# La Xénotransplantation

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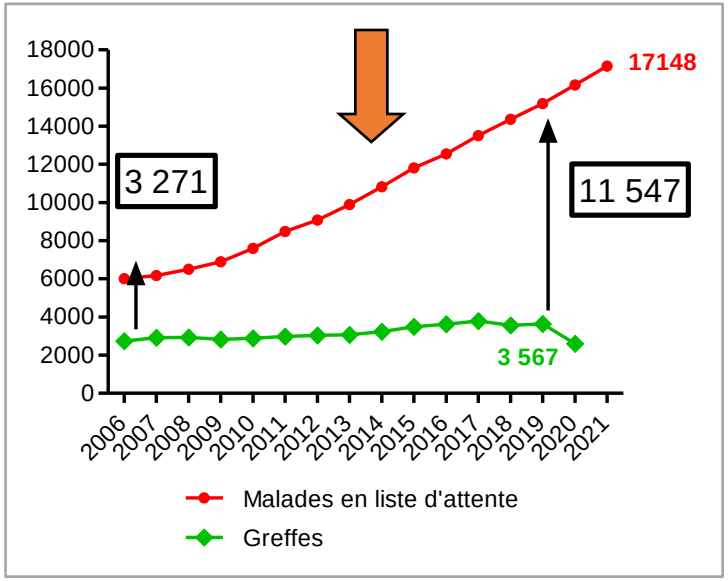
# Current Problems of the transplantation

Organ shortage




Immunosuppression

Long term side effects:

Infection and Cancer Risk

Chronic rejection
Ab mediated rejection

Low control of Immunosuppression

***Xenotransplantation*** ⟷ ***Tolerance Induction***
***Desensitization***

# Les raisons du regain d'intérêt récent pour la xénotransplantation

- La pénurie d'organes
- L'émergence de nouvelles technologies : thérapie génique, transgénèse, clonage,

# Historique de la Xénotransplantation

Les années 1900 : Matthieu Jaboulay, Alexis Carel … mise au point de l'anastomose vasculaire, premières xénotransplantations chez l'humain

| DONNEUR | ORGANE | SURVIE | ANNEE | AUTEURS |
|---|---|---|---|---|
| Chimpanzé | REIN | 9 mois | 1964 | Reemtsma |
| Monkey | REIN | 10 jours | 1964 | Reemtsma |
| Babouin | REIN | 4 jours | 1964 | Hitchcock |
| Babouin | REIN | 2 mois | 1964 | Starzl |
| Chimpanzé | COEUR | Insuffisance cardiaque | 1964 | Hardy |
| Chimpanzé | FOIE | 14 jours | 1969-1974 | Starzl |
| Babouin | COEUR | Rejet suraigu | 1977 | Barnard |
| Chimpanzé | COEUR | 4 jours | 1977 | Barnard |
| Babouin | COEUR | 4 semaines | 1985 | Bailey |
| Babouin | FOIE | 70 jours | 1992 | Starzl |

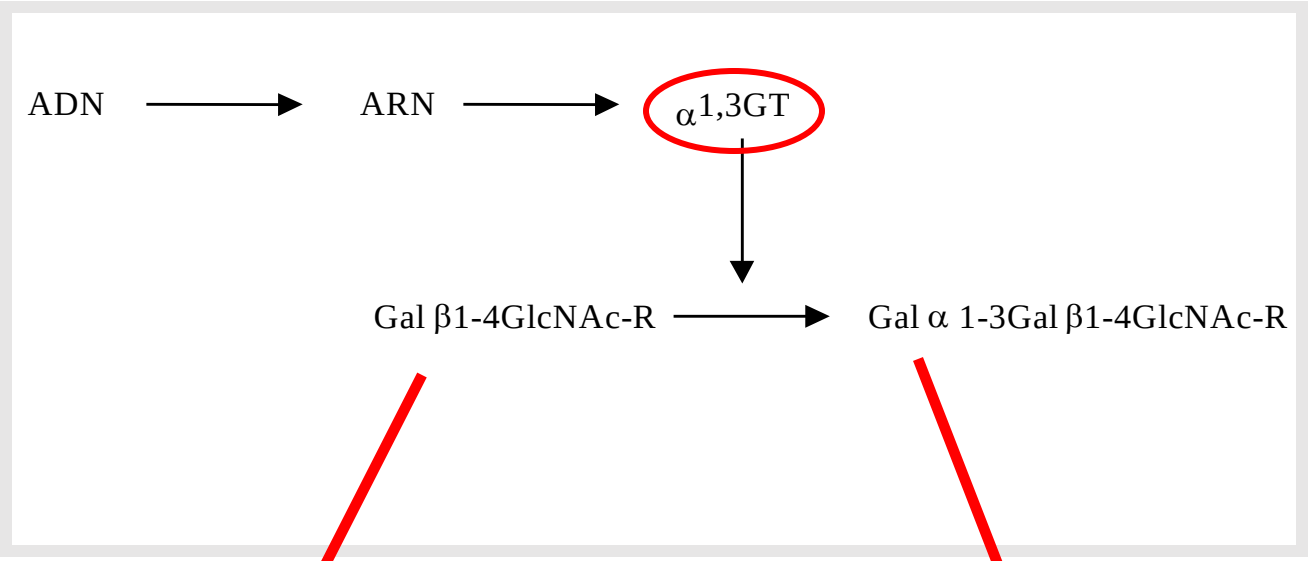
# Particularités des primates

- Intérêt en tant que modèle préclinique … s'il n'y a pas de modèle alternatif
- La proximité d'espèce = 99% d'homologie génomique entre un chimpanzé et un humain
  - Le 1% fait la différence …
- Le chimpanzé (et autres espèces en voie de disparition, Grands singes anthropoides, éléphants) : Annexe 1 de la convention de Washington
- -→ Utilisation d'autres primates

# Le choix de l'animal donneur

- Les primates : NON
  - les rétrovirus (proximité d'espèce)
  - problèmes éthiques
- Le porc : OUI
  - culture et facilité d'élevage
  - taille et physiologie compatibles
  - accès à la transgénèse
  - risque viral demeure

# Spécificité des humains et des primates de l'ancien monde




Humains
Babouins
Macaques

Autres mammifères, quelques exceptions (capybara)

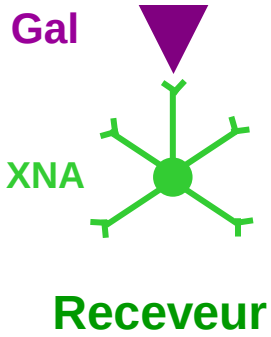
# Galα1-3Gal : Ag xénogénique

**Porc**

**Galα1-3Galβ1-4GlcNAc (Gal)**

**Homme/Primate de l'ancien monde**

**Fucα1-2Galβ1-4GlcNAc (H)** ⇢ **Groupe sanguin A ou B**



# Distribution de l'épitope Gal et Ac anti-Gal

| Species | Gal expression | Anti-Gal Ab production |
|---|---|---|
| **Non-primates mammals** (pig, mice, rabbit, rat) | + | - |
| **New world monkeys** (South America) (marmouset, tamarin, capucin) | + | - |
| **Old world monkeys** (Asia et Africa) (baboons, macaques**)** | - | + |
| **Large monkeys** (chimpanzee, gorilla, gibbon, orang-outan) | - | + |
| **Human** | - | + |

# Xénotransplantation

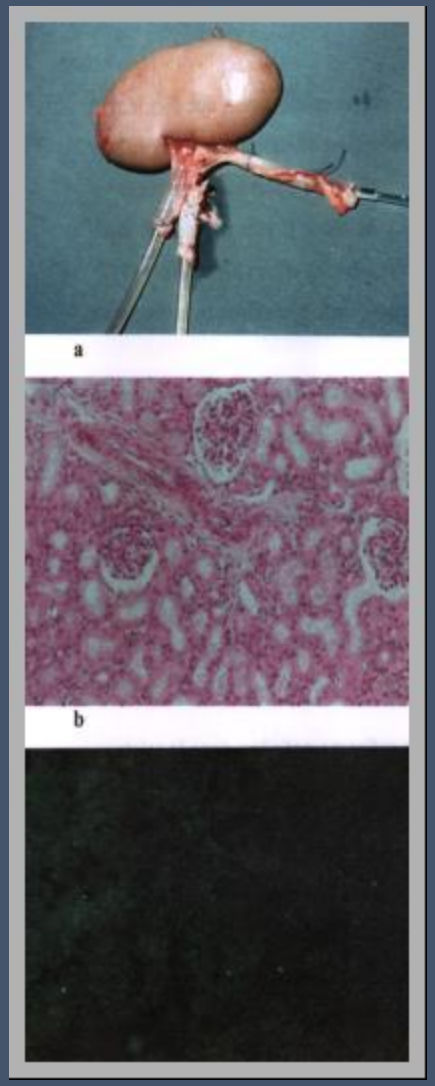
- **Combinaison discordante : porc sur homme/primate**

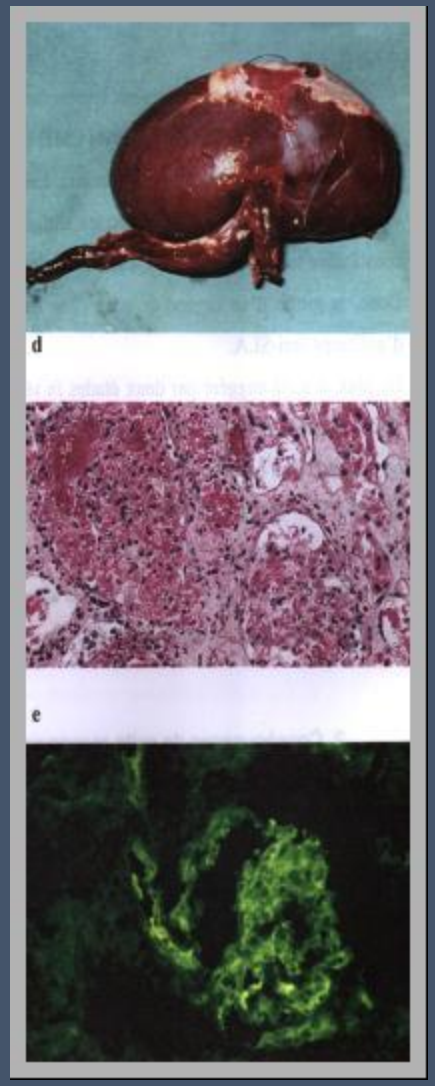
**Ac préformés** → **Rejet hyperaigu** → **Rejet vasculaire aigu**

