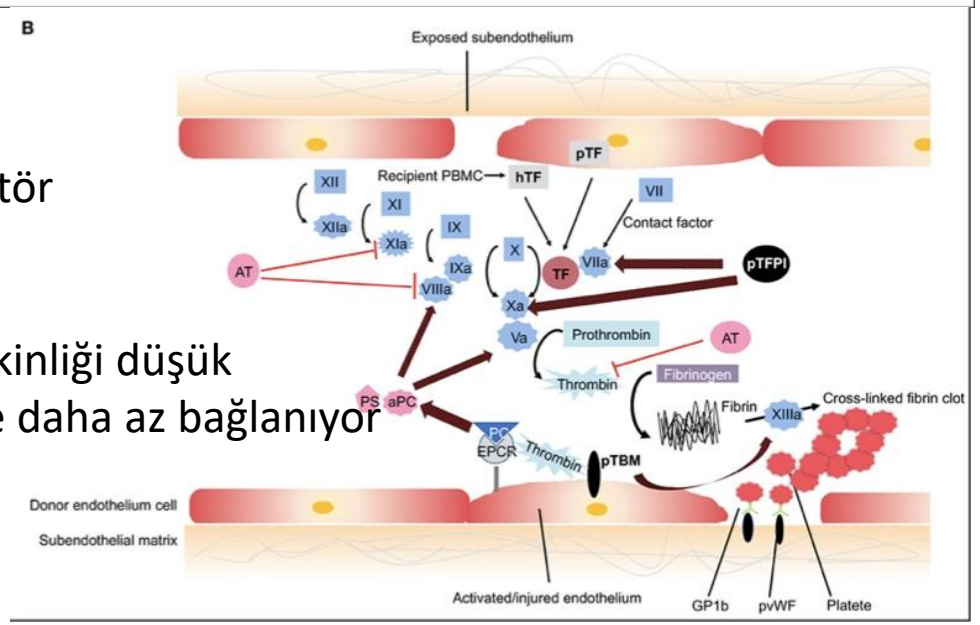
# Koagülasyon Disregülasyonu

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..



# Xenotransplantasyon Bariyer

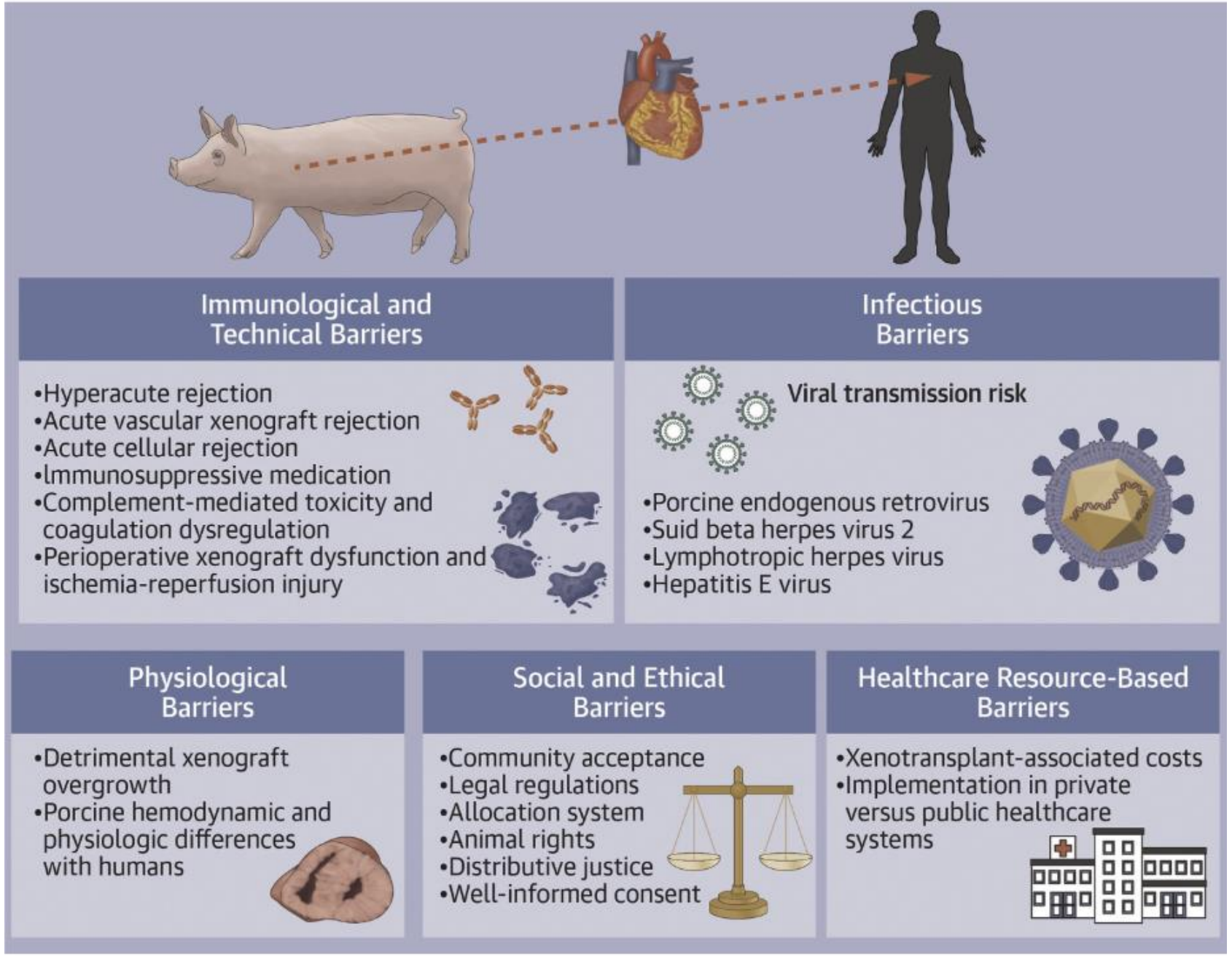
| | |
|---|---|
| 1. Hiperakut rejeksiyon | Dakikalar - Saatler |
| 2. Akut humoral rejeksiyon | Saatler-Günler |
| 3. T hücreli rejeksiyon | Günler Haftalar |
| 4. Kronik rejeksiyon | Haftalar -Aylar |

Koagülasyon Disregülasyonu

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

Kompleman Regülatör proteinler

Lu T, Front in Imm, 2020



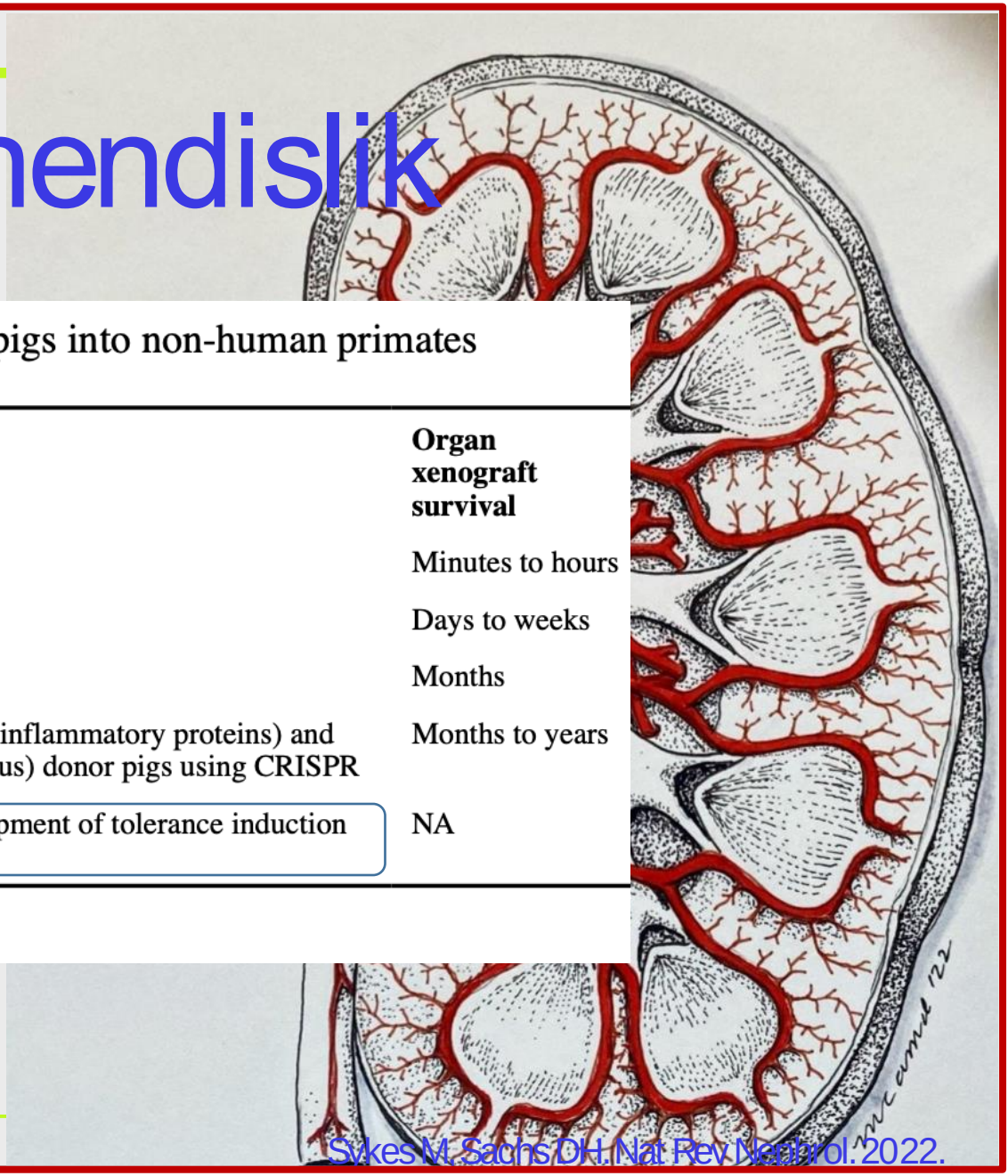

J, et al. J Am Coll Cardiol Basic Trans Science. 2022;7(7):716-729.

# 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.

# 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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- 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).

