

Lo xenotrapianto: stato della ricerca

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Approccio al problema del rigetto di uno xenotrapianto

Ricevente

Donatore

Produzione di animali “ingegnerizzati”

Production of “engineered” animals

- Insertion of one or more new genes in the pig genome
 - Modulation of the COMPLEMENT cascade
 - Modulation of the CLOTTING cascade
(regular TG, intrabodies, antisense RNA)
- Deletion of one or more genes in the pig genome
 - α 1,3Gal-KO

Xenotrapianto: questioni fondamentali

- Immunologia
- Fisiologia
- Zoonosi
- Aspetti etici

Xenotrapianto: questioni fondamentali e ingegnerizzazione

- Immunologia

- Fisiologia

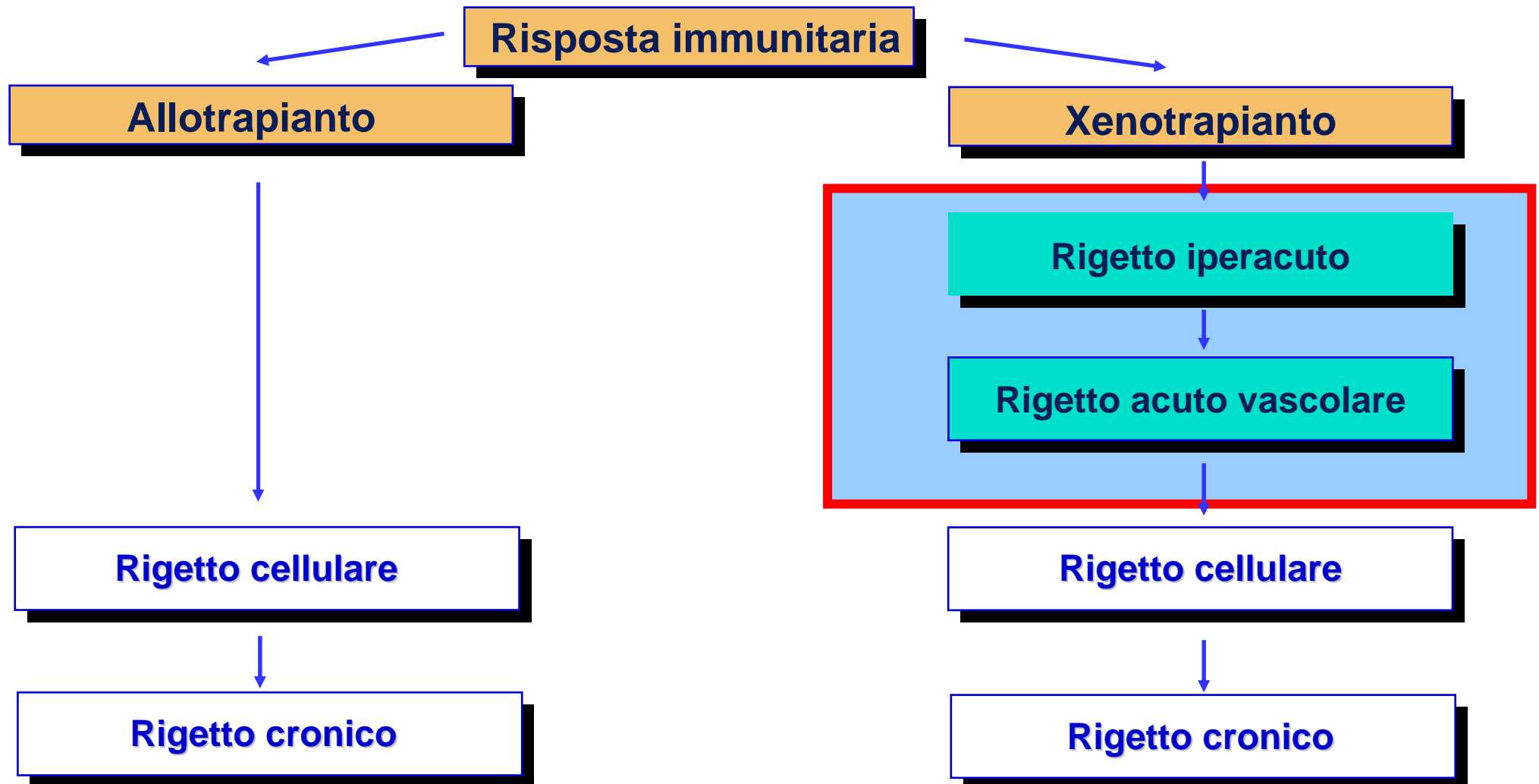
- Zoonosi

- Aspetti etici

Xenotrapianto: questioni fondamentali

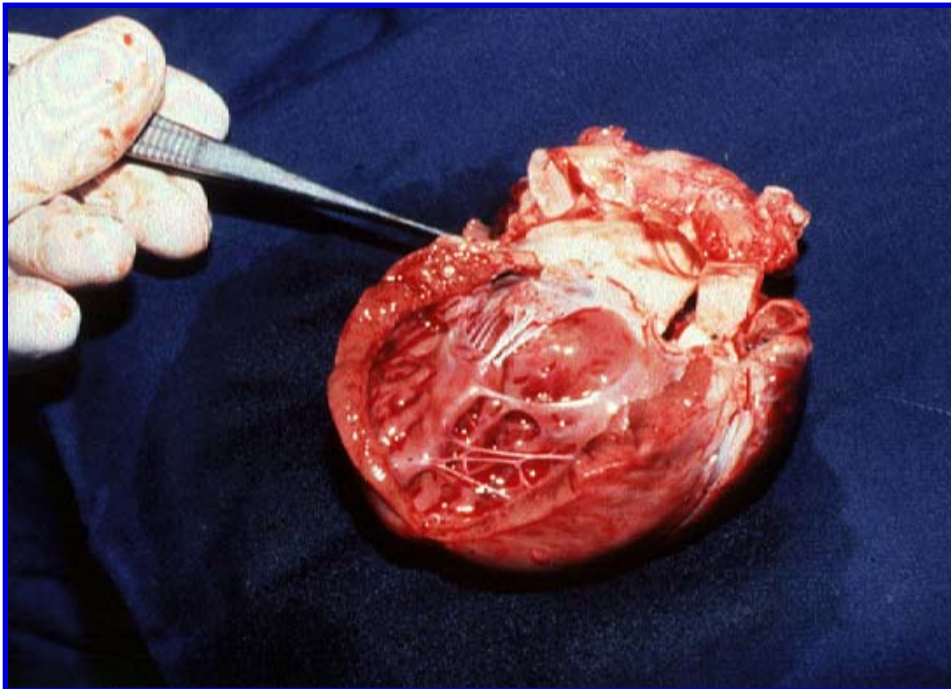
- Immunologia
- Fisiologia
- Biosicurezza
- Aspetti Etici