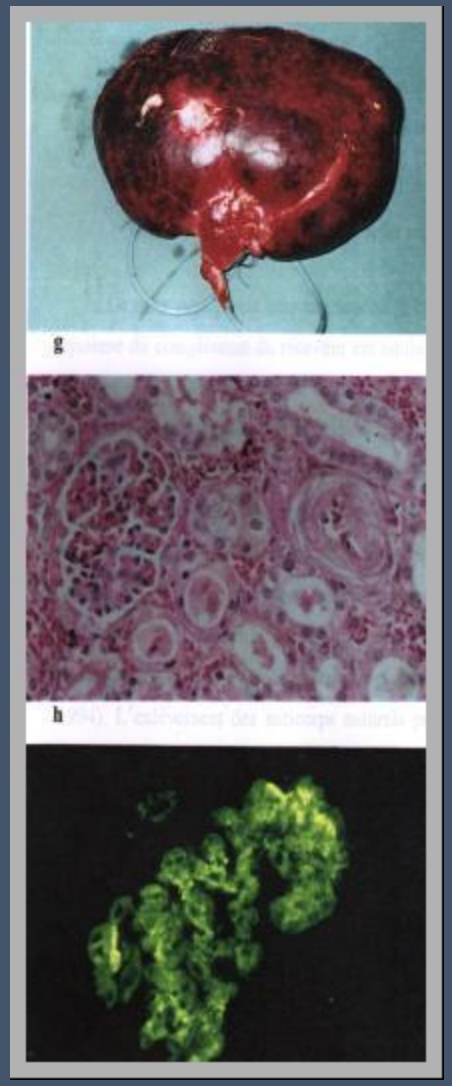
- **Combinaison concordante : hamster sur rat**

**Pas Ac préformés** → **Rejet vasculaire aigu**

**Rein normal** **Rejet hyperaigu** **Rejet vasculaire aigu**




**macroscopie**

**histologie**

**IgM**

# Le rejet hyperaigu

**Ac xénoréactifs**
**Complément**

Recrutement leucocytaire

**C3a/C5a**

**Défenses cellules endothéliales au repos: ADPase, Thrombomoduline, TFPI**

**Médiateurs d'inflammation**
**C3a/C5a, histamine, LT4**

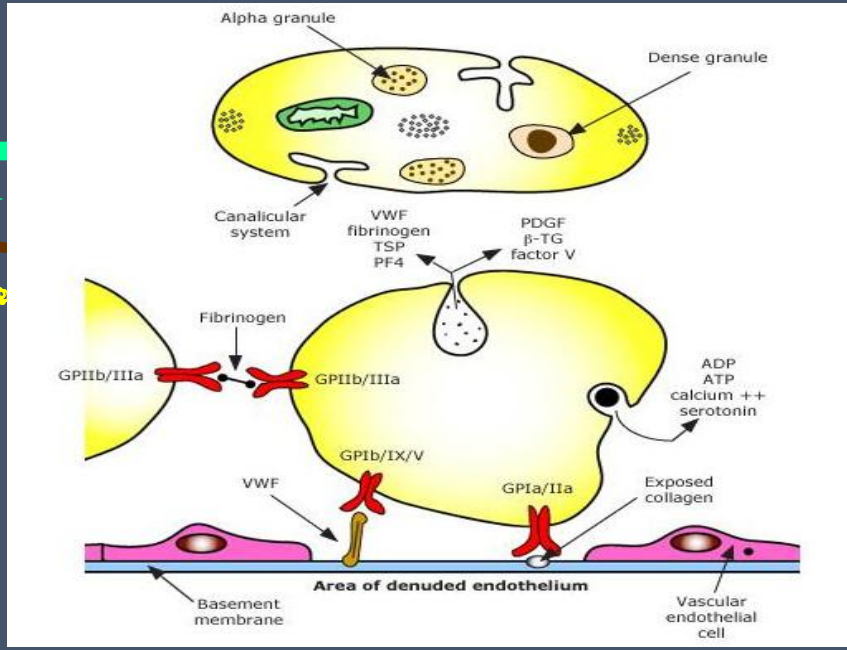
PAF

P-sélectine

galα1-3gal

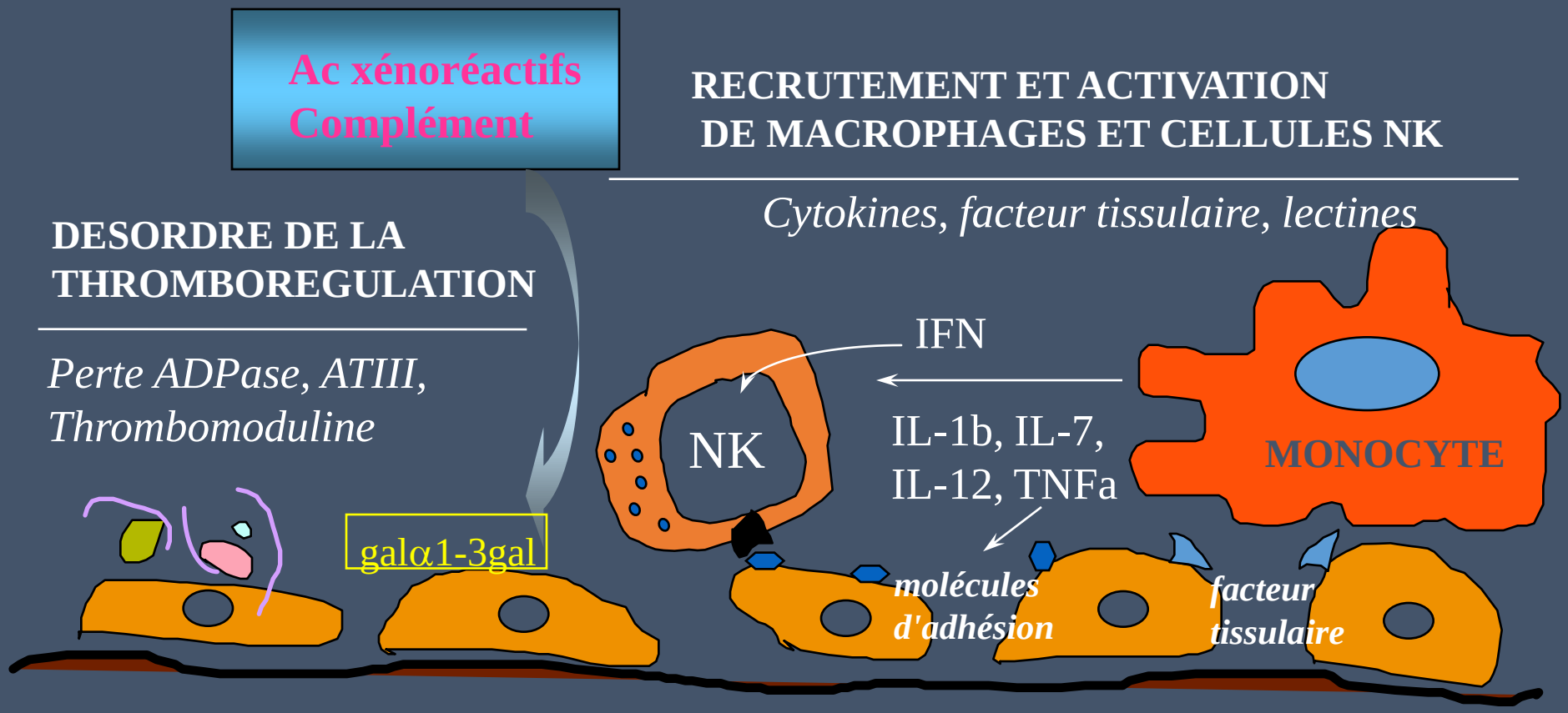
gpIb

**vWF**




Activation endothéliale de type I:
rétraction endothéliale, P-sélectine

# Le rejet vasculaire aigu




**ACTIVATION ENDOTHELIALE DE TYPE II**

*adhésion leucocytaire, réponse procoagulante, cytokines (IL-8, MCP...)*

# LES MODELES ANIMAUX

# Protocoles sur des primates sans ou avec immunodépression (1)

| Author/Year | Recipient | Porcine Graft | Treatment | Graft survival |
|---|---|---|---|---|
| Calne/1968 | Baboon | Liver | CS + AZA | 6h-3 days |
| Calne/1970 | Rhesus | Liver | ALG | <12h |
| Cooper/1988 | Baboon | Heart | None<br>SPx<br>CsA+ CS | <8h<br><8h<br>2h-5days |
| Alexandre/1989 | Baboon | Kidney | None | <2h |
| Fischel/1992 | Rhesus | Heart | None | 2h |
| Leventhal/1993 | Baboon | Heart | None | 90min |
| Kawauchi/1994 | Japanese macaques | Heart | None<br>SPx + FK506 | <14 min<br>30 min |
| Ye/1994 | Baboon | Heart | CsA+CyP+MTX+CS<br>Idem+melibiose | 15 min<br>12-18h |
| Kaplon/1995 | Baboon | Heart | None | <82h |
| Kaplon/1995 | Cynomolgus | Lung | None | 7-9h |
| Sablinski/1995 | Cynomolgus | Kidney | None | <1h |
| Kobayashi/1996 | Baboon | Heart | CsA±CyP±CS±MTX± | 15-40 min |
| Michler/1996 | Baboon | Heart | None | 15-96h |

From *Lambrigts et al. Transplantation, 1998; 66: 547*

# Protocoles sur des primates sans ou avec immunodépression (2)

| Author/Year | Recipient | Porcine graft | Treatment | Graft survival |
|---|---|---|---|---|
| Sanfilippo/1996 | Cynomolgus | Heart | None<br>CsA+CyP+CS | 1h<br>1h |
| White/1996 | Cynomolgus | Heart | None | 45 min |
| Zaidi/1997 | Cynomolgus | Heart | CsA+CyP+CS | <30 days |
| Luo/1997 | Baboon | Liver | CsA+CyP+CS | 2h |
| Minanov/1997 | Baboon | Heart | None<br>CsA+MMF+CS | <4days<br><6days |
| Yeatman/1997 | Baboon | Lung | None | <30 min |
| Shah/1997 | Baboon | Lung | None | <4h |
| Blum/1997 | Cynomolgus | Lung | Thromboxane R blocker ± NO | 2-15 min |
| Kawauchi/1997 | Monkey | Heart | SPx + FK506 | 5-11 days |
| Salerno/1997 | Rhesus | Heart | None | < 2h |
| Daggett/1997 | Baboon | Lung | None | <30 min |
| Lawson/1997 | Baboon | Kidney | CsA+CyP+CS | 45 min |
| Itescu/1997 | Baboon | Heart | CsA+CyP+CS | 6 days |

From *Lambrigts et al. Transplantation, 1998; 66: 547*

# Protocoles avec déplétion des Ac anti-porc (1)

| Author/Year | Recipeint | Porcine organ | Treatment | Graft survival |
|---|---|---|---|---|
| Cooper/1988 | Baboon | Heart | IA | 20h-5 days |
| Alexandre/1989 | Baboon | Kidney | PE+ALG+CsA+CS+SBGS | |
| Fischel/1991 | Rhesus | Heart | PE+SPx+ALG+CsA+AZA +CS | 1-8 days |
| Fischel/1992 | Rhesus | Heart | PE<br>IA<br>IA+ALG+CsA+AZA+CS<br>PE+ALG+CsA+AZA+CS | <12h<br><80h<br>120h<br>192h |
| Roslin/1992 | Baboon | Heart | IA+TLI+CsA+CS | 6-15 days |
| Brewer/1994 | Baboon | Heart | IA+TLI<br>PE+TLI | 6-15 jours<br>1-8 days |
| Fukushima/1994 | Baboon | Heart | CsA+DSG+FUT175+IA+S Px±PE | <16 days |
| Kawauchi/1994 | Japanese macaque | Heart | PE<br>PE+SPx+FK506+FUT175 | 8 min<br>86-270 min |
| Leventhal/1994 | Baboon | Heart | PE+SPx+DSG | <50h |
| Leventhal/1995 | Baboon | Kidney | IA+SPx+CyP+DSG+ ATG+CS | 13 days |
| Gianello/1995 | Baboon | Kidney | PE+ALG+CsA+CS | 1-23 days |