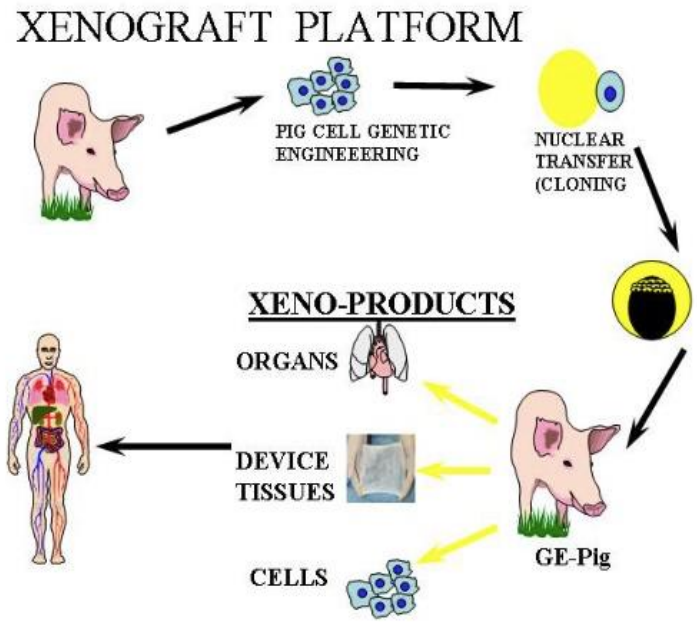

**TABLE 1 |** Genetically modified pigs currently available for xenotransplantation research.

| 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 |

# Kliniğe geçmeden önceki insan çalışması

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ORIGINAL ARTICLE



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

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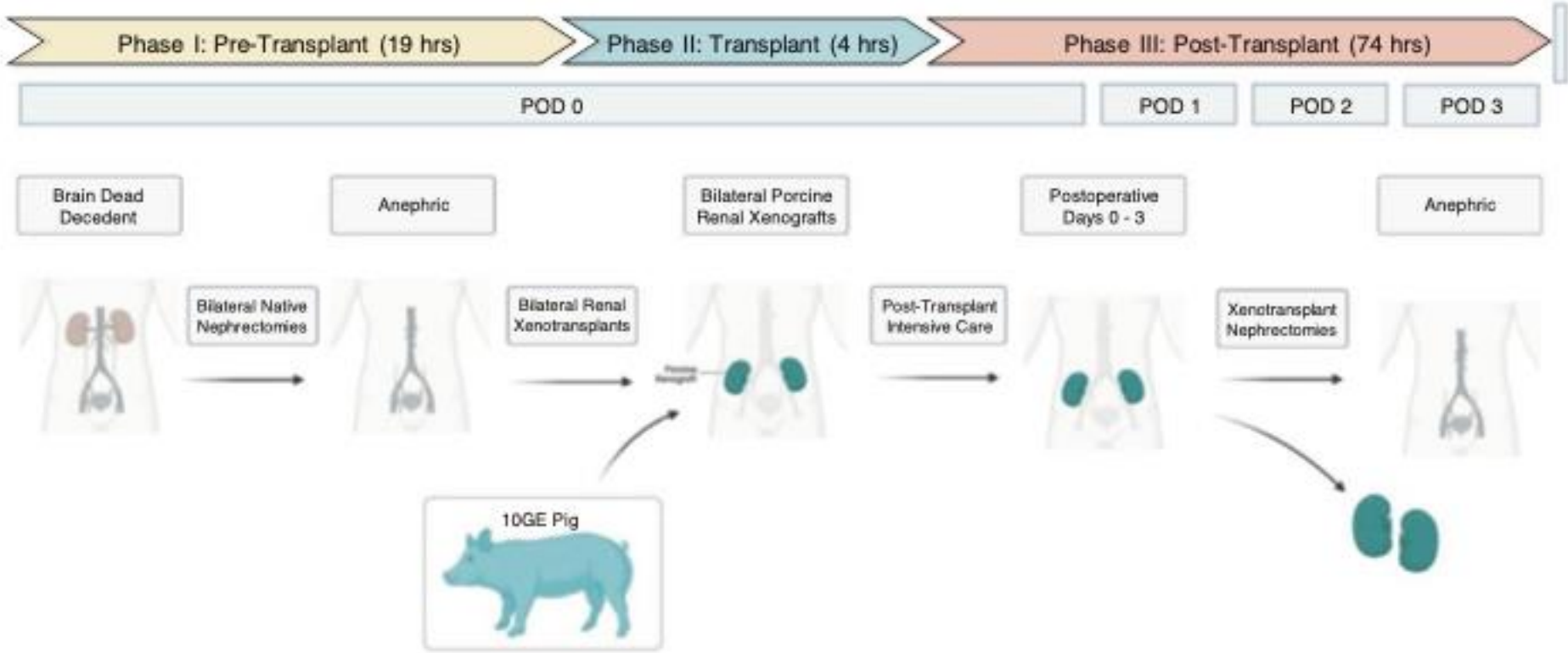
# Domuz Kaynağı

- Porcine renal xenografts were procured from genetically engineered (GE) pigs provided by Revivicor, Inc.
- **GE domuzlar 10 genetik modifikasyon içeriyor (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**




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

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

# İmmunsupresyon

## İndüksiyon

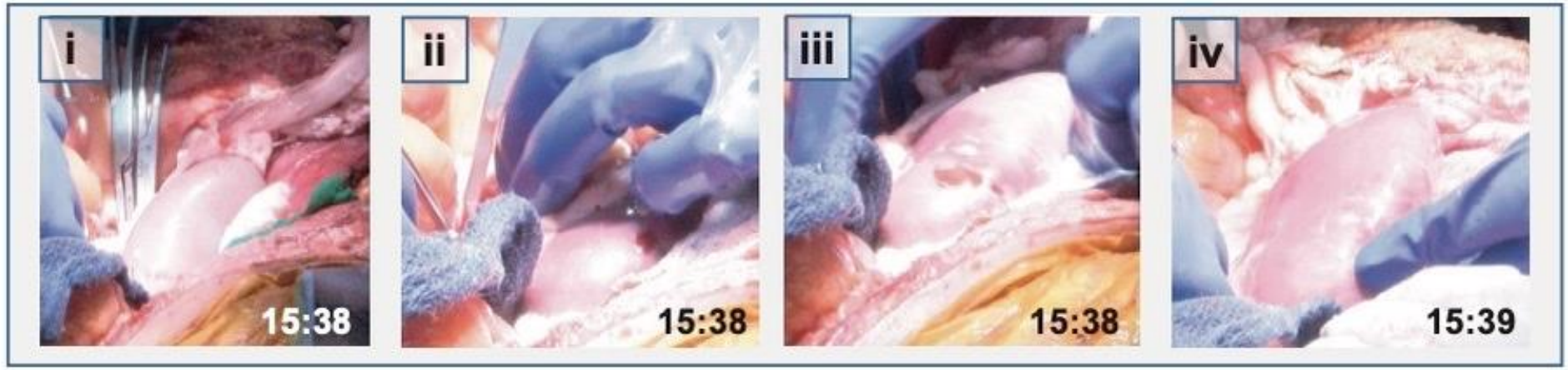
- Metil prednizolon (MP)
- ATG (6 mg/kg)
- Anti-CD20

## İdame

- MP
- MMF
- Tacrolimus

(A)

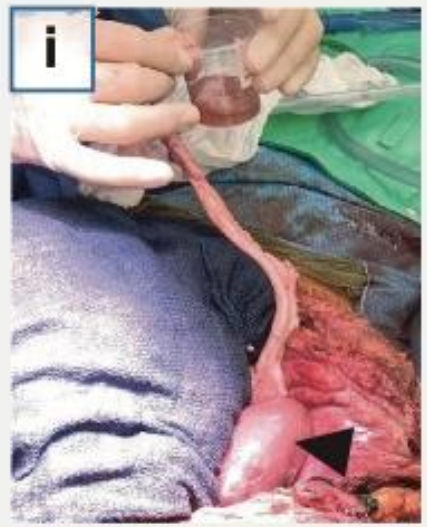
## Right Porcine Renal Xenograft Reperfusion

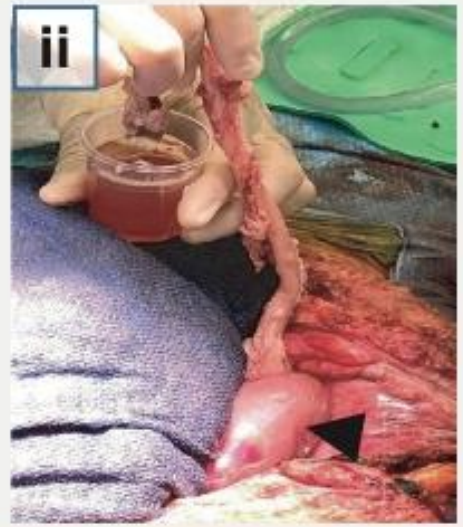


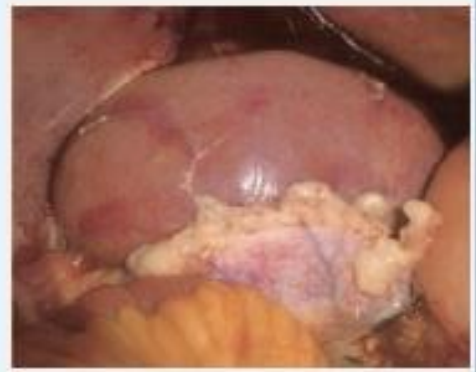
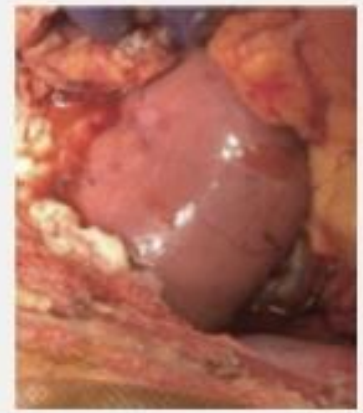
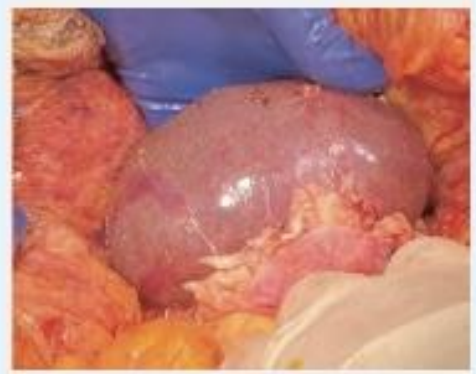
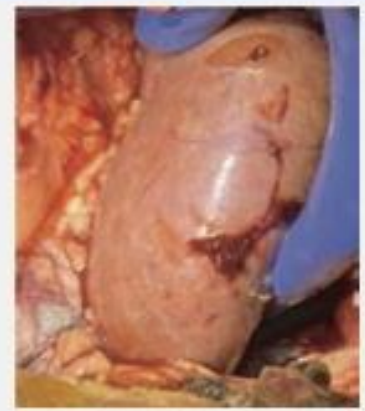

(B)

## 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 |

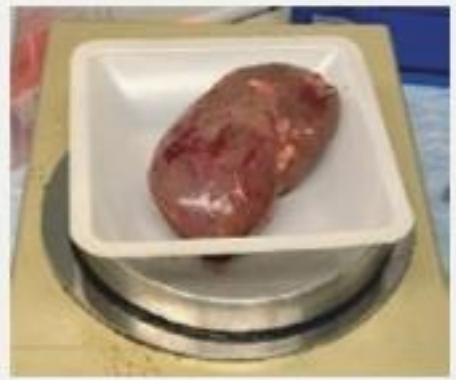




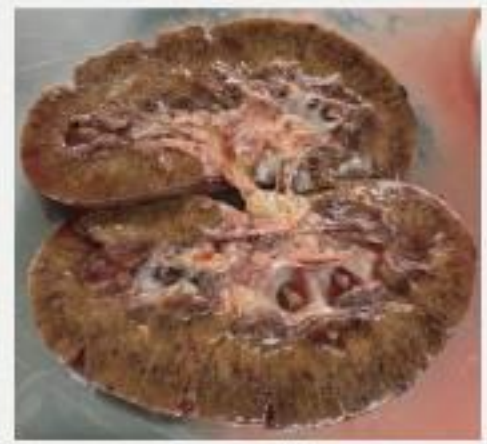
| | Right | Left |
| --- | --- | --- |
| POD 1 | **19 h 32 min (Day 1: 10:06)**  | **16 h 41 min (Day 1: 09:56)**  |
| POD 3 | **72 h 54 min (Day 3: 16:32)**  | **71 h 56 min (Day 3: 16:19)**  |