Rigetto di allotrapianti e xenotrapianti

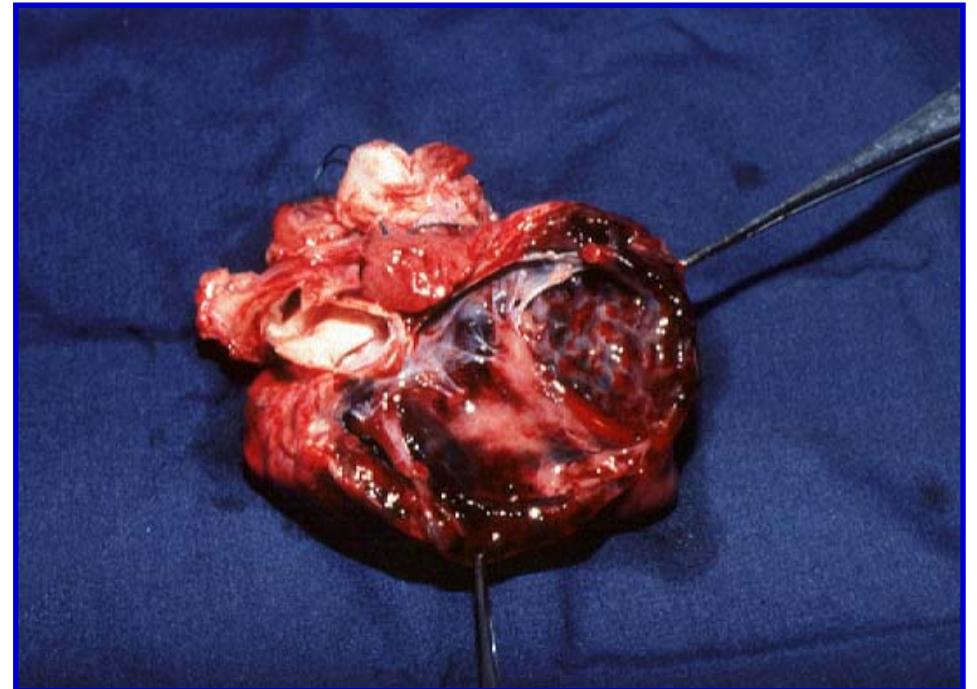


Xenotrapianto da maiale a primate non umano