From *Lambrigts et al. Transplantation, 1998; 66: 547*

# Protocoles avec déplétion des Ac anti-porc (2)

| Author/Year | Recipient | Porcine organ | Treatment | Graft survival |
|---|---|---|---|---|
| Sablinski/1995/ 1997 | Cynomolgus | Kidney | IA+WBI+TI+ATG+ Donor bone marrow+SPx+IgMmAb +CsA | <15 days |
| Cooper/1996 | Baboon | Heart | IA+CsA+CyP+CS | <115 min |
| Kobayashi/1996 | Baboon | Heart | FUT175+K76COOH | 4-10h |
| Kobayashi/1996 | Baboon | Heart | CVF+SPx+CsA±CyP+ CS±MTX | 6-25 days |
| Sanfilippo/1996 | Cynomolgus | Heart | sCR1 | 48-90h |
| Pruitt/1996 | Cynomolgus | Heart | sCR1 (continuous) | 11 days |
| Bollinger/1996 | Cynomolgus | Heart | sCR1 (continuous) + CsA+CyP+CS | 21-31 days |
| Marsh/1997 | Cynomolgus | Heart | intermTItent sCR1 +CsA+CyP+CS | 6 weeks |
| Kroshus/1997 | Baboon | Heart | IA+SPx+CsA+CS+MT X | 5 days |
| Matsumiya/1997 | Baboon | Heart | IA+SPx+FUT175 ±FK506+MTX+ATG | 4-14 days |
| Xu/1997 | Baboon | Heart | IA+TLI+CsA+MTX+A TG+ISP | <19 days |
| Meyer/1997 | Baboon | Kidney | IA | 2 days |

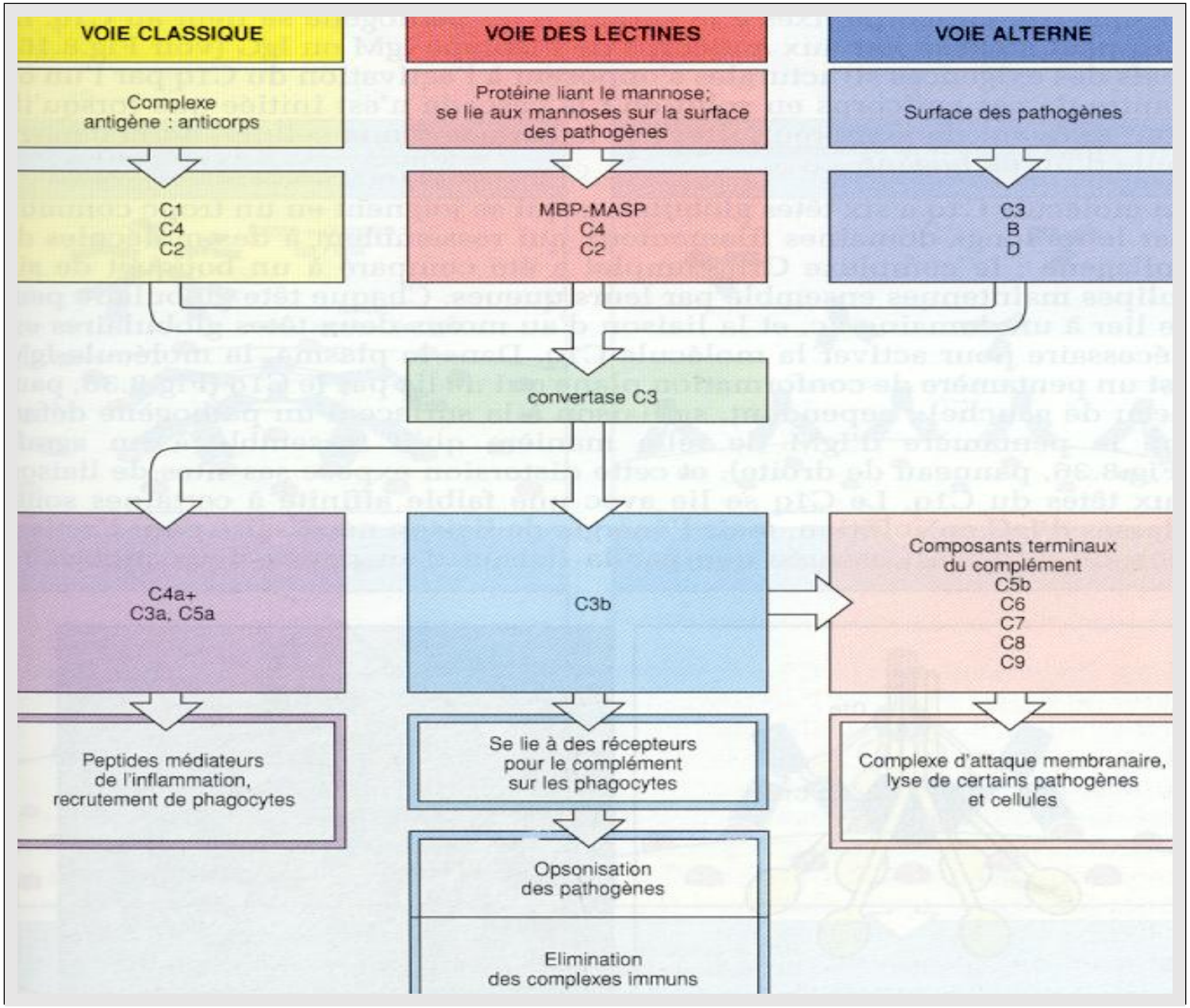
From *Lambrigts et al. Transplantation, 1998; 66: 547*

# Protocoles avec déplétion ou inhibition du complément

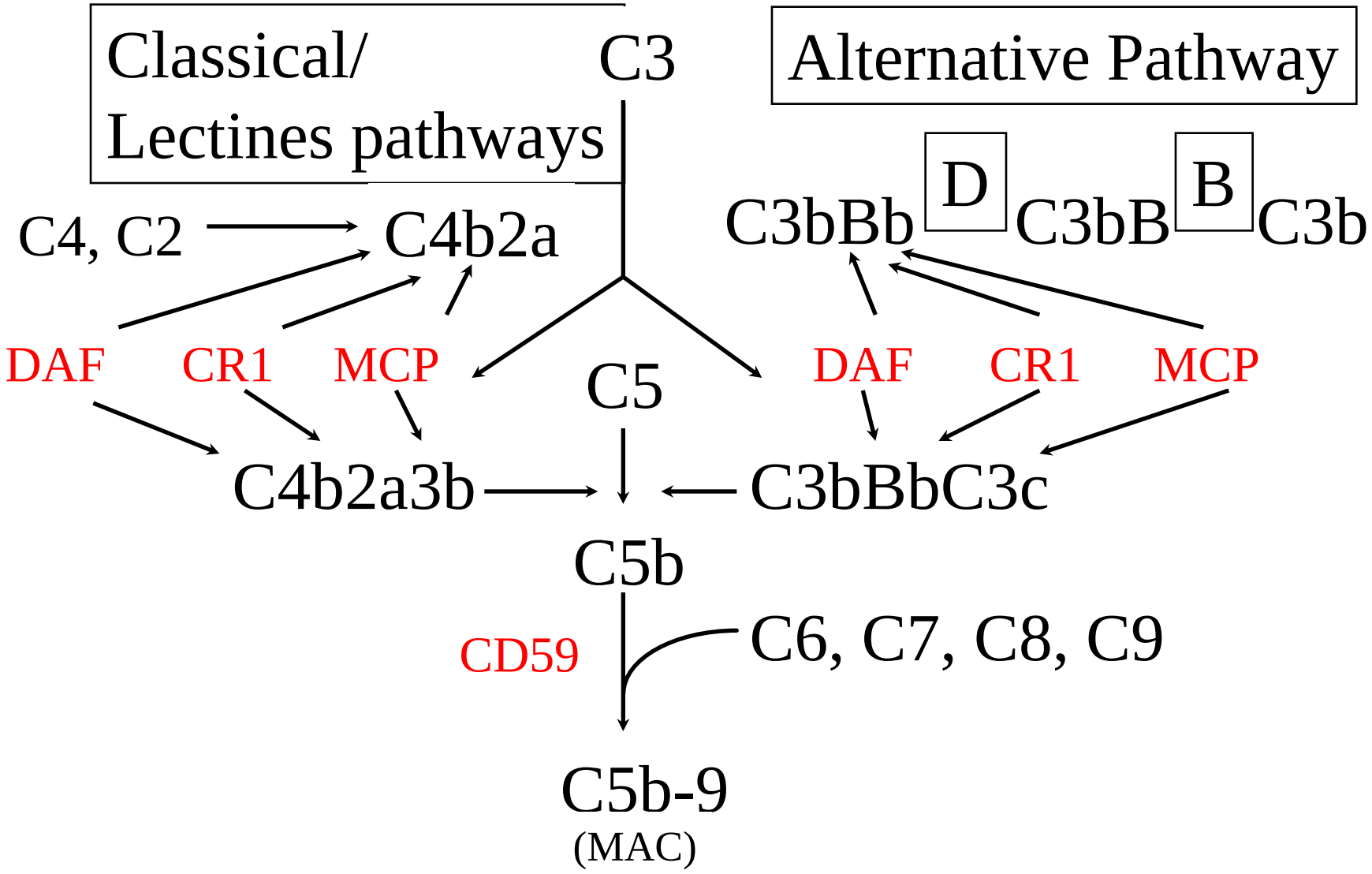
| Author/Year | Recipient | Porcine graft | Treatment | Graft survival |
|---|---|---|---|---|
| Leventhal/1993 | Baboon | Heart | CVF | 68-92h |
| Fukushima 1994 | Baboon | Heart | CsA+DSG+FUT175+SPx | 9.8h |
| Leventhal/1994 | Baboon | Heart | CVF+SPx+DSG+CyP + ALG+CsA+CS | <24h<br>8-17 days |
| Pruitt/1994 | Cynomolgus | Heart | SCRI | 5-7 days |
| Kobayashi/1996 | Baboon | Heart | FUT175+K76COOH | 4-10h |
| Sanfilippo/1996 | Cynomolgus | Heart | sCR1 | 48-90h |
| Pruitt/1996 | Cynomolgus | Heart | sCR1 (continuous) | 11 days |
| Bollinger/1996 | Cynomolgus | Heart | sCR1 (continuous) + CsA+CyP+CS | 21-31 days |
| Kobayashi/1997 | Baboon | Heart | CVF+SPx+CsA±CyP+CS±MTX | 6-25 days |
| Marsh/1997 | Cynomolgus | Heart | intermittent sCR1 +CsA+CyP+CS | 6 weeks |