Explant

**Ex vivo (Day 3: 23:25)**



**Ex vivo (Day 3: 23:22)**



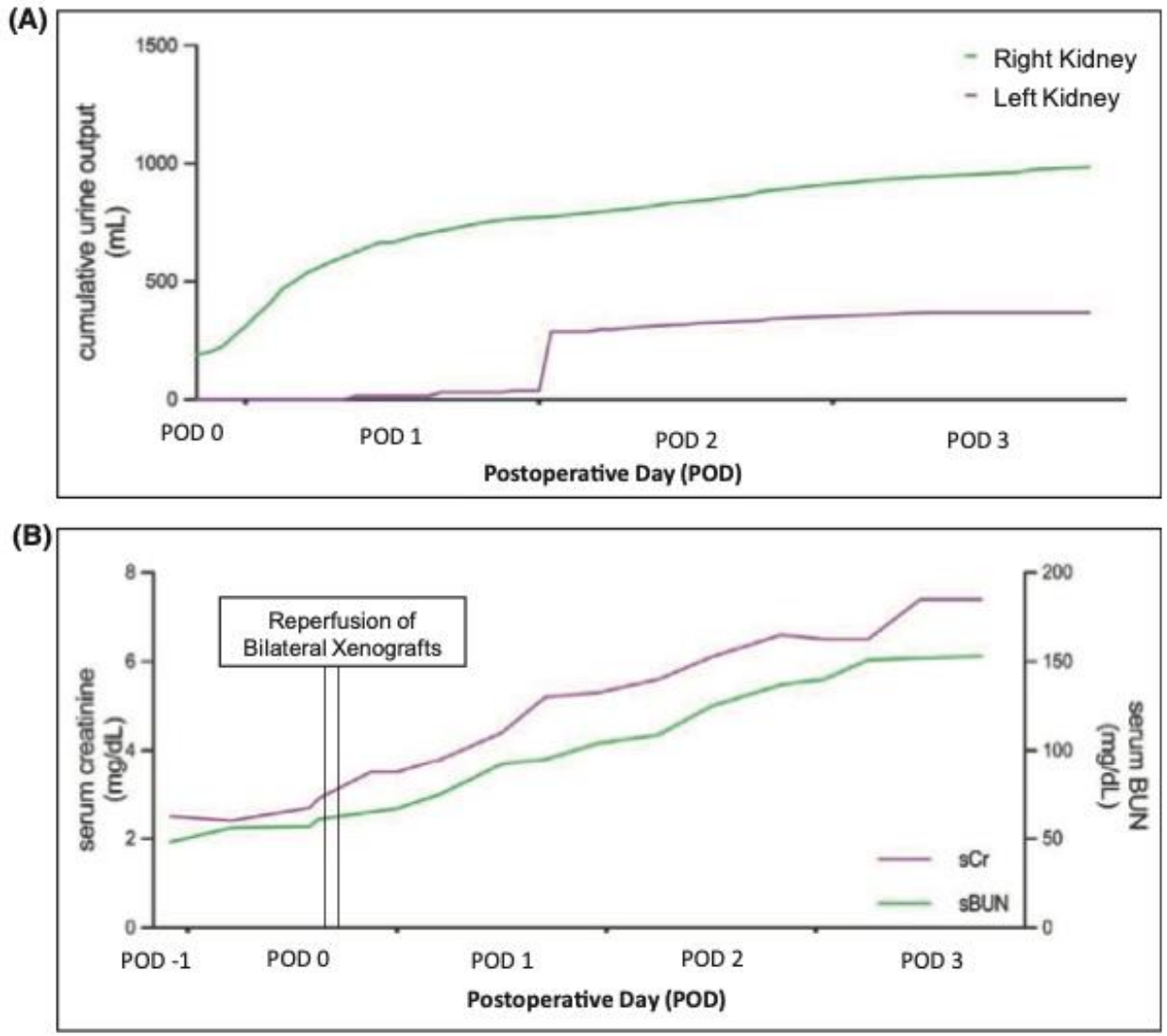



**FIGURE 6** Porcine renal xenotransplant function in the human decedent. (A) Cumulative posttransplant urine output from transplantation to study end from right and left xenografts. (B) BUN and creatinine in the decedent's serum. Results prior to POD 0 reflect function of decedent's native kidneys prior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

The NEW ENGLAND JOURNAL of MEDICINE

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.)

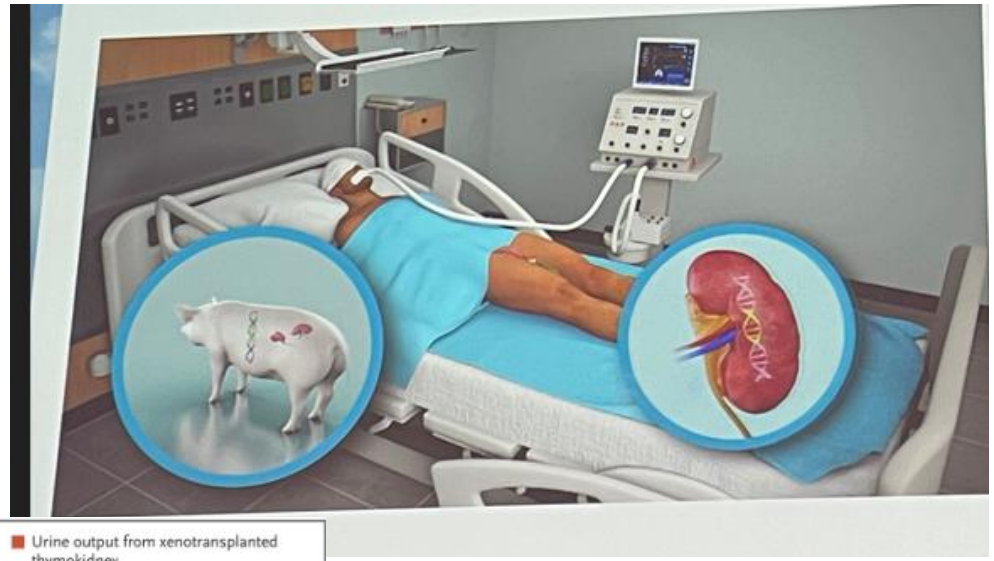
From the New York University (NYU) Langone Transplant Institute (R.A.M., J.M.S., B.E.L., V.S.T., M.M., E.W., N.L., C.D., R.A.D., B.S., G.B., G.P., N.M.A., S.A.M., Z.A.S.), the Departments of Pathology (M.W.), Anesthesia (P.S.), Biochemistry and Molecular Pharmacology (J.D.B.), and Cardiothoracic Surgery (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.

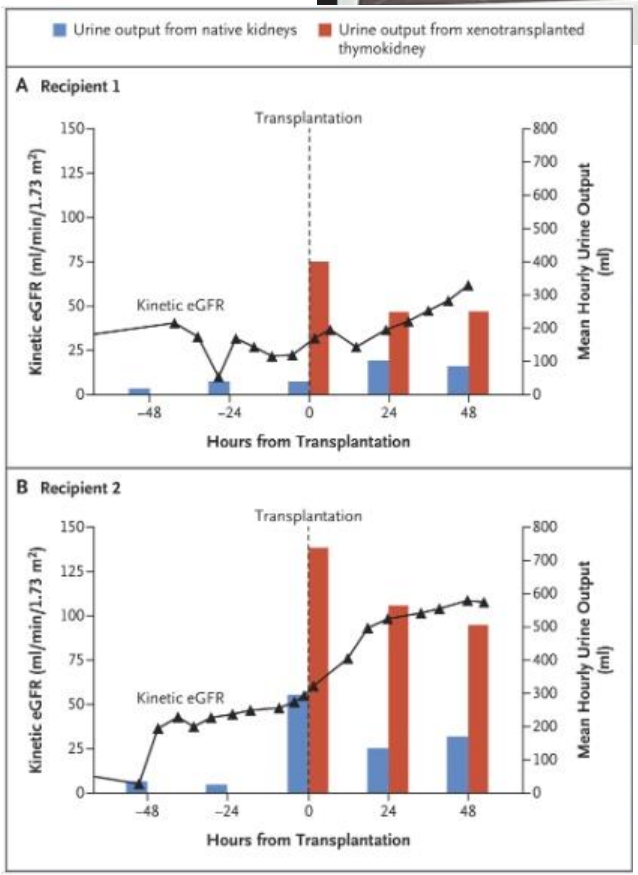
N Engl J Med 2022;386:1889-98.
DOI: 10.1056/NEJMoa2120238