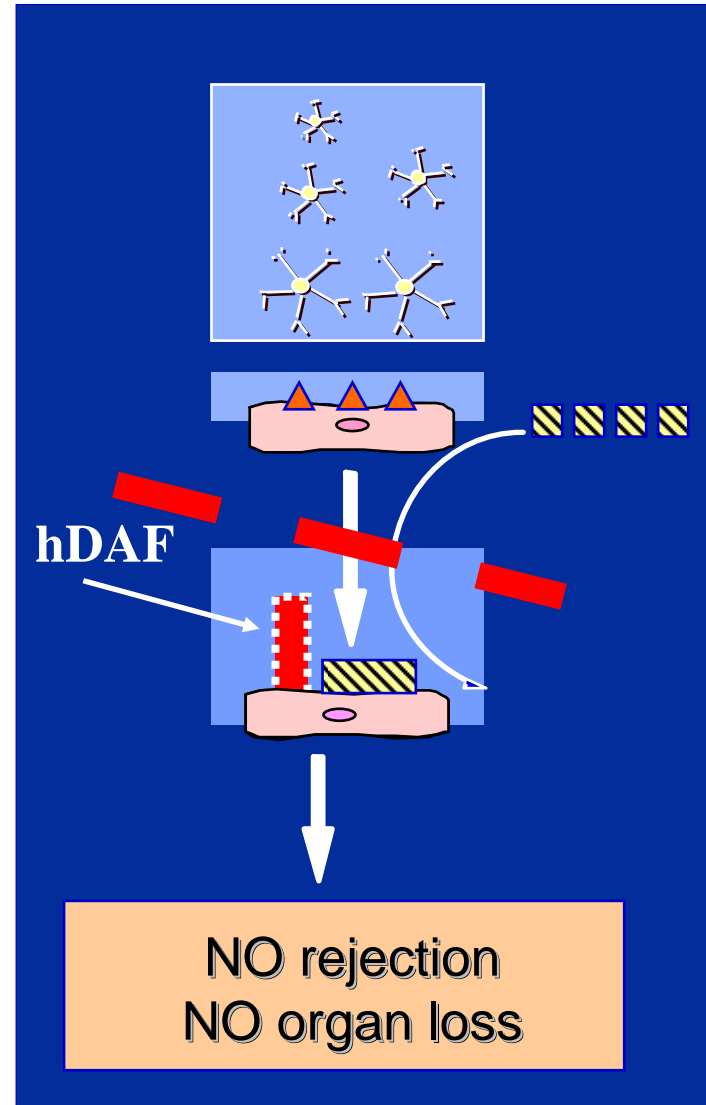
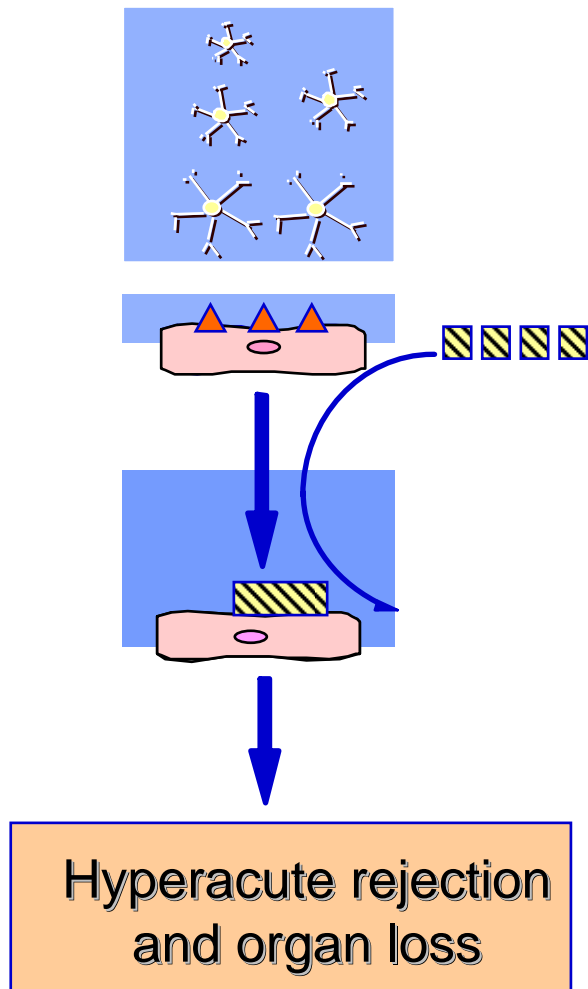
ORGANO NON TRAPIANTATO