From *Lambrigts et al. Transplantation, 1998; 66: 547*

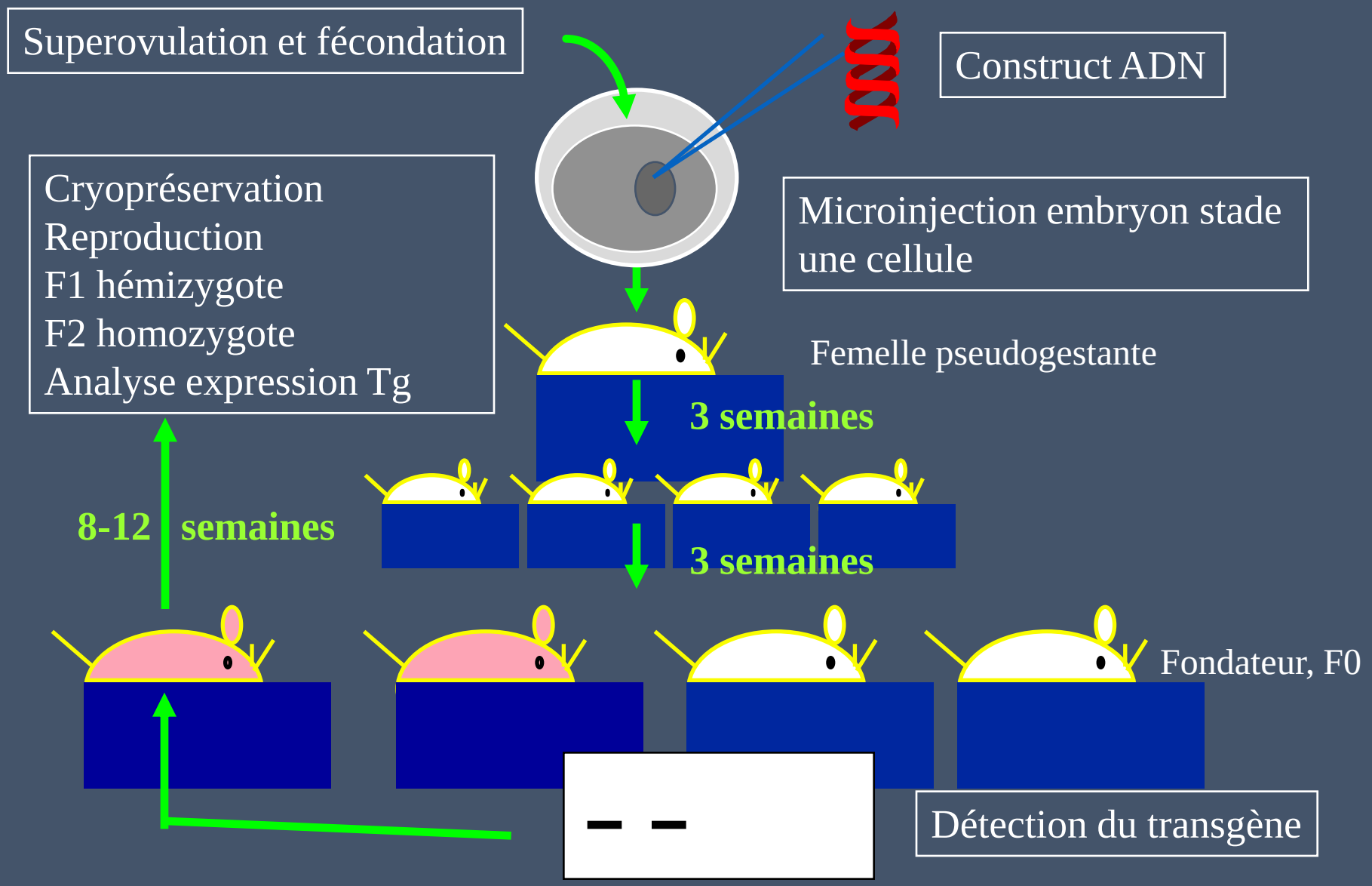
# Le système du complément



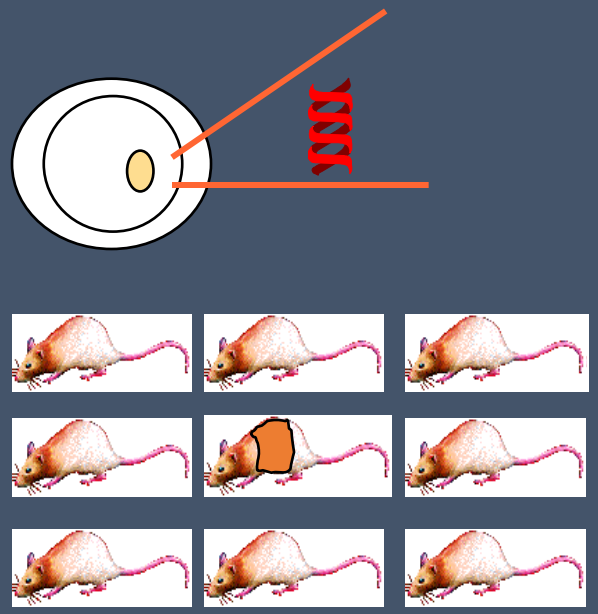
# Complement Activation



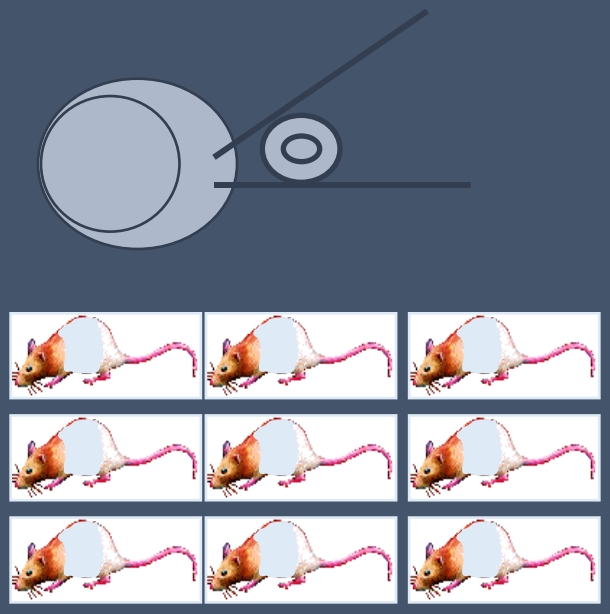
# Transgénèse



## DNA Microinjection



## Nuclear Transfer



| | | |
|---|---|---|
| Time | longer | shorter |
| Gene Integration | non-controled | controled |
| New gene | surexpression | surexpression |
| | knock-down: | knock-out |

antisense, intrabodies, intrakines, toxines ou genes suicide
competition enzymatique, mutants dominant negatifs

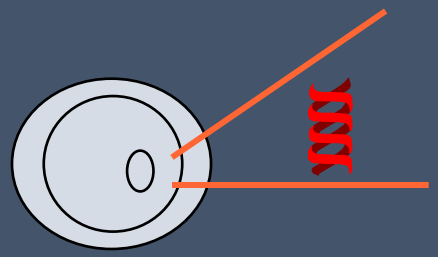
# Animaux transgéniques

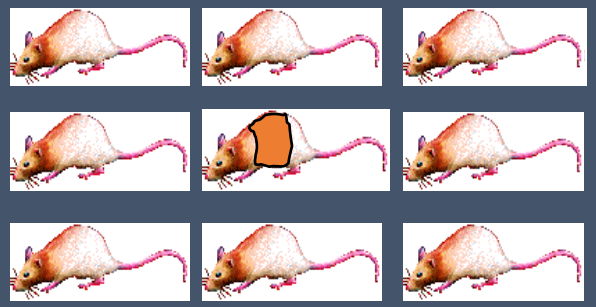
- **CD55**
- **CD59/CD55**
- **CD59/CD46**
- $\alpha 1-2$ **Fucosyl Transferase I and II**
- $\alpha$ **Galactosidase**
- **Sialyl Transferase ($\alpha$ 2.3, $\alpha$ 2.6)**
- **N-Acetyl GnT-III**
- **Anti Galactosyl Transferase ScFv**
- **I$\kappa$B**
- **Molécules antiapoptotiques**
- **Microenvironnement anticoagulant**
- **CTLA4-Ig**

# Protocoles utilisant des organes de porcs transgéniques

| Author/Year | Recipient | Porcine graft | Treatment | Graft survival |
|---|---|---|---|---|
| McCurry/1995 | Baboon | Heart | hDAF+hCD59+SPx+CyP+ AZA+CS+IA | 4-30h |
| Norin/1996 | Baboon | Lung | hCD59 | <12h |
| White/1995 | Cynomolgus | Heart | HDAF | 5 days |
| Cozzi/1995/1997 | Cynomolgus | Heart | hDAF+CsA+CyP+CS | 6-62 days |
| Kroshus/1997 | Baboon | Heart | hCD59+IA+SPx+CsA+ CS+MTX | <10 days |
| Lin/1997 | Baboon | Heart | hCD59/hDAF+IA+CsA +CyP+CS | <29 days |
| Daggett/1998 | Baboon | Lung | hDAF/hCD59<br>hDAF/hCD59+IA | <4h<br><24h |
| Zaidi/1998 | Cynomolgus | Kidney | hDAF+CsA+CyP+CS | 6-35 days |
| Waterworth/1998 | Baboon | Heart | HDAF | 2-21 days |
| Yeatman/1998 | Baboons | Lung | hDAF+hCD59 | <3h |
| Bhatti/1997 /1999 | Baboon | Heart | hDAF+CsA+CyP+CS<br>hDAF+CsA+CyP+CS+ SPx | 3 months |
| Vial/2000 | Baboon | Lung | hDAF+CsA+CyP+CS | 39 days |
| Cowan/2000 | Baboon | Kidney | CD55/HT<br>CD55/CD59/HT | 30h<br>5 days |
| McGregor 2003 | Baboon | Heart | CD46 +FK<br>Rapa/αCD20/NEX1285 | 4.5 months |

## DNA Microinjection





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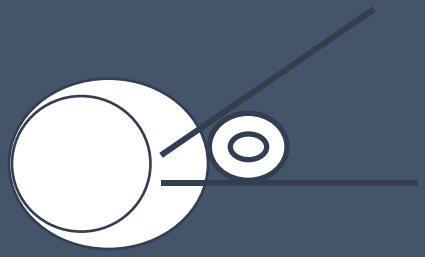

| | | |
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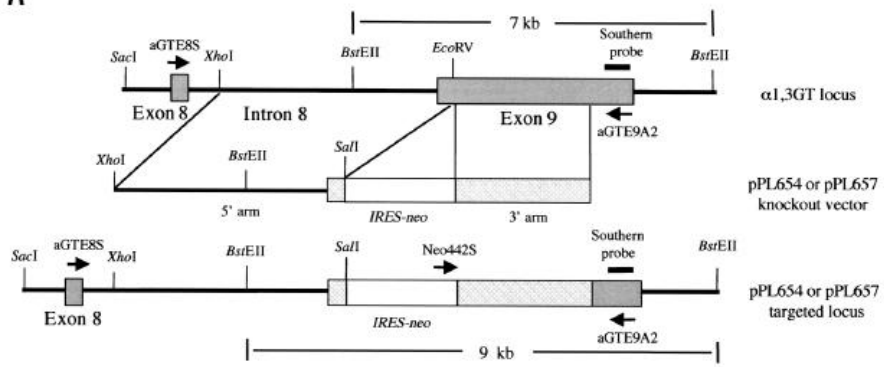
antisense, intrabodies, intrakines, toxines ou genes suicide
competition enzymatique, mutants dominant negatifs