# Mayıs 2022






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

1000 mg/gün Prednisolon
2x1000 mg MMF IV

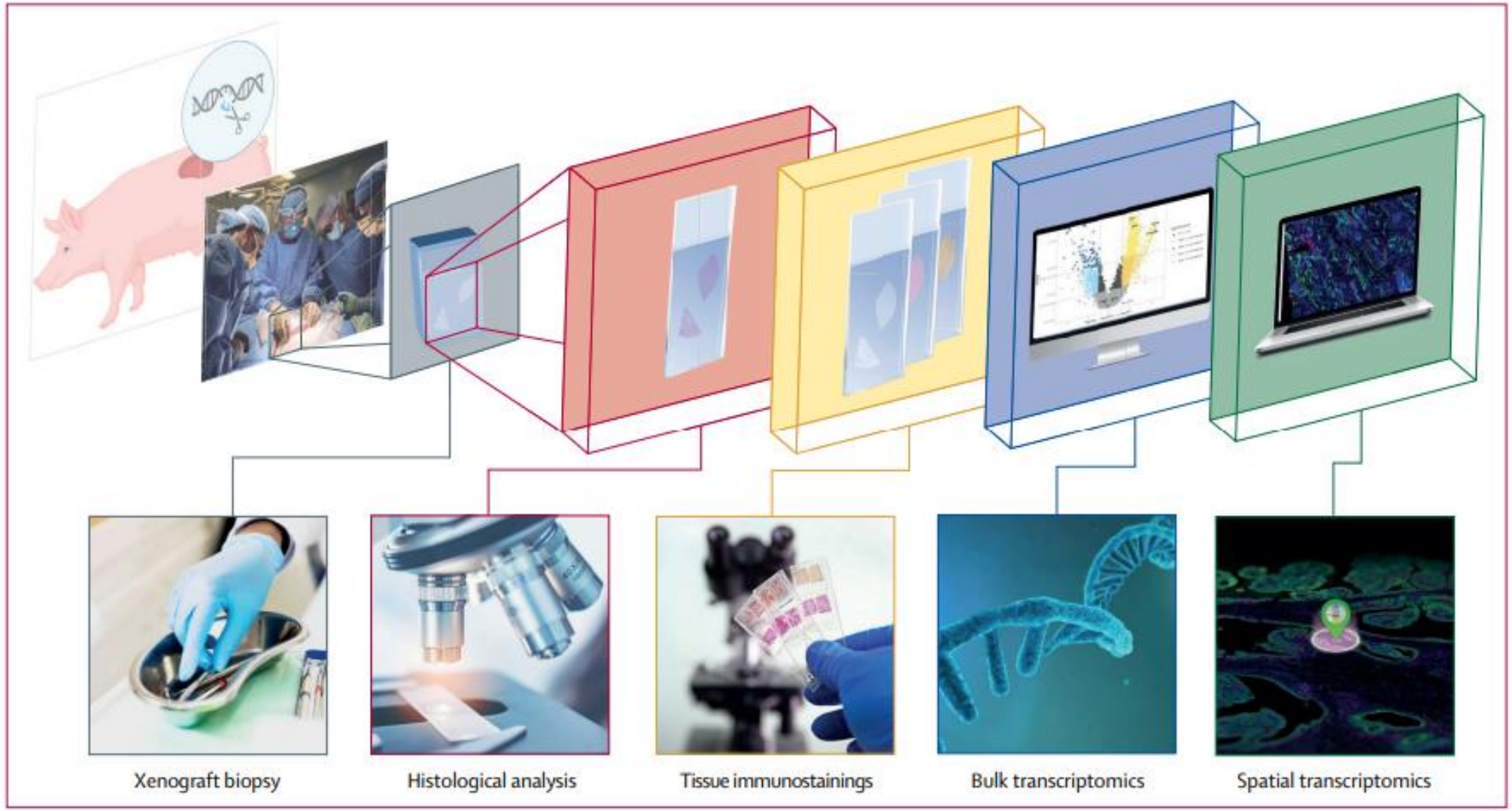



***Figure 1:*** **Multimodal deep phenotyping of pig kidney xenografts**
We performed a deep phenotyping of xenograft biopsies using a four-step strategy combining a morphological evaluation by histological analysis, immunophenotyping