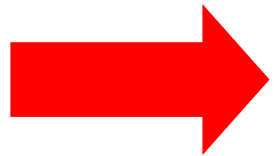
RIGETTO IPERACUTO



Hyperacute rejection



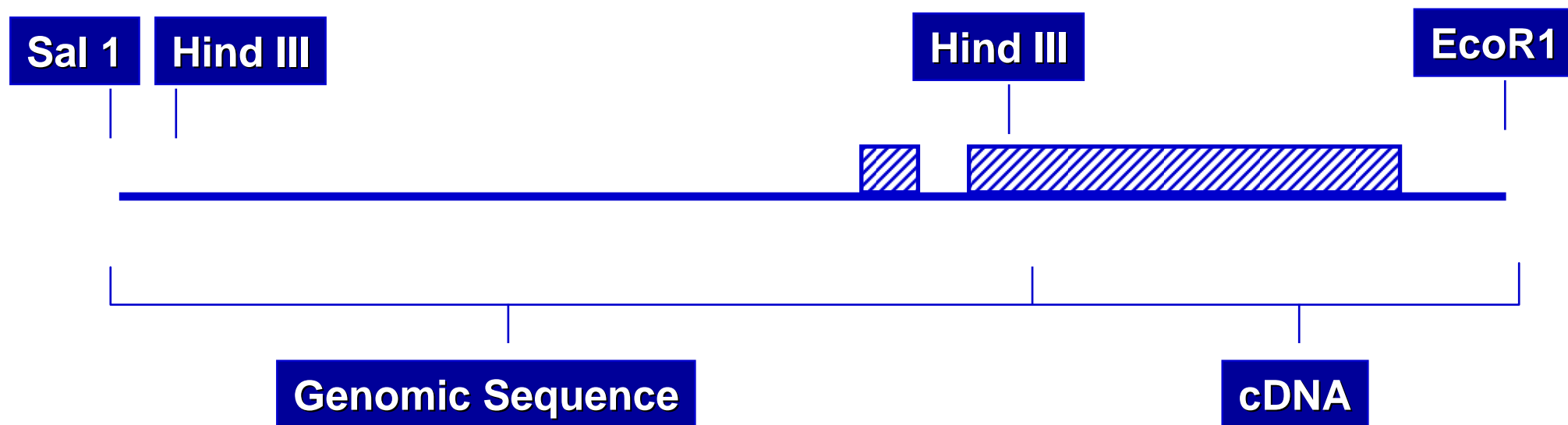
Production of “engineered” animals



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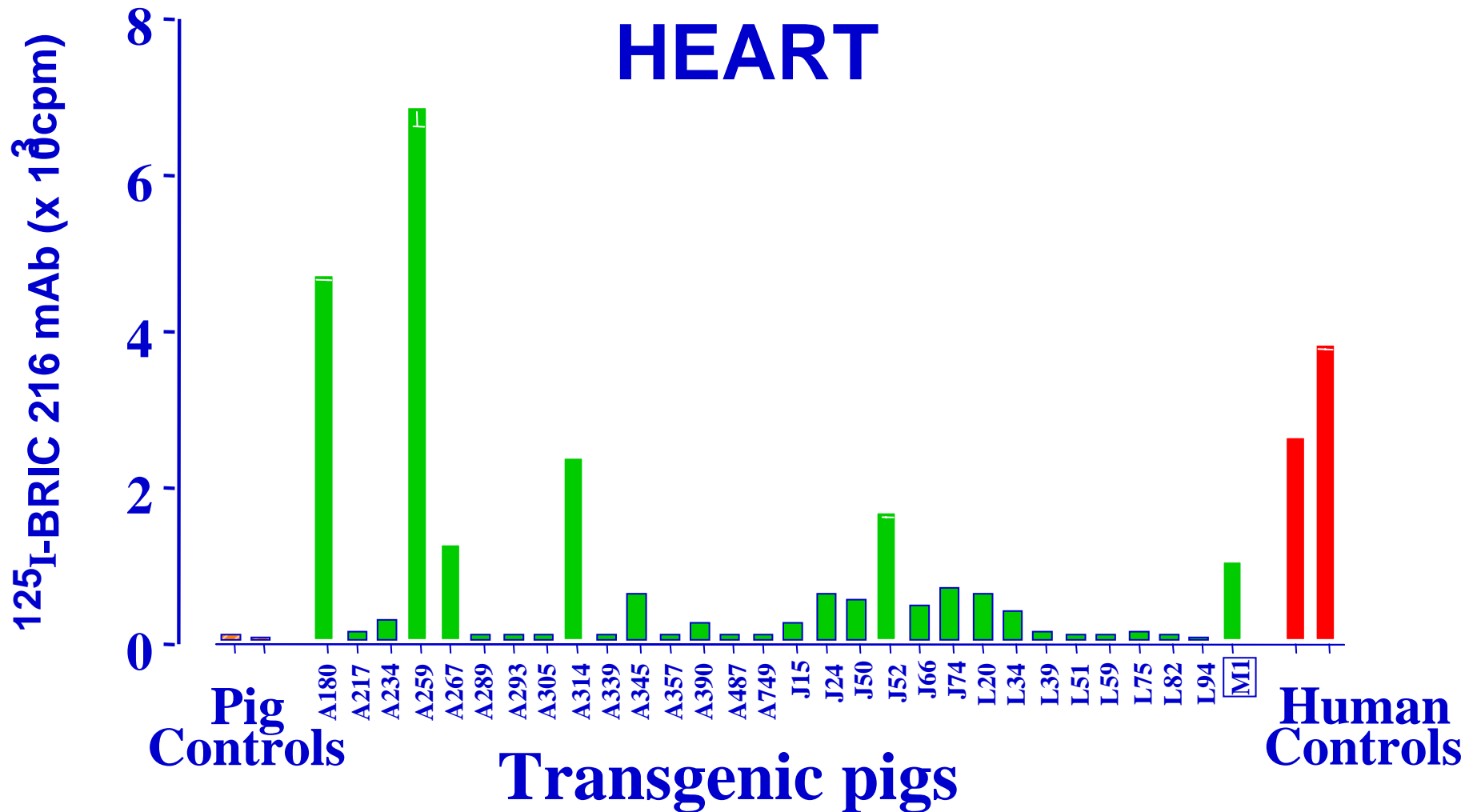
Human DAF Minigene