# Les porcs Gal KO

- Production of $\alpha$ 1-3 galactosyltransferase Knockout pigs through nuclear transfer cloning. Liangxue Lai et al. Immerge Biotherapeutics. Science January 2002
- Targeted disruption of 1-3 galactosyltransferase gene in cloned pigs. Yifan Dai et al. PPL Therapeutics. Nature Biotechnology March 2002
- Production of $\alpha$1,3-Galactosyltransferase-Deficient Pigs.
  Phelps CJ et al. PPL Therapeutics. Science January 2003

  Groupe devenu Revivicor (Pittsburg, Fujizawa …)
- Le groupe de Tony D'Apice à Melbourne (pas de publications)

# Les porcs Gal KO



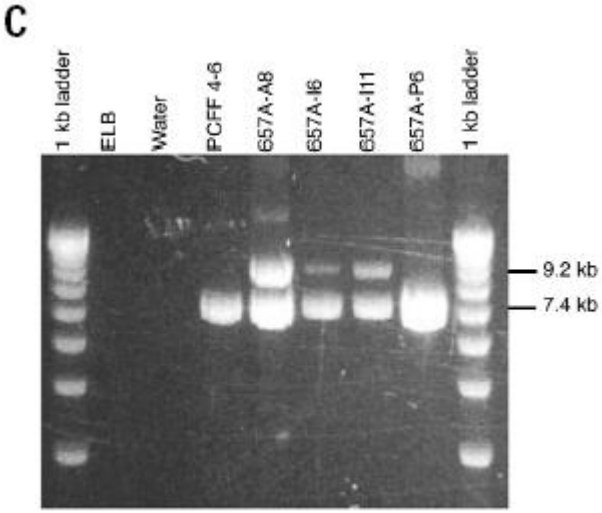




Figure 3. Five α1,3GT gene knockout piglets at 2 weeks of age.

αGT KO hétézygote

KO , 2eme allèle

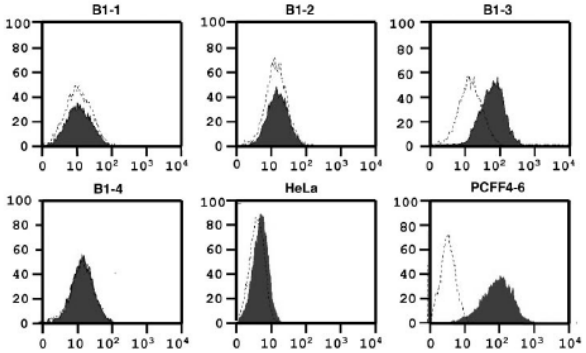



Fig. 1. Flow cytometry analysis of 680B1-1 to B1-4 cells with GS-IB4 lectin staining. Horizontal and

αGT KO homozygote

Dai Y, Nature Biotechnology, 20, March 2002

Phelps, Science January 2003

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

Kenji Kuwaki[1], Yau-Lin Tseng[1], Frank J M F Dor[1], Akira Shimizu[2], Stuart L Houser[3], Todd M Sanderson[1,2], Courtney J Lancos[1], Derek D Prabharasuth[1], Jane Cheng[2], Kathleen Moran[2], Yosuke Hisashi[1], Nicolas Mueller[4], Kazuhiko Yamada[1], Julia L Greenstein[2], Robert J Hawley[2], Clive Patience[2], Michel Awwad[2], Jay A Fishman[4], Simon C Robson[5], Henk-Jan Schuurman[2], David H Sachs[1], David K C Cooper[1,6]



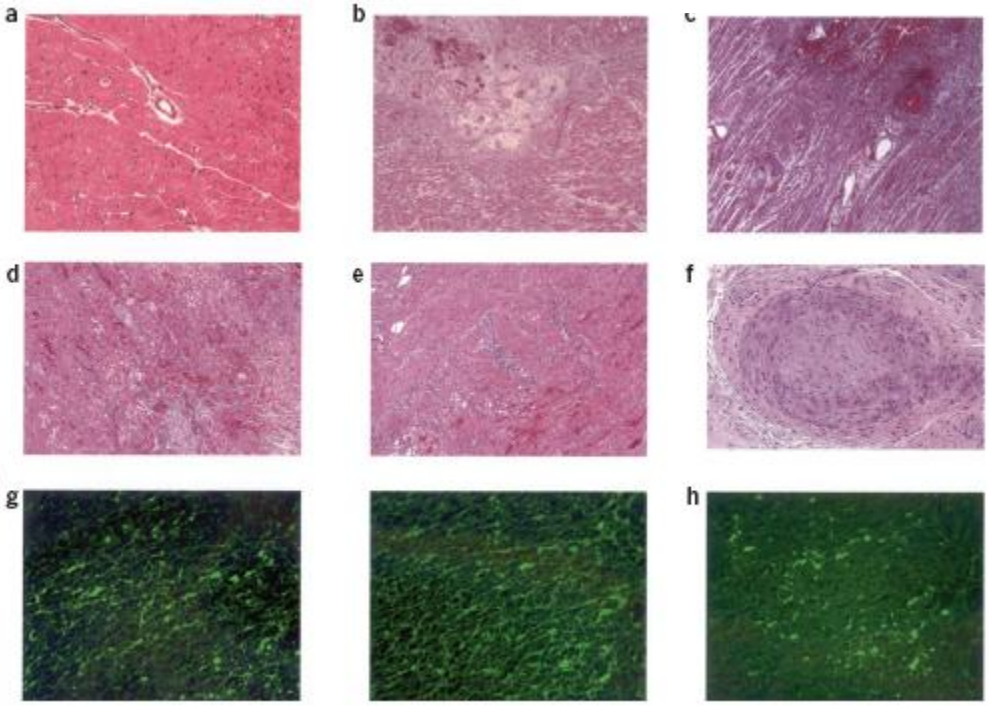
**Hearts from α1,3-galactosyltransferase knockout pigs (GalT-KO, $n = 8$) were transplanted heterotopically into baboons using an anti-CD154 monoclonal antibody–based regimen. The elimination of the galactose-α1,3-galactose epitope prevented hyperacute rejection and extended survival of pig hearts in baboons for 2–6 months (median, 78 d); the predominant lesion associated with graft failure was a thrombotic microangiopathy, with resulting ischemic injury. There were no infectious complications directly related to the immunosuppressive regimen. The transplantation of hearts from GalT-KO pigs increased graf survival over previous studies.**

on flow cytometry and a low level of cytotoxicity (>20% at 1:2–1:16) to GalT-KO peripheral blood mononuclear cells (PBMC). Pre- and immediate post-transplantation treatment reduced the T cell count to <500 cells/mm$^3$, but no considerable reduction of B cells (CD20$^+$) was observed.

Both GalT-low pig hearts were rejected within 20 min and showed histopathological features consistent with hyperacute rejection, with diffuse moderate-to-severe IgM deposition, and focal IgG and C4d deposition.

No GalT-KO heart underwent hyperacute rejection, and there were no major complications directly related to the immunosuppressive regimen. Of >100 blood cultures drawn over the course of the experiments, only three were positive; no case was associated with clinical infection. Three baboons died when donor heart contractions remained good, one on day 56 from bleeding and two by euthanasia on days 23 (anemia) and 16 (ischemic limb) (Table Ib). In five baboons, pig heart contractions ceased on days 59, 67, 78, 110 and 179, respectively (median 78 d; mean 99 d); we excised the hearts and continued to monitor the baboons. Graft survival of 179 d is the longest

| | | | | | | |
|---|---|---|---|---|---|---|
| Group 1: GalT-KO pig donors ($n = 8$) | | | | | | |
| B214 | 14 | + | – | – | 59 | TM, focal AHXR, alive |
| B216 | 14 | + | – | – | >56 | Died, heart beating, TM |
| B218 | – | + | – | – | 67 | TM, focal AHXR, alive |
| B223 | – | + | 1–12 | (late) | 110 | MI, TM, AHXR, ACR, vasculopathy, alive |
| B225 | – | + | 1–12 | – | >23 | MI, euthanized, heart beating, mild TM |
| B226 | 4 | + | – | + | >16 | Euthanized, heart beating, minimal TM |
| B228 | 4 | – | – | + | 179 | TM, focal AHXR, alive |
| B229 | 4 | + | 1–12 | + | 78 | MI, TM, focal AHXR, alive |
| Group 2: GalT-low pig donors ($n = 2$) | | | | | | |
| B220 | – | + | – | – | <1 | Hyperacute rejection |
| B222 | – | + | – | – | <1 | Hyperacute rejection |



# Porcs Gal KO

nature medicine



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

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**We transplanted kidneys from α1,3-galactosyltransferase knockout (GalT-KO) pigs into six baboons using two different immunosuppressive regimens, but most of the baboons died from severe acute humoral xenograft rejection. Circulating induced antibodies to non-Gal antigens were markedly elevated at rejection, which mediated strong complement-dependent cytotoxicity against GalT-KO porcine target cells. These data suggest that antibodies to non-Gal antigens will present an additional barrier to transplantation of organs from GalT-KO pigs to humans.**

suppression) treatment strategy of immunosuppression that has been used clinically[12].

We performed six kidney transplants in baboons using GalT-KO pigs as donors. We treated three of the baboons with a multiagent regimen that included a short course of thymoglobulin (ATG) followed by daily doses of tacrolimus, mycophenolate mofetil and steroids. We induced immunosuppression in the other three with a single high dose of ATG followed by monotherapy with tacrolimus ('light therapy'; **Table 1a** and **Supplementary Methods** online).

Both endothelial cells and lymphocytes, isolated from the GalT-KO donor pigs, had undetectable Gal expression measured by FACS when compared with the negative controls (**Supplementary Fig. 1** online), confirming that the GalT-KO donor pigs were truly Gal-negative.