with multiple immunostainings, gene expression profiling with bulk transcriptomics, and whole-transcriptome digital spatial profiling and cell deconvolution. Parts of figure created with BioRender.com.

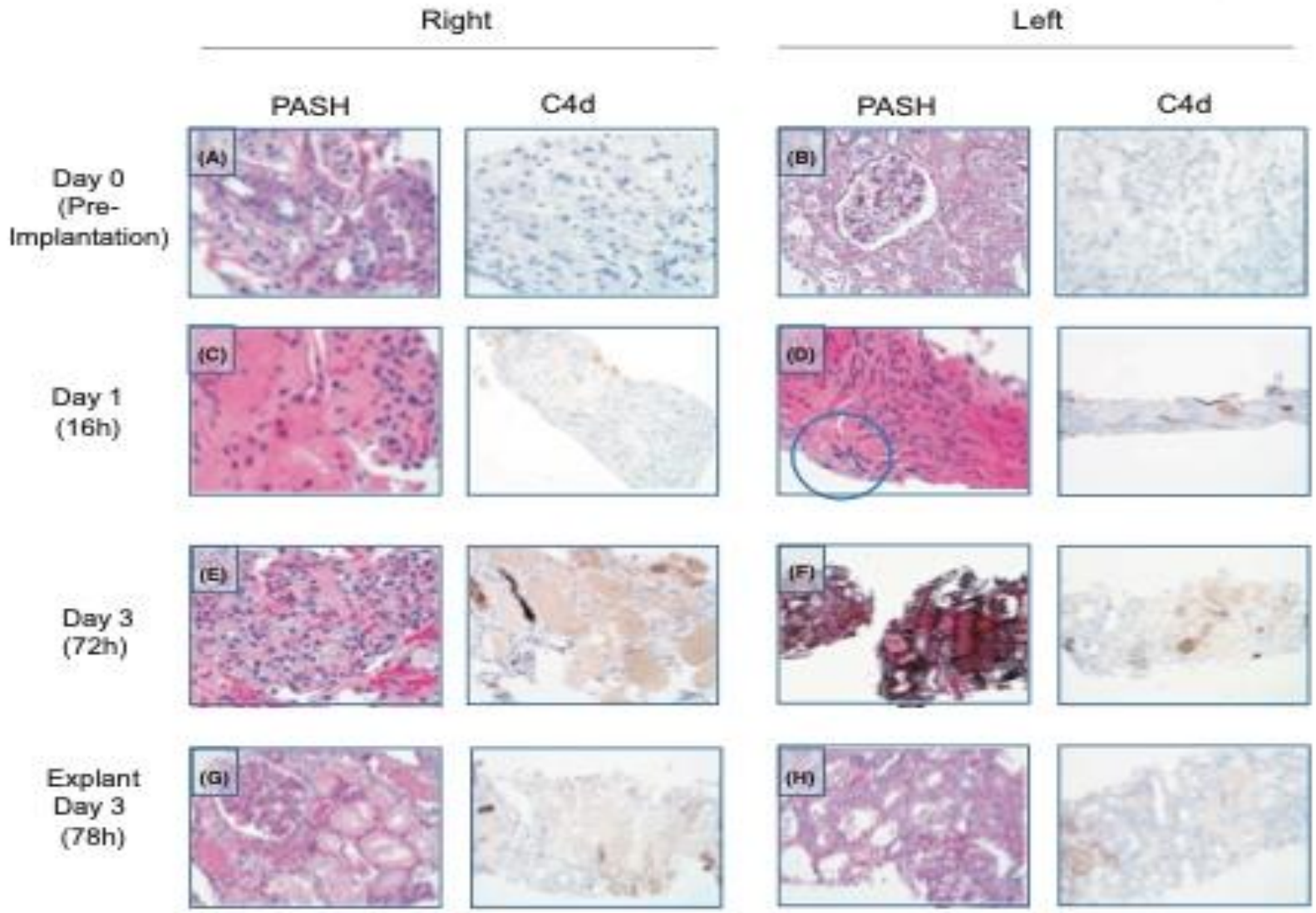
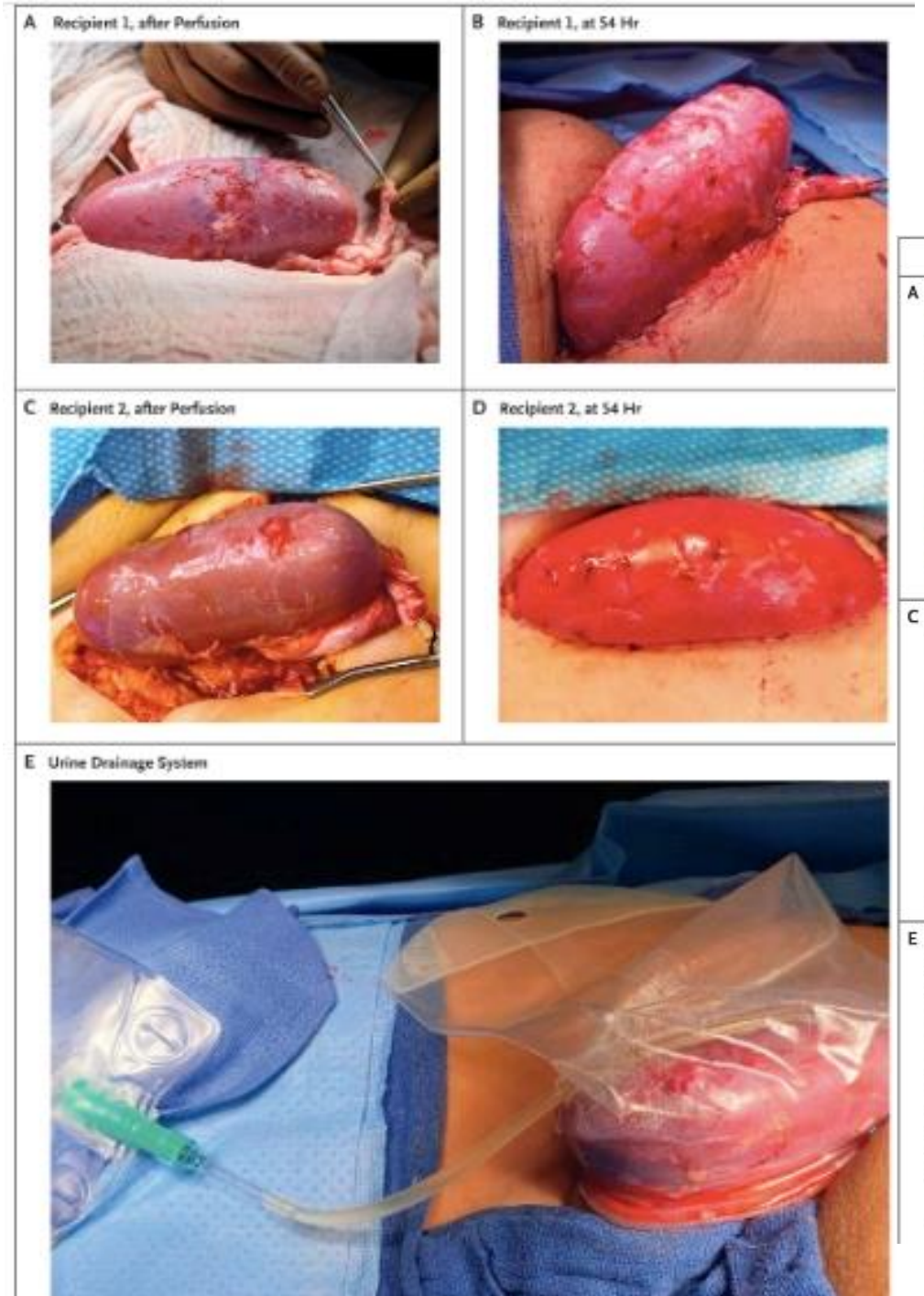
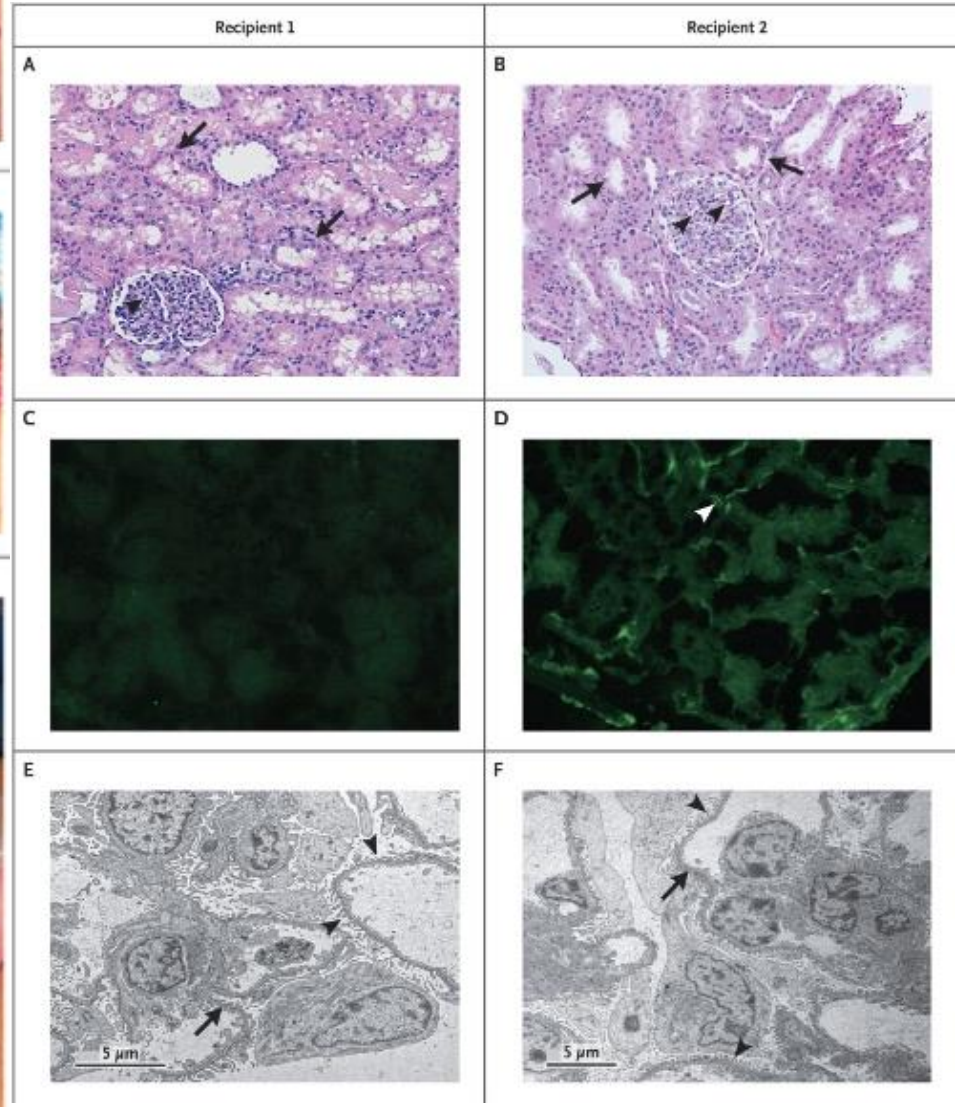
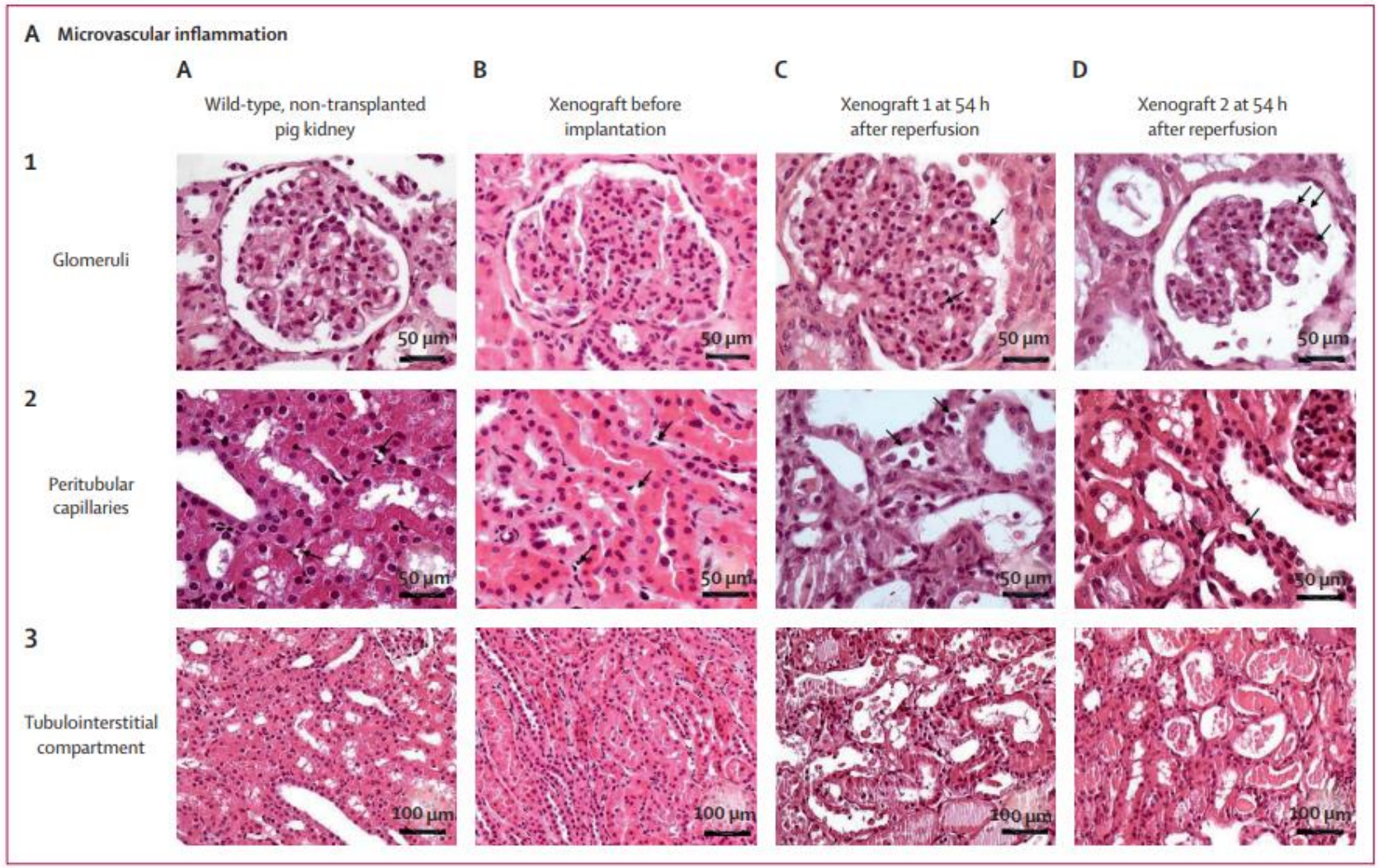



FIGURE 7 Serial histologic examination of the porcine kidney xenografts. All biopsies represent core biopsies. (A, B, G, and H) Were obtained *ex vivo*. (C, D, E and F) Were obtained *in vivo*. Sections are stained with PASH and are 10X, except for (C and D) (40X) and (F) (silver stain). C4d negative 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




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






(Figure 2 continues on next page)

## Histopatolojik bulgular -

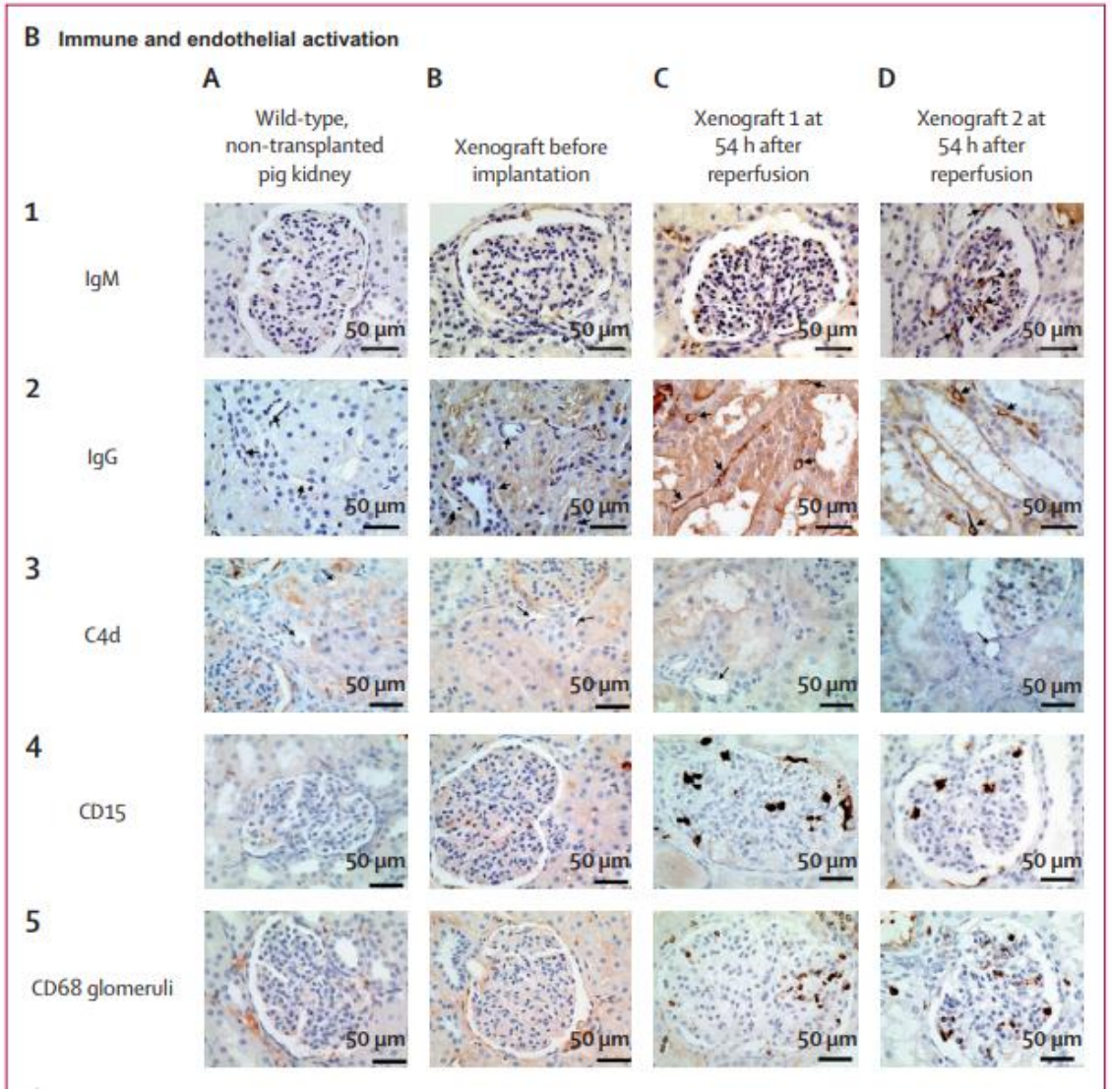
### post-operatif 1. gün

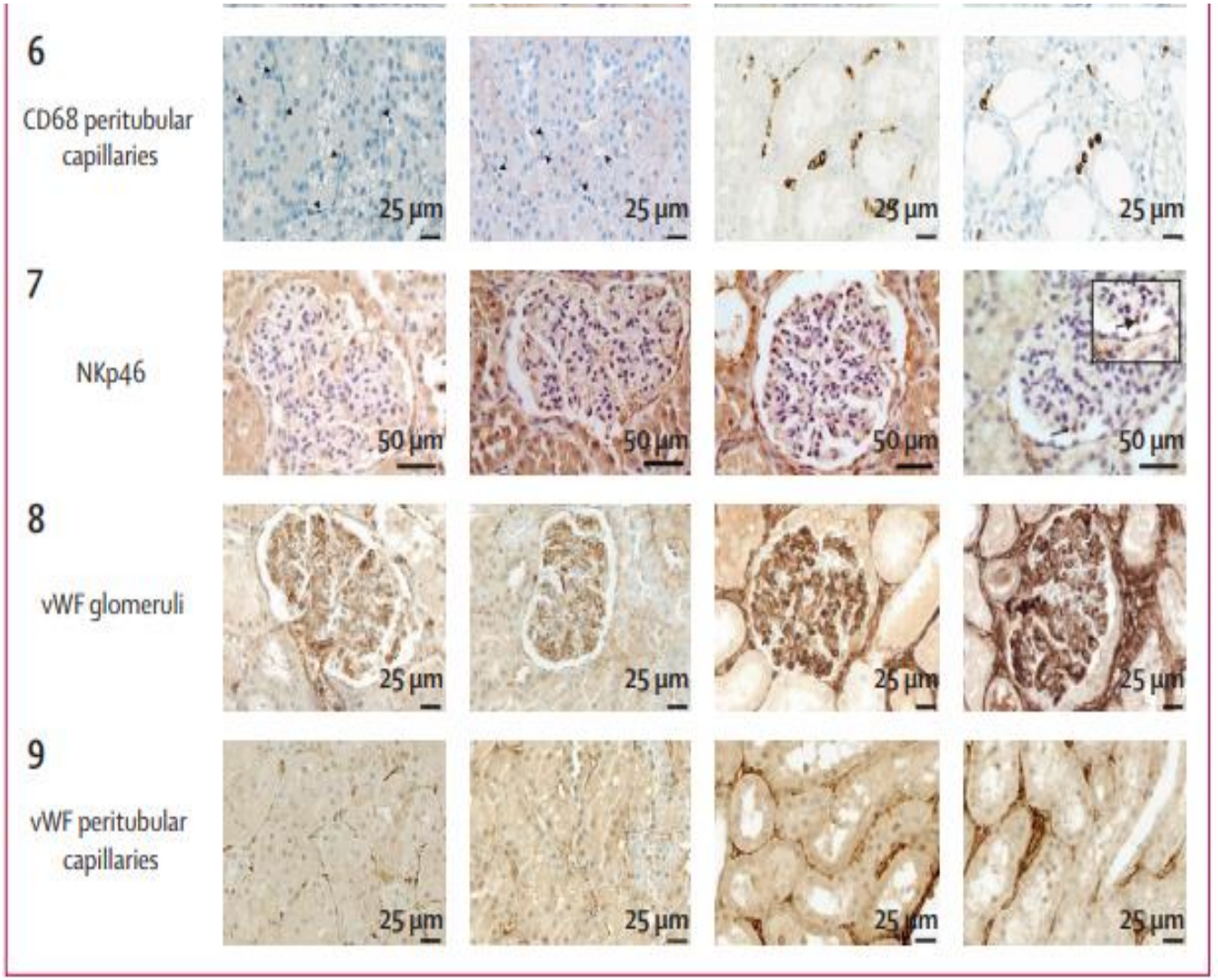
- thrombotik mikroangiopati,  TMA
- Diffuse glomeruler kapiller konjesyon  KONJESYON
- Endotel hücre şişmesi
- . (FİBRİN TROMBUS) Tam obliterasyona yakın

### post-operative 3. gün

- İlerleyici tubular hasar ve tubuler nekroz  (ATN)
- TMA görünümünde ek bulgular
- C4d ve IgM, IgG, IgA, C1q, and C3 negatif .
- Kortikal nekroz ve interstisyel hemoraji yok
- glomeruler kapiller konjesyon da azalma .
- Post terminasyon sonrası böbrek dokusu incelemesinde insan transgenlerinde artış

# Immun response – Loupy& Montgomerry NEJM




Histological and immunological changes occurring in xenografts after transplantation

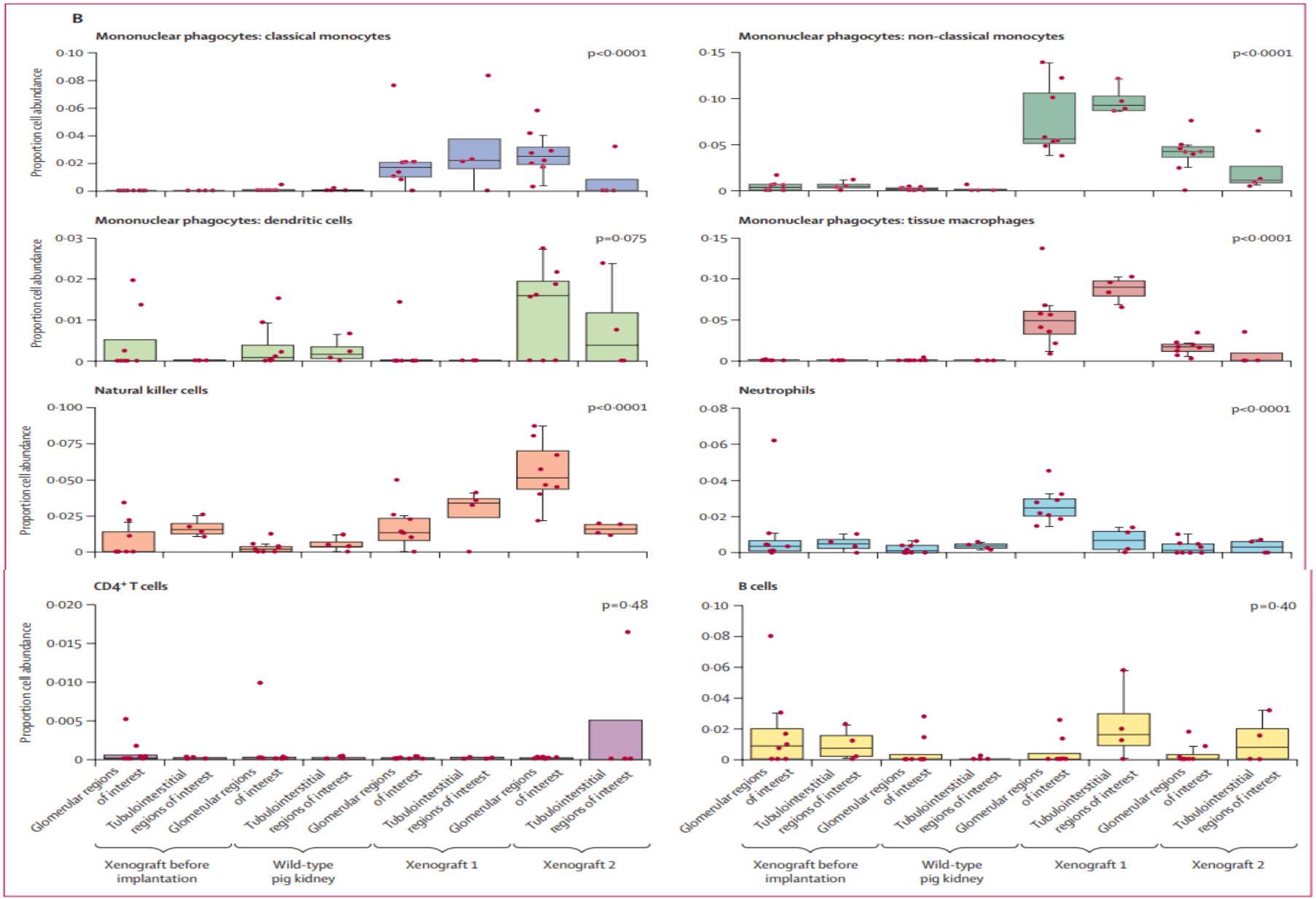



*Figure 5*: **Cell-type spatial profiling of genetically modified pig kidney xenografts**