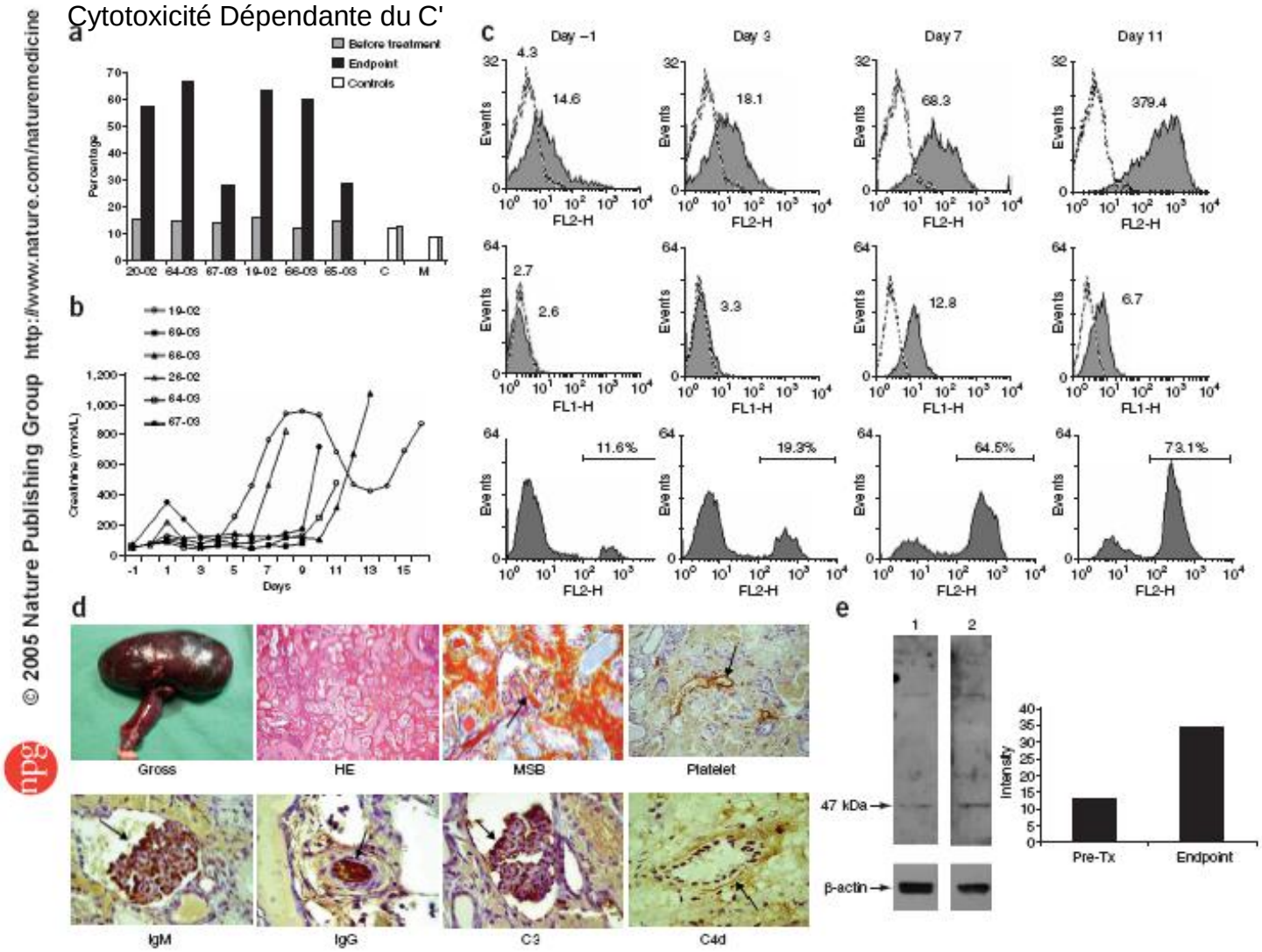
All recipient baboons had low levels of preformed non-Gal–specific IgG and IgM (**Table 1b**) before transplantation. Furthermore, sera collected from the recipient baboons before transplantation showed similar levels of complement-dependent cytotoxicity (CDC) against GalT-KO porcine lymphocytes as controls (**Fig. 1a**). None of the GalT-KO porcine grafts in this study developed hyperacute rejection.

**Table 1b** summarizes the clinical events and terminal graft histology for each animal. Using either immunosuppressive protocol, survival was limited between 8 and 16 d. Four baboons developed renal failure resulting from severe AHXR (**Fig. 1b**), despite the fact that peripheral lymphocyte counts were well controlled at a level of less than $0.5 \times 10^9$ cells/l (**Supplementary Fig. 2** online). AHXR was coincident with

*Nature Medicine* **11**, 1295 - 1298 (2005)

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

Chen et al




*Nature Medicine* **11**, 1295 - 1298 (2005)

Survie d'une xénogreffe cardiaque porc sur primate

à plus de 3 ans

nature COMMUNICATIONS

ARTICLE

Received 20 Jan 2016 | Accepted 23 Feb 2016 | Published 5 Apr 2016

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# Chimeric 2C10R4 anti-CD40 antibody therapy is critical for long-term survival of *GTKO.hCD46.hTBM* pig-to-primate cardiac xenograft

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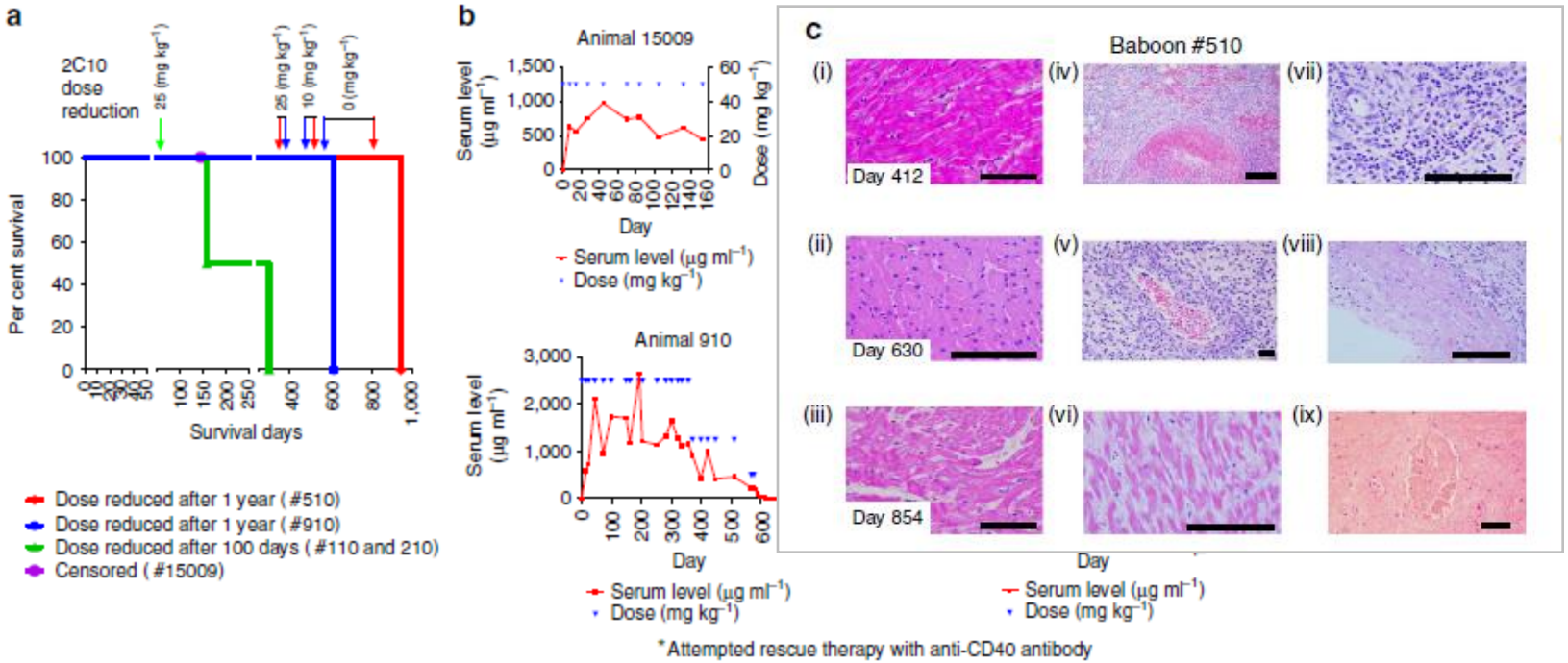
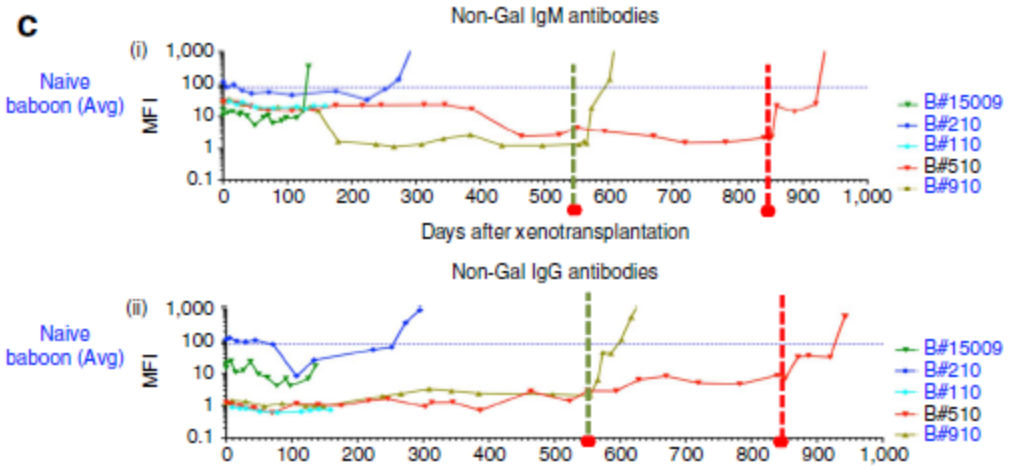
# Survie d'une xénogreffe cardiaque porc sur primate > 3 ans




Mohiuddin et al. NATURE COMMUNICATIONS | 7:11138 | DOI: 10.1038/ncomms11138 | 2016

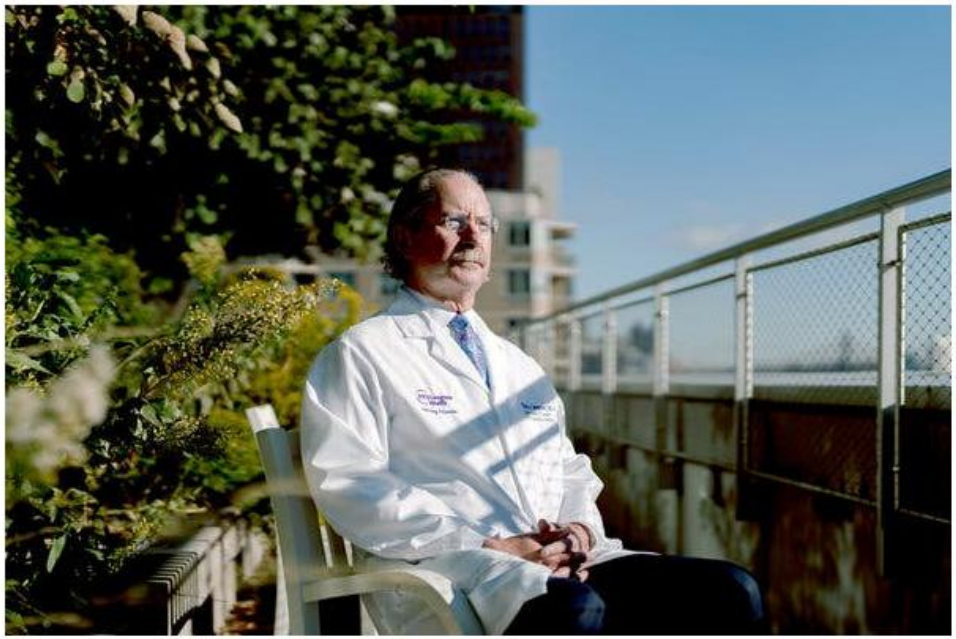
# Évolution des survies avec la technologie et l'immunosuppression

| Années | Expérimentations | Survies |
|---|---|---|
| 1900 -1960 | - | Minutes |
| 1960 - 1990 | Immunosuppression<br>Echanges plasmatiques | Jours - Semaines |
| 1990 - 2000 | Porc transgéniques | Semaines - Mois |
| 2000 - 2013 | Porcs clonés – Gal KO | Mois |
| 2013 - | Gal KO transgéniques<br>Anti CD40 | Années |

The New York Times

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

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.