# Xenograft Transplantasyon sonrası moleküler profil

**Humoral yanıtla ilgili gen expresyonunda artışlar**

**(HLA-B),**

**IFNγ yanıtı (IFI30, B2M, and C1QC), monocyte and macrophage activation (CD74),**

**complement activasyonu (C1QB),**

**endothel aktivasyon (POSTN ve LDB2),**

**natural killer cell burden (FCGR3A and HLA-E),**

**innate immune sistem (CST3, PTPRBve TXNIP),**

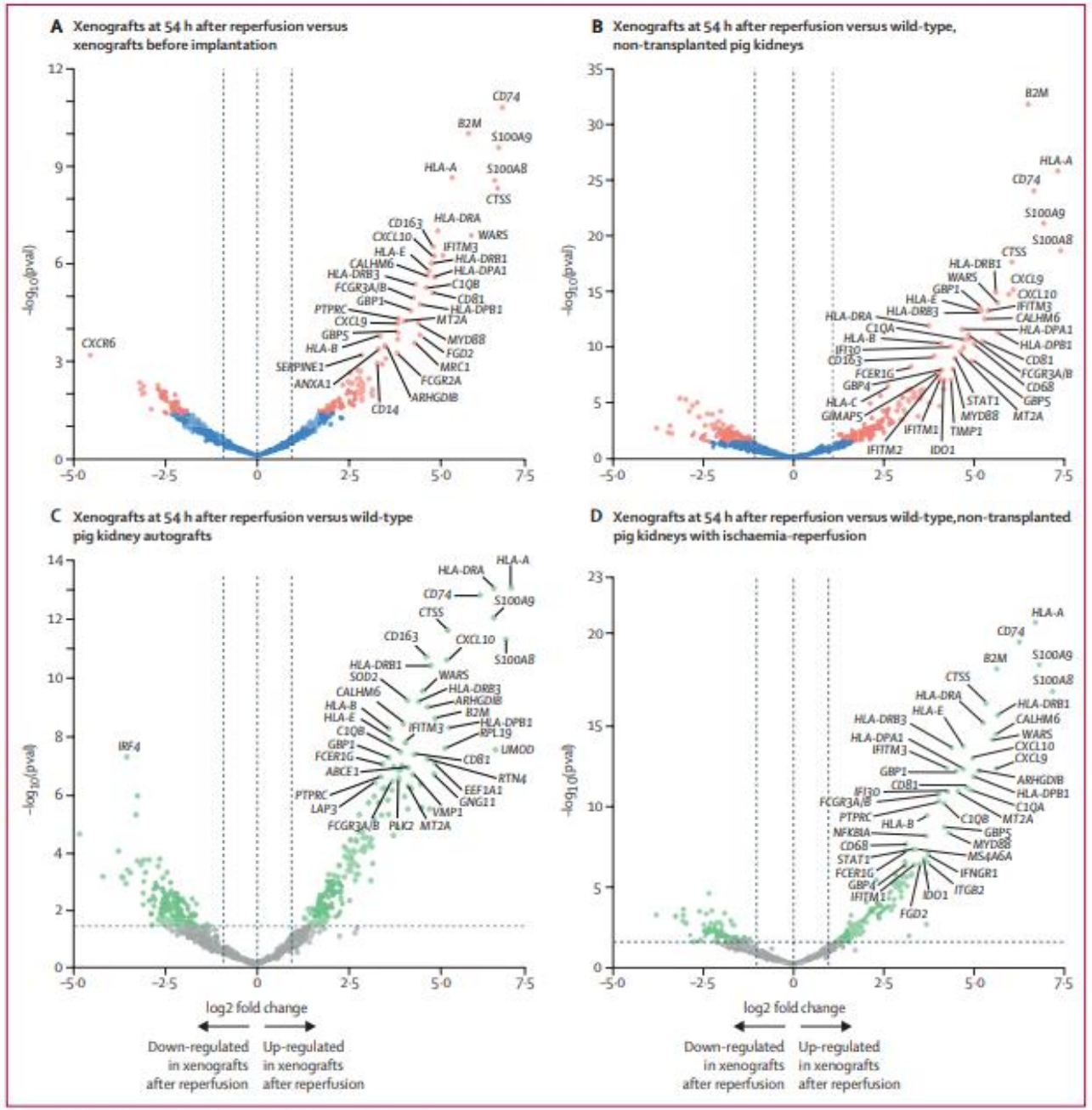



Figure 3: Molecular profiling of xenografts after transplantation

https://doi.org/10.1038/s41591-024-02972-1

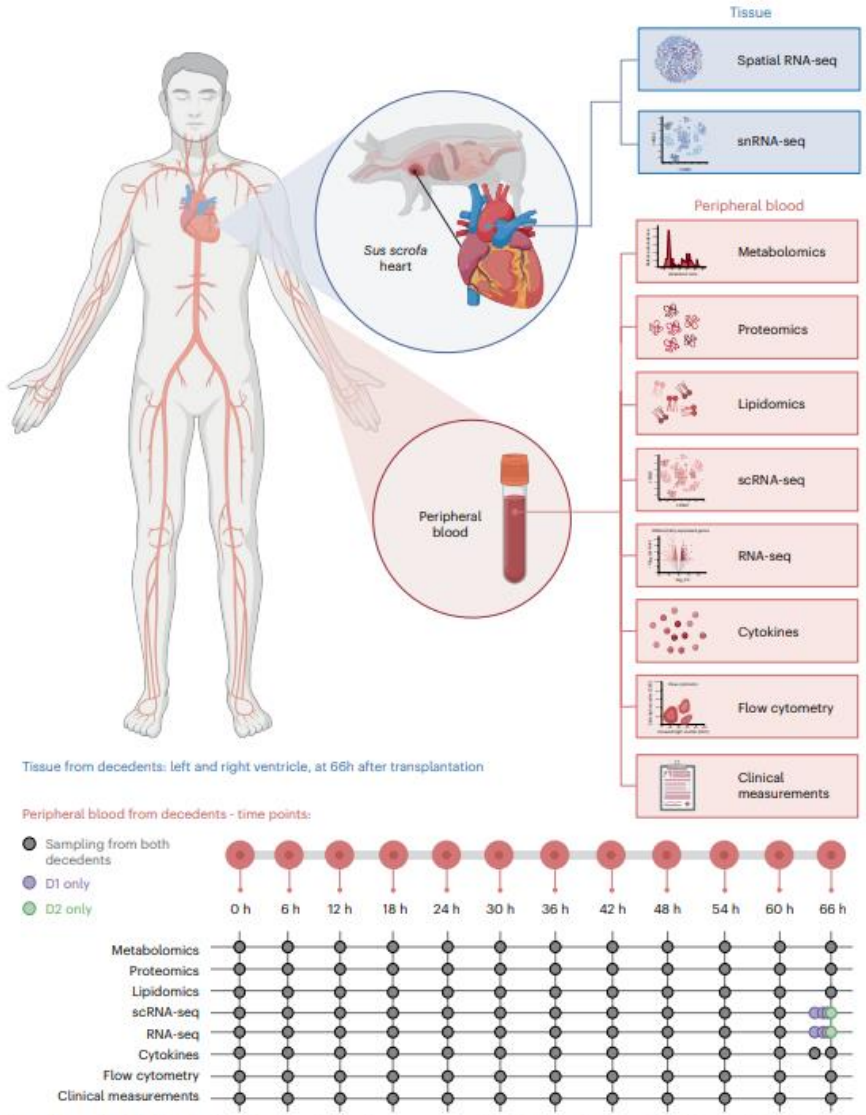
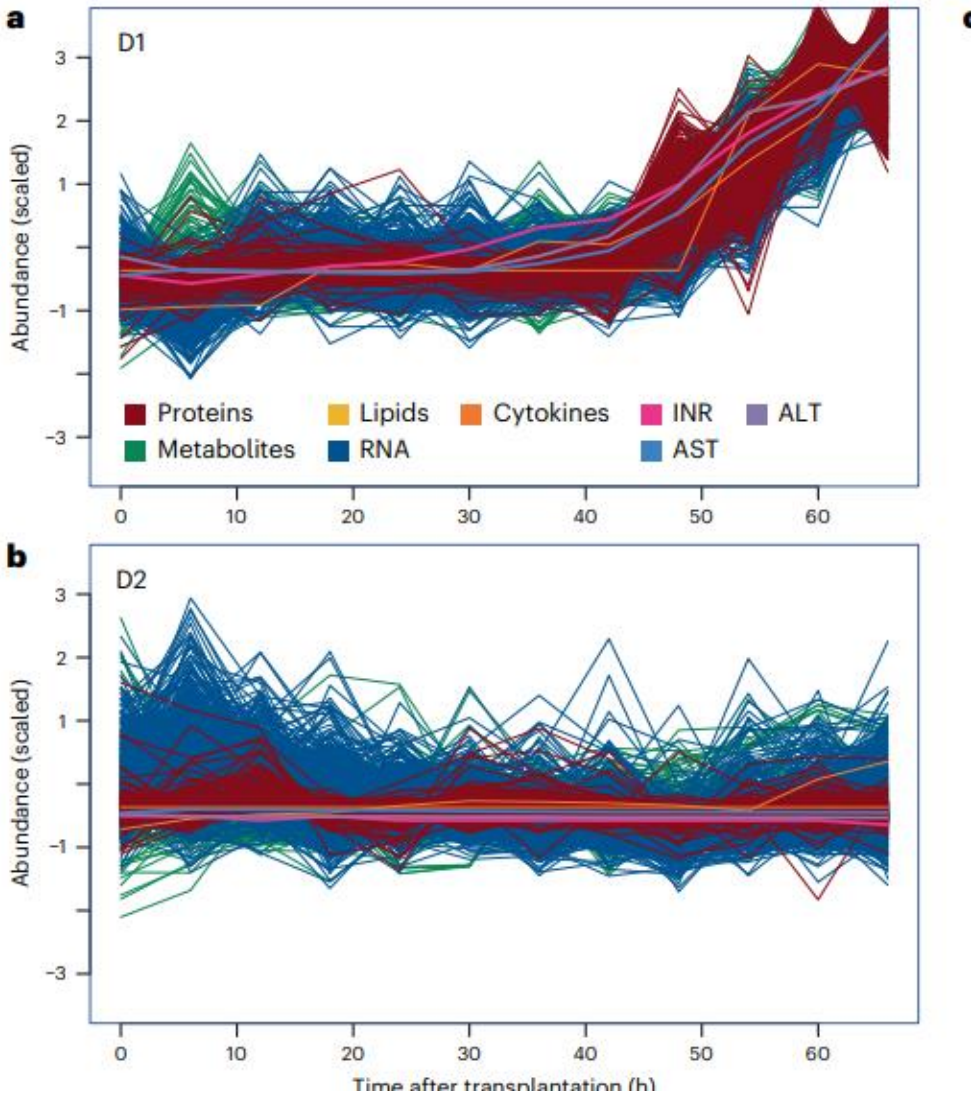



**Fig. 1 | Study design and sampling timeline.** For both decedents, left and right ventricle tissue samples were obtained 66 h after transplantation for snRNA-seq and spatial transcriptomic analyses. Peripheral blood samples were collected at 6-h intervals for comprehensive analysis, including metabolomic, proteomic, lipidomic, cytokine profiling, bulk RNA-seq, scRNA-seq and execution after transplantation. The timing at which each assay from blood was carried out is indicated with a gray dot for sampling from both decedents and purple or green dots for sampling from only D1 or D2, respectively. Routine clinical measurements including INR, ALT and AST, in addition to arterial





| Date | Tx Site | Length (Days) | Organ | Company/(Gene Edits) | PubMed ID/Note | |
|---|---|---|---|---|---|---|
| **Decedent Studies** | | | | | | |
| Sept 25th 2021 | NYU, USA | 3 | Kidney | UT, (1, α-Gal) | 35584156 | |
| Sept 30th 2021 | U-Alabama, USA | 3 | Kidney | UT, (10) | 35049121 | |
| Nov 2021 | NYU, USA | 3 | Kidney | UT, (1, α-Gal) | 35584156 | |
| Jan 2022. | U-Alabama, USA | 3 | Kidney | UT, (10) | | |
| Nov 2022 | Hainan, MU, China | 12 | Kidney | Clonorgan (4) | 39317190 | |
| Spring 2023 | U-Alabama, USA | 7 | Kidney | UT, (10) | 37585176 | |
| June 2023 | Hainan, MU, China | 6 | Kidney | Clonorgan (5) | 39317190 | |
| Juy 2023 | NYU, USA | 61 | Kidney | UT, (1, α-Gal) | pending | |
| Mar 2024 | Xijing Hospital, China | 9 | Kidney | Qihan Biotech (3) | pending | |
| June 2022 | NYU, USA | 3 | Heart | UT, (10) | 37488288 | |
| July 2022 | NYU, USA | 3 | Heart | UT, (10) | 37488288 | |
| Dec 2023 | U.Penn, USA | 3 | Liver | eGenesis (69) | pending | *ex vivo* pig liver support |
| Mar 2024 | Xijing Hospital, Chin | 10 | Liver | Clonorgan (6) | 38514875 | |
| April 2024 | U.Penn, USA | 4 | Liver | eGenesis (69) | pending | *ex vivo* pig liver support |
| May 2024 | U.Penn, USA | 4 | Liver | eGenesis (69) | pending | *ex vivo* pig liver support |
| Spring 2025 | U.Penn, USA | 5 | Liver | eGenesis (69) | pending | *ex vivo* pig liver support |
| **Living Humans** | | | | | | |
| Jan 2022 | U.Maryland | 60 | Heart | UT, (10) | 37393920 | Cause of death still not unclear |
| Sept 2023 | U.Maryland | 40 | Heart | UT, (10) | 39779924 | Patient died with suspected AbMR |
| Mar 16th 2024 | MGH, USA | 65 ** | Kidney | eGenesis (69) | 39927618 | Xenograft was functioning at time of death |
| April 12th 2024 | NYU, USA | 47** | Kidney | UT, 1 (α-Gal) | pending | Xenograft removed (LVAD complications) |
| Nov 25th 2024 | NYU, USA | 130** | Kidney | UT, (10) | pending | Xenograft lasted 130 days. Pt back on dialysis |
| Jan 25th 2025 | MGH, USA | 110+ | Kidney | eGenesis (69) | pending | ** As of 5/15/205 |
| Mar 6th 2025 | Xijing Hospital, China | 30+ | Kidney | Qihan Biotech/eGenesis | pending | ** As of last media report 4/25/2025 |
| May 17th 2024 | Anhui Univ, China | >10** | Liver | Yunnan Univ (10) | pending | Patient is reported to have died ater 100 days |

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.)