# Porcs Gal KO / hTgs


The New York Times

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

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 R. Montgomery,
NYU Langone Health

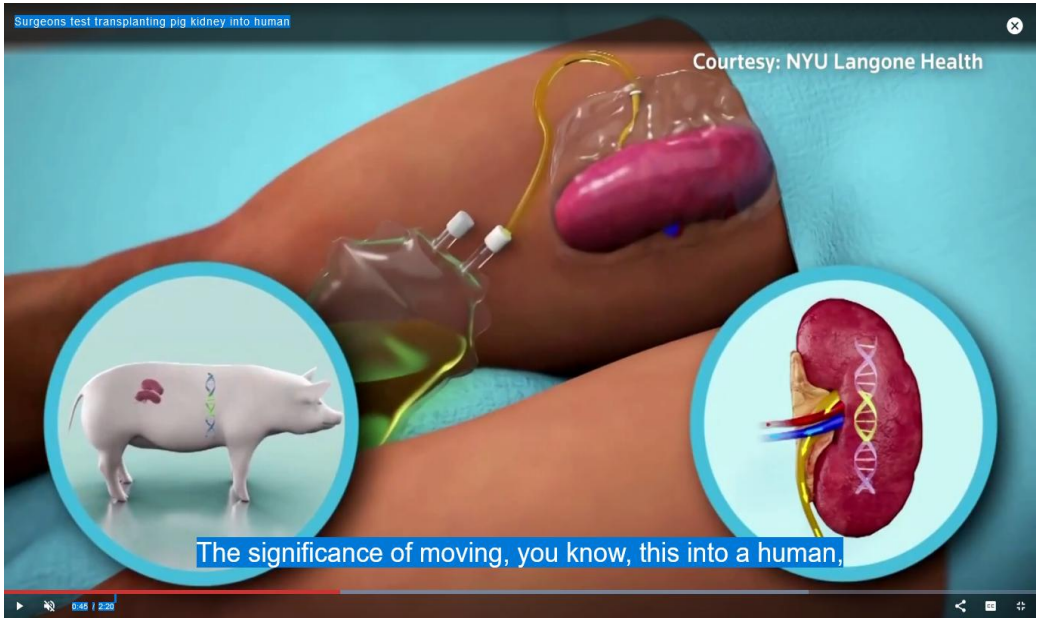
The New York Times

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

- Pig organ source: ***Revivicor*** (Blacksburg, VA)

KO for 4 pig genes and Tg for 6 human genes

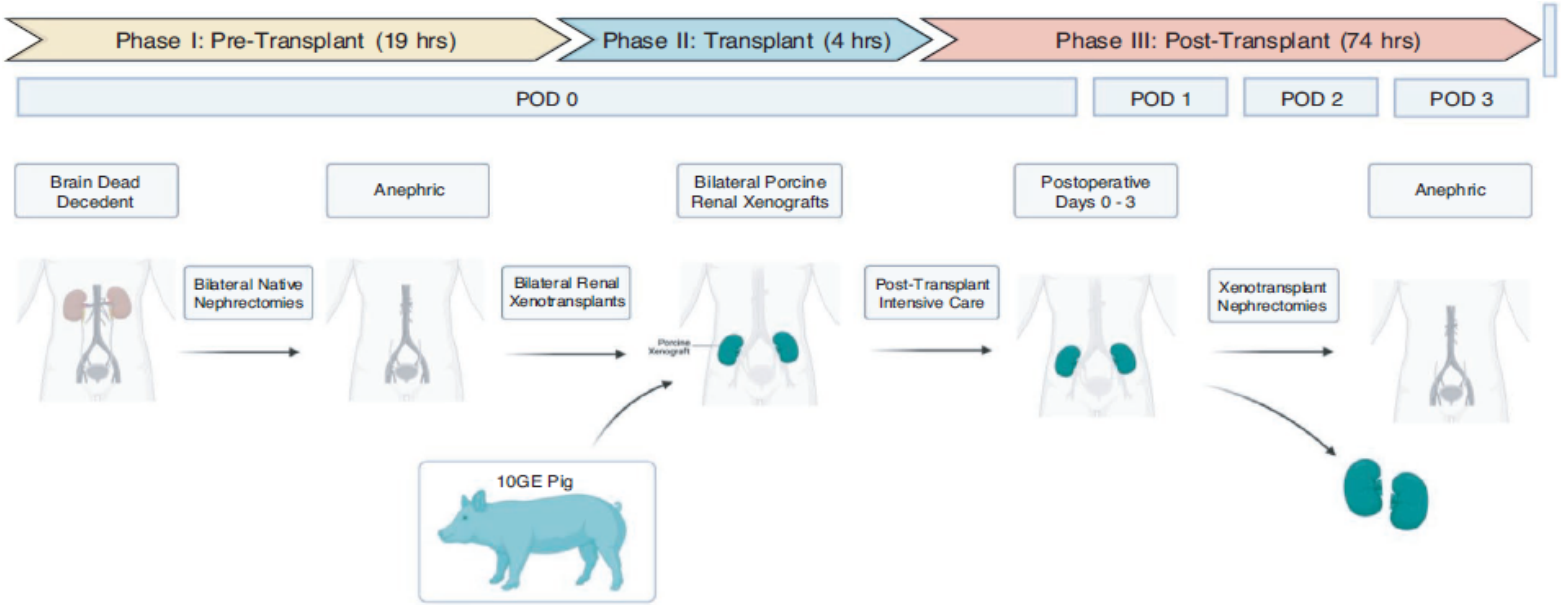
- Pig kidney attached to the blood vessels in the leg of a brain-dead patient on a ventilator
- organ produced urine: functioning normally
- **with no signs of rejection**.
- procedure performed in September, 25, 2021 but results not yet published in a peer-reviewed journal.



# Porcs Gal KO / hTgs

## 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] | Dominique 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]

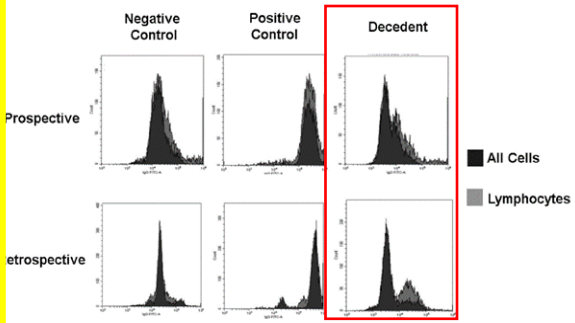


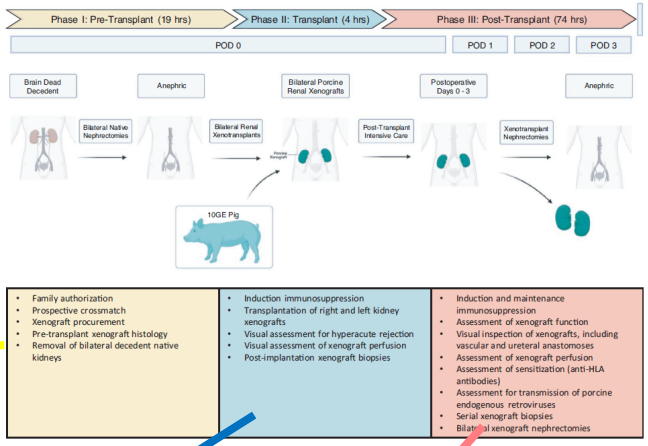

| | | |
|---|---|---|
| • Family authorization<br>• Prospective crossmatch<br>• Xenograft procurement<br>• Pre-transplant xenograft histology<br>• Removal of bilateral decedent native kidneys | • Induction immunosuppression<br>• Transplantation of right and left kidney xenografts<br>• Visual assessment 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 |

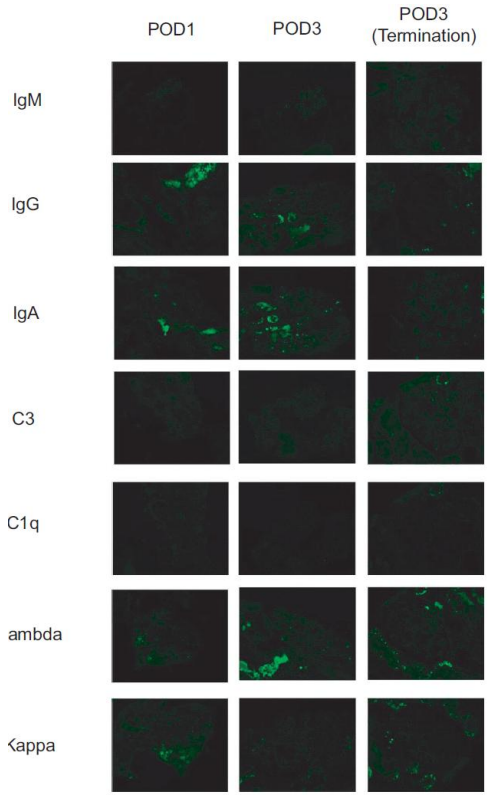
American Journal of Transplantation, 2022

# Porcs Gal KO / hTgs

- Brain-dead recipient had a negative crossmatch with the pig donor




- Pig organ source: ***Revivicor*** (Blacksburg, VA)
  - KO for 3 pig genes: ***GGTA1, CMAH, GHR***
  - Tg for 6 human genes: ***hCD55, hCD46, hTBM, hEPRC, hCD47, hHO1***
  - free for porcine CMV & PERV C

- **Induction :** methylprednisolone, Thymoglobuline, anti-CD20
- **Maintenance** : FK506, MMF, prednisolone
- urine produced within 23 min.
- No sign of hyperacute rejection.
- Mild/moderate acute tubular injury
- No evidence of endothelial injury, fibrin thrombi, or IgG, IgM, or C4d deposition

AJT, 2022

# News in focus

UNIV. MARYLAND SCHOOL OF MEDICINE

Surgeons at the University of Maryland Medical Center transplanted a genetically altered pig heart into David Bennett.

## FIRST PIG-TO-HUMAN HEART TRANSPLANT: WHAT CAN SCIENTISTS LEARN?

Researchers hope a person who has lived for more than a week with a genetically modified pig heart will advance the field of xenotransplantation.