# 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

# 2. Genetiği Değiştirilmiş Domuzdan İnsana kalp nakli : 6 hafta sonra ex

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
Toplam 69 gen edit

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

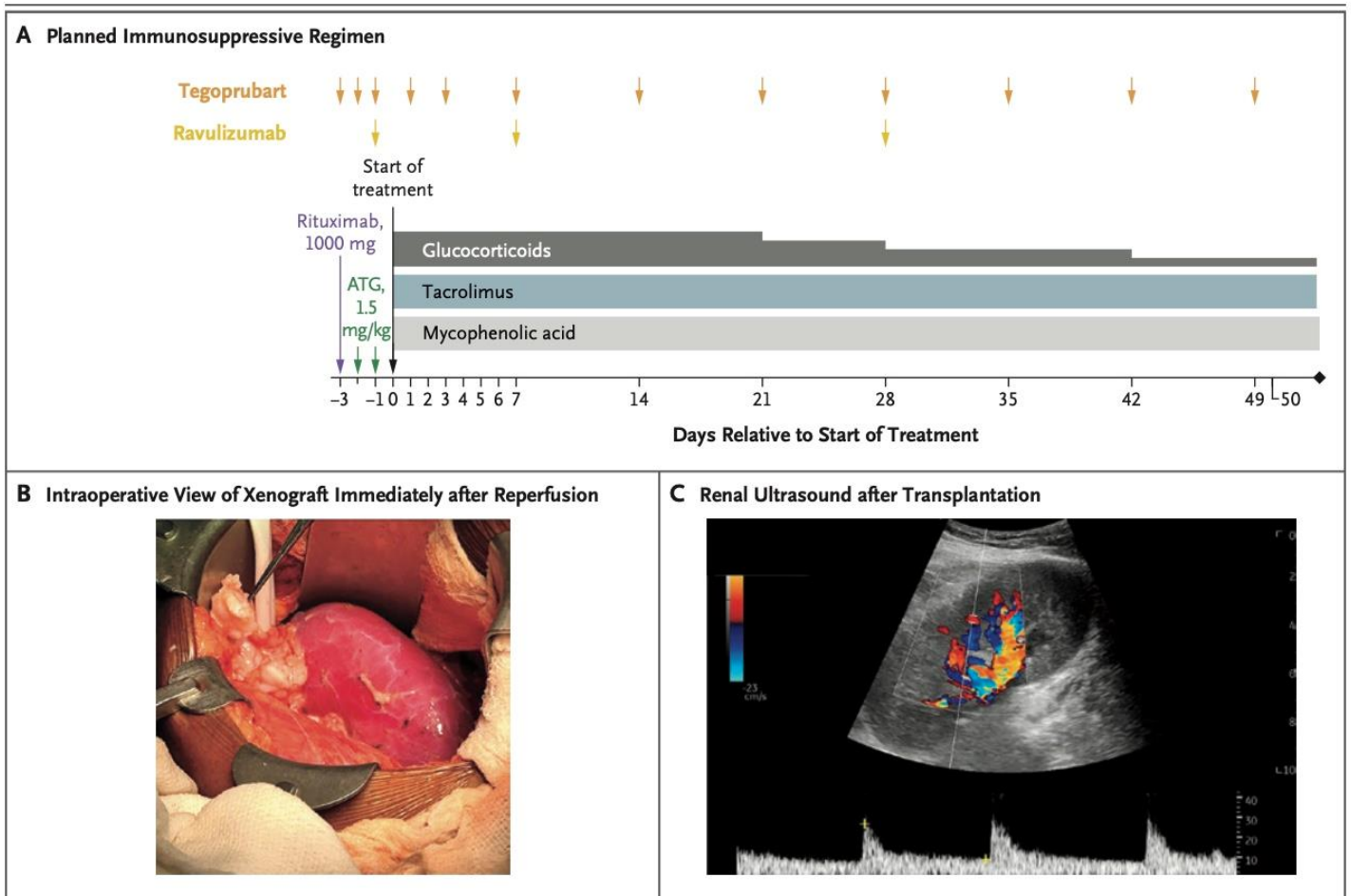
# 4.4.2024

## World's first pig kidney transplant recipient discharged from hospital

# İmmunsupresyon

- ATG
- RTX
- Anti-CD 154 monoklonal Ab (Tegoprubart)
- Anti C5 Ab (Ravulizumab)
- Tac-MMF-PRednizolon

8. Gün TCM Rejeksiyon 2A
Tociluzumab
ATG-Steroid



The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

# Xenotransplantation of a Porcine Kidney for End-Stage Kidney Disease

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**52 gün sonra ani Kardiak nedenli Ex**

## SUMMARY

Xenotransplantation offers a potential solution to the organ shortage crisis. A 62-year-old hemodialysis-dependent man with long-standing diabetes, advanced vasculopathy, and marked dialysis-access challenges received a gene-edited porcine kidney with 69 genomic edits, including deletion of three glycan antigens, inactivation of porcine endogenous retroviruses, and insertion of seven human transgenes. The xenograft functioned immediately. The patient's creatinine levels decreased promptly and progressively, and dialysis was no longer needed. After a T-cell–mediated rejection episode on day 8, intensified immunosuppression reversed rejection. Despite sustained kidney function, the patient died from unexpected, sudden cardiac causes on day 52; autopsy revealed severe coronary artery disease and ventricular scarring without evident xenograft rejection. (Funded by Massachusetts General Hospital and eGenesis.)

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# eGENESIS Kidney

**She donated a kidney years ago. Last month, she received a kidney transplanted from a gene-edited pig**

By Jen Christensen, CNN
7 minute read · Updated 3:35 PM EST, Tue December 17, 2024

T. Looney, 54Y,
1999'da annesine böbrek vermiş
Gebelikte Preeklampsi ve KBH gelişmiş
2016'da Diyalize başlanmış

90 günü geçen ve Genetiği değiştirilmiş Domuz böbreği ile yaşayan ilk insan
UKIDNEY

30 Ocak 2025- 7 Şubat 2025

# Genetiği değiştirilmiş domuz üretimi Bioteknoloji Firmaları

**Revivicor (UT) (NYU)**
**eGenesis (Mass Gen.)**
**+ 4 company**

10 mutasyon genetikUKidney 10 mut.+59 gen edit

1 mutasyon + Uthmyokidney yok

125/yıl domuz üretebilecek kapasite ?

2 kalp ve 1 böbrek/canlı alıcı 1 böbrek/canlı alıcı (taburcu

ancak

- Kalp alıcıları 45 ve 60 günde ex 52. günde ex greft fonksiyonel

4 böbrek/kadavra alıcı (2 ay fonksiyon) T. Andrews, Halen hayatta +180

Pisano, U thymokidney, 1.5 ay greft, 2.5 ay survi

T. Looney, Ukidney, Canlı alıcı halen hayatta +120

# FDA

- United Therapeutics
- *eGENESİS ?*
  - 2025 için UT firmasına
    - Tahmini 50 hasta alımı planlanması için onay verdi

**UNITED THERAPEUTICS CORPORATION ANNOUNCES FDA CLEARANCE OF ITS INVESTIGATIONAL NEW DRUG APPLICATION FOR THE UKIDNEY XENOTRANSPLANTATION CLINICAL TRIAL**

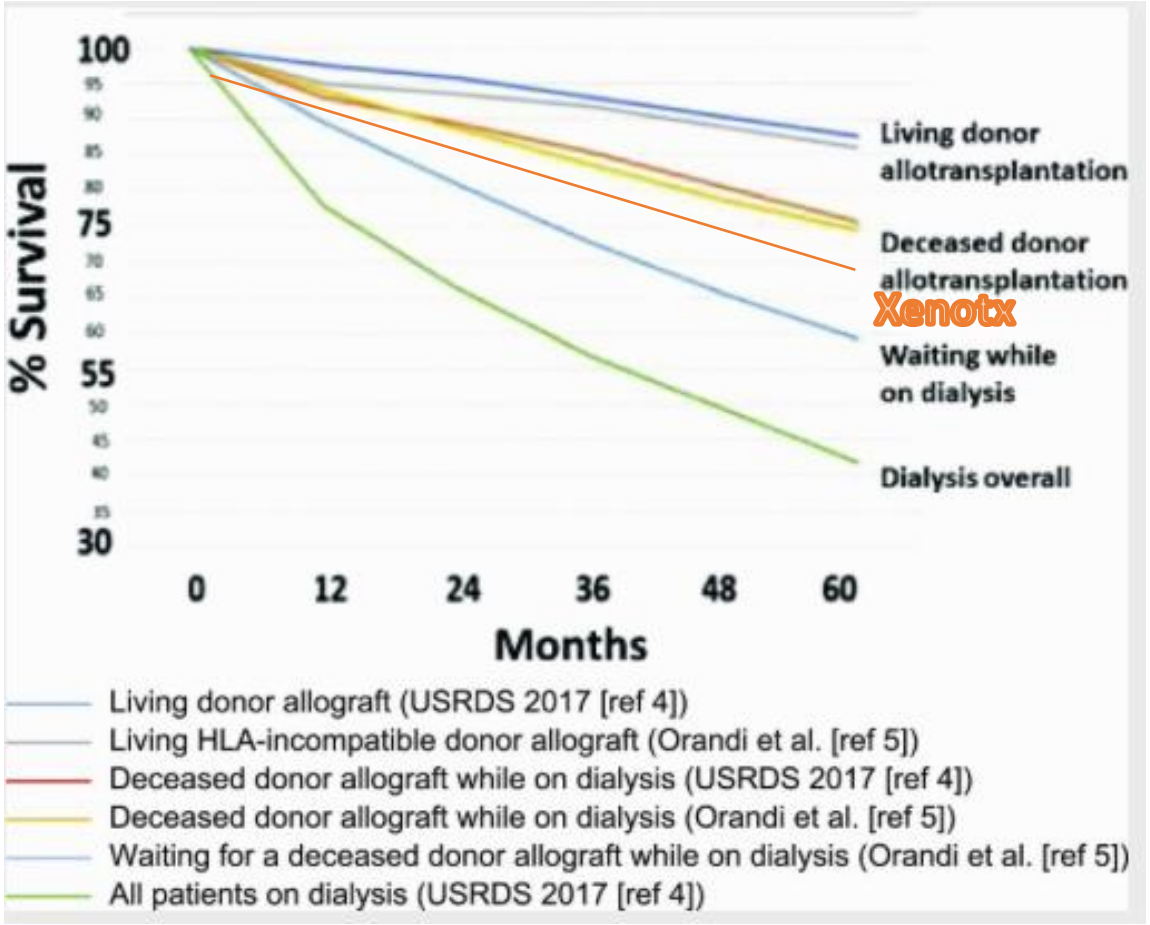
# Xenotransplantasyon Klinik Çalışma

- Domuzdan baboon a başarılı nakil
- Domuzdan beyin ölümü olan insana başarılı nakil
- Domuzdan yaşam beklentisi düşük insana nakil

- 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 ?
    - Massachusetts GH
    - NYU- Langone
    - Johns Hopkins

# Böbrek Nakli

- **Xenotransplantasyon sonuçları : beklenti**




USRDS,2020

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

# ‘Amazing feat’: US man still alive six months after pig kidney transplant

**The first six months after an organ transplant are the riskiest for recipients.**

By Rachel Fieldhouse