**By Sara Reardon**

The first person to receive a transplanted heart from a genetically modified pig is doing well after the 7 January procedure in Baltimore, Maryland. Surgeons hope that the advance will enable them to give more people animal organs, but many ethical and technical hurdles remain.

"It's been a long road to get to this point, and it's very exciting we are at a point where a group was ready to try this," says Megan Sykes, a surgeon and immunologist at Columbia University in New York City. "I think there's going to be a lot of interesting things to be learned."

Physicians and scientists worldwide have for decades been pursuing the goal of transplanting animal organs into people, known as xenotransplantation.

This month's procedure marks the first time that a pig organ has been transplanted into a human who has a chance to survive and recover. In 2021, surgeons at New York University Langone Health transplanted kidneys from the same line of genetically modified pigs into two legally dead people with no discernible brain function. The organs were not rejected, and functioned normally while the recipients were sustained on ventilators.

Aside from that, most research has so far taken place in non-human primates. But researchers hope that the 7 January operation will kick-start clinical xenotransplantation and help to push it through myriad ethical and regulatory issues.

Transplant surgeons might now apply to give more people animal organs.

UNIV. MARYLAND SCHOOL OF MEDICINE

"From 4 patients, we'd learn a lot that we wouldn't learn from 40 monkeys," says David Cooper, a transplant surgeon at Massachusetts General Hospital in Boston. "It's time we move into the clinic and see how these hearts and kidneys do in patients."

Xenotransplantation has seen significant advances in recent years with the advent of CRISPR–Cas9 genome editing, which made it easier to create pig organs that are less likely to be attacked by human immune systems. The latest transplant, performed at the University of Maryland Medical Center, used organs from pigs with ten genetic modifications.

The researchers had applied to the US Food and Drug Administration (FDA) to do a clinical trial of the pig hearts in people, but were turned down. According to Muhammad Mohiuddin, the University of Maryland surgeon who leads the research team behind the transplant, the agency was concerned about ensuring that the pigs came from a medical-grade facility, and wanted the researchers to transplant the hearts into baboons first.

But 57-year-old David Bennett gave Mohiuddin's team a chance to jump straight to a human transplant. Bennett had been on cardiac support for almost two months and couldn't receive a mechanical heart pump because of an irregular heart beat. Neither could he receive a human transplant, because he had a history of not complying with doctors' treatment instructions. Given that he otherwise faced certain death, the researchers got permission to give Bennett a pig heart.

The surgery went well and "the heart function looks great", Mohiuddin says. He and his team will monitor Bennett's immune responses and the performance of his heart. They will continue working towards controlled clinical trials, but Mohiuddin says they might apply to conduct more emergency procedures if the right patients come along.

If Bennett's procedure proves successful and more teams try similar surgery, regulators and ethicists will need to define what makes a person eligible for a pig organ, says Jeremy Chapman, a retired transplant surgeon at the University of Sydney in Australia. Waiting a long time for an organ isn't enough to justify the highly experimental and possibly risky procedure, he says. That's especially true with other organs; most people waiting for kidney transplants can be put on dialysis, for example.

Chapman likens the process to the use of experimental cancer drugs that are too dangerous to test in people with other options. Regulators will need to decide what chance of success outweighs the risk of making a person wait for a human organ, he says.

## 'Crazy, exciting week'

For now, transplantation is limited by the supply of pigs as well as regulatory hurdles. There is currently just one company — Revivicor in Blacksburg, Virginia, owned by United Therapeutics — that has suitable facilities and clinical-grade pigs.

"This has been a crazy, exciting week," says Revivicor chief executive David Ayares. The company's pigs are being raised at a facility near Birmingham, Alabama, but Revivicor is building a larger facility in Virginia that it hopes will eventually supply hundreds of organs per year.

To make the pig heart used in the transplant, the company knocked out three pig genes that trigger immune attacks, and added six human genes that help the body to accept the organ. A final modification aims to prevent the heart from responding to growth hormones, ensuring that it remains human-sized.

Although the combination seems to have worked, it's unclear how many of the modifications are necessary. "There's a lot more science needed in assessing each genetic modification," says Sykes, who adds that "we need that information" because the modifications also have the potential to be harmful in people.

Mohiuddin says that because each transplant into a baboon costs around US$500,000, testing multiple combinations would be prohibitively expensive. Cooper and others say that the future of xenotransplantation probably includes tailoring the modifications to suit particular organs and recipients.

It might be some time before other organs are ready for clinical use. Waiting lists are shorter for liver transplants, making it harder to justify people receiving a pig organ. And although people who require lung transplants often die on the waiting list, Sykes says that fragile pig lungs have proved tricky to transplant into primates and are often rejected.

## Limits of animal models

Cooper, Chapman and others say that it is important to study the transplants in humans rather than baboons. The differences between species "preclude us moving further with that model to predict the clinical outcome", Chapman says.

Non-human primates tend to have antibodies that humans don't, and some of these attack proteins on pig organs, so a lot of work has gone into making the organs suitable for baboons, not people. Furthermore, researchers need to be able to study the physiology of the pig heart — whether it will beat at the same rate as a human heart, for instance — and whether people who are ill will react to the transplant in the same way as healthy baboons.

Several other companies are engineering pigs for solid-organ transplants with different genetic modifications, although none yet has medical-grade facilities, as United Therapeutics does. eGenesis in Cambridge, Massachusetts, is making pigs that cannot pass on retroviruses that are present in all pig genomes. And NZeno in Auckland, New Zealand, is breeding miniature pigs whose kidneys remain human-sized without growth-hormone modifications. Chapman suspects that many more organizations are genetically modifying pigs for transplant but have yet to disclose commercially sensitive information.

Ayares and United Therapeutics declined to say how much each pig costs to produce, although they acknowledge that the animals are expensive. But as more companies get into the game, Cooper expects that the cost will drop and the FDA and other regulators will loosen some of their requirements for clean facilities. Infection with pathogens from pig organs doesn't yet seem to be a problem, although Bennett and any future recipients will need to be monitored.

# Porcs Gal KO / hTgs

**Faculty Scientists and Clinicians Perform Historic First Successful Transplant of Porcine Heart into Adult Human with End-Stage Heart Disease-** *UMSOM, Baltimore (10/01/22)*

UNIVERSITY *of* MARYLAND
SCHOOL OF MEDICINE

B.P. Griffith, MD, M.M., Mohiuddin, MD, xenotransplantation laboratory at UMSOM

-Pig organ source: ***Revivicor*** (Blacksburg, VA), KO for 4 pig genes and Tg for 6 human genes

- Pig heart *perfused in the XVIVO Heart Box*, before surgery.

- Transplanted in a patient with end-stage heart disease

- Use of a new IS drug (Kiniksa Pharmaceuticals)

- Procedure performed in January, 7, 2022 but results not yet published in a peer-reviewed journal.

- Patient death at 2 months, without rejection

- Thanks to Bennett, "we have gained invaluable insights learning that the genetically modified pig heart can function well within the human body while the immune system is adequately suppressed," said Mohiuddin. "We remain optimistic and plan on continuing our work in future clinical trials."

MIT Technology Review

Featured Topics Newsletters Events

- Bartley Griffith , webinaire by the ATC on April 20th : *porcine CMV*

  *“maybe was the actor, or could be the actor, that set this whole thing off.”*
- Porcine CMV est supposé non transmissible chez l’homme
- Mauvaises survies de greffons cardiaques CMV+ chez le babouin (Munich) : forte réplications virales dans les greffons
- Donneurs censés être « free of pathogens », mais la technique de détection utilisée , était manifestement insuffisamment sensible
- Recherche de pathogènes et porcin cell free DNA quotidienne
- Détection CMV à J20

MIT Technology Review

Featured Topics Newsletters Events

- Aggravation état général à J43 avec fièvre et dyspnée
- Cidofovir , IVIg : amélioration 24h puis dégradation profonde
- Biopsie : absence de signe de rejet mais œdème ++ (syndrome de fuite capillaire ?) et fibrose : insuffisance cardiaque diastolique terminale

# Aspects sécuritaires

- Le risque viral: les rétrovirus du porc (PERV)
  - risque individuel
  - risque collectif
- Les PERV sont capables in vitro d'infecter des cellules humaines
- Autres virus : NIPAH ou virus H5N1, responsable de la grippe aviaire, décelée dans des échantillons prélevés sur des porcs en 2001 et 2003

# Humains et PERV

- Rétrovirus endogènes intégrés au génome sous forme d'ADN proviral se transmettant de façon mendelienne : > 50 copies de PERV dans les chromosomes de porc

- Les PERV peuvent infecter les cellules humaines in vitro
- *Patience C, Takeuchi Y, Weiss RA: Infection of human cells by an endogenous retrovirus of pigs. Nat Med 1997, 3: 282-6.*
- *Martin U, Kiessig V, Blusch JH, Haverich A, von der Helm K, Herden T, Steinhoff G: Expression of pig endogenous retrovirus by primary porcine endothelial cells and infection of human cells. Lancet 1998, 352: 692-4.*

- Risque individuel des receveurs
- Risque collectif

Science

REPORTS

Cite as: D. Niu *et al.*, *Science* 10.1126/science.aan4187 (2017).

# Inactivation of porcine endogenous retrovirus in pigs using CRISPR-Cas9

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Xenotransplantation is a promising strategy to alleviate the shortage of organs for human transplantation. In addition to the concern on pig-to-human immunological compatibility, the risk of cross-species transmission of porcine endogenous retroviruses (PERVs) has impeded the clinical application of this approach. Earlier, we demonstrated the feasibility of inactivating PERV activity in an immortalized pig cell line. Here, we confirmed that PERVs infect human cells, and observed the horizontal transfer of PERVs among human cells. Using CRISPR-Cas9, we inactivated all the PERVs in a porcine primary cell line and generated PERV-inactivated pigs via somatic cell nuclear transfer. Our study highlighted the value of PERV inactivation to prevent cross-species viral transmission and demonstrated the successful production of PERV-inactivated animals to address the safety concern in clinical xenotransplantation.

# Modèles porc-primate et physiologie

- Rein:
  - Un rein de porc peut supporter une fonction rénale normale chez un primate pendant au moins 3 mois et vraisemblablement plus
  - anémie
  - hypophosporémie
  - protéinurie
- Coeur:
  - Hétérotopique dans la majorité
  - Orthotopique: jusqu'à 39 jourss
    *Vial CM..J Heart Lung Transplant* 2000 Feb;19(2):224-9
  - Pas de réel travail physiologique pour l'instant
- Ilôts de Langerhans et Cellules neuronales

# Conclusions

- Les modèles porc-primate sont les meilleurs modèles expérimentaux pour évaluer la faisabilité de la xénotransplantation clinique
- Le rejet hyperaigu peut être prévenu
- Évolution vers des porcs multi-KO et multi-transgéniques
- Le rejet vasculaire aigu est encore l'obstacle majeur: évolution des traitements IS
- Le risque infectieux est présent- Porc PERV KO
- Les résultats physiologiques manquent encore
- Nouvelles avancées des Américains chez l'Homme pour organes vascularisés
- Réactivation de la dynamique : Chine +++, Allemagne, EU (?)