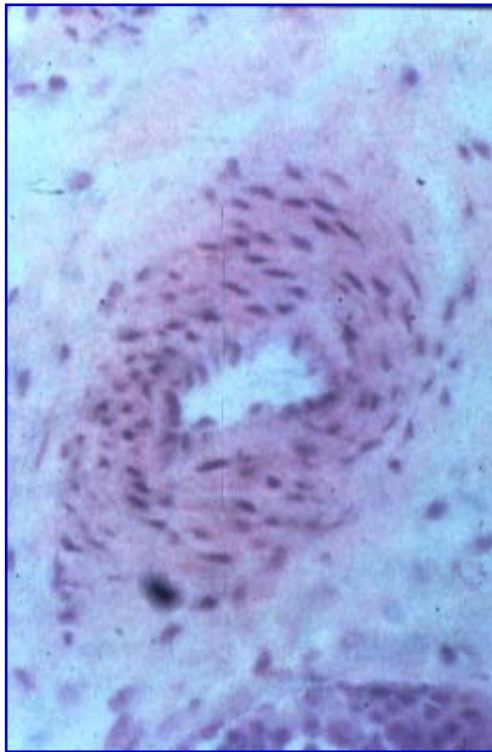
Microiniezione

Maiale transgenico

hDAF expression in 30 lines of transgenic pigs



Maiale transgenico A74



H + E



hDAF



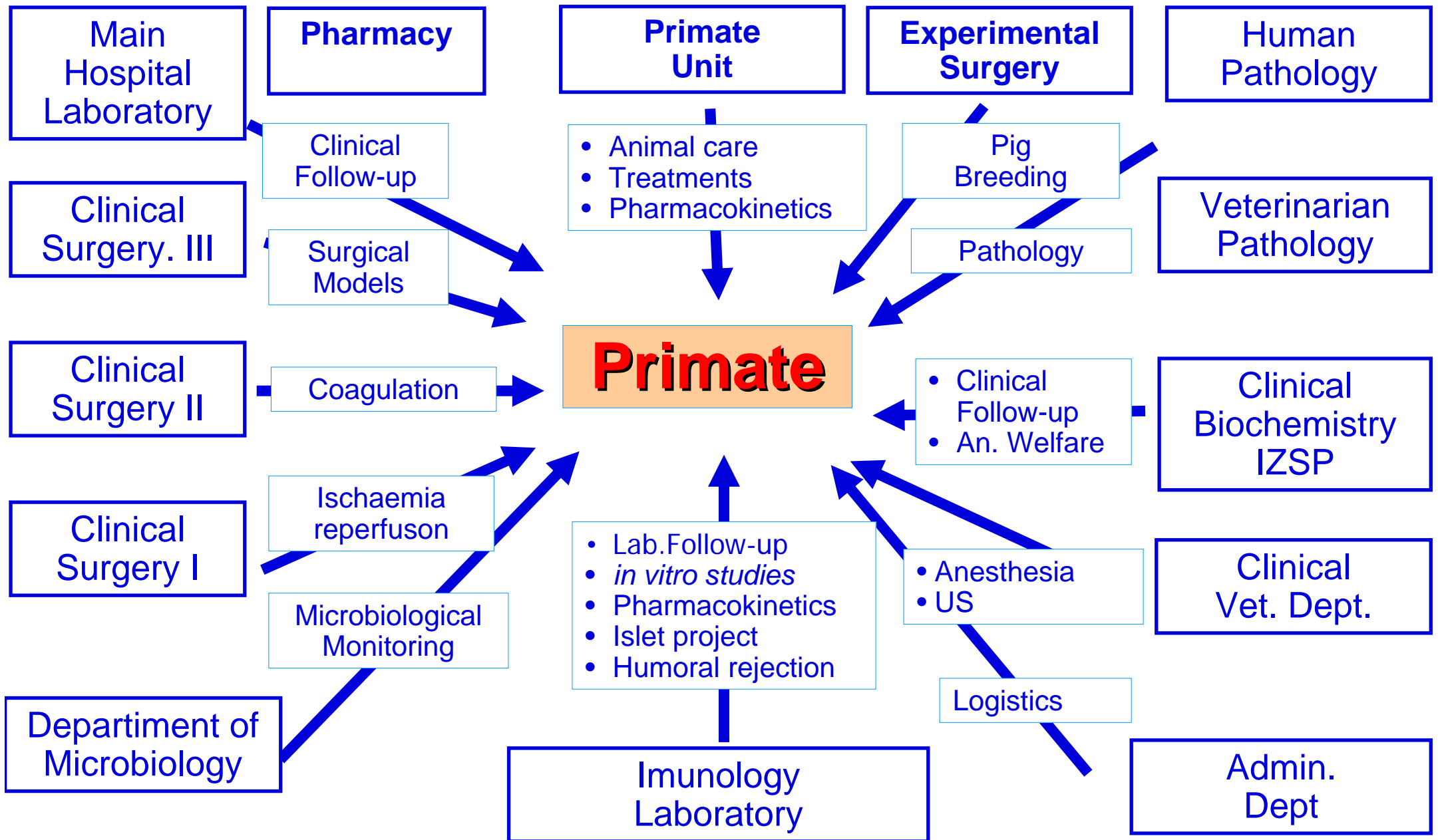
Von Willebrand
factor

Engineered organs: do they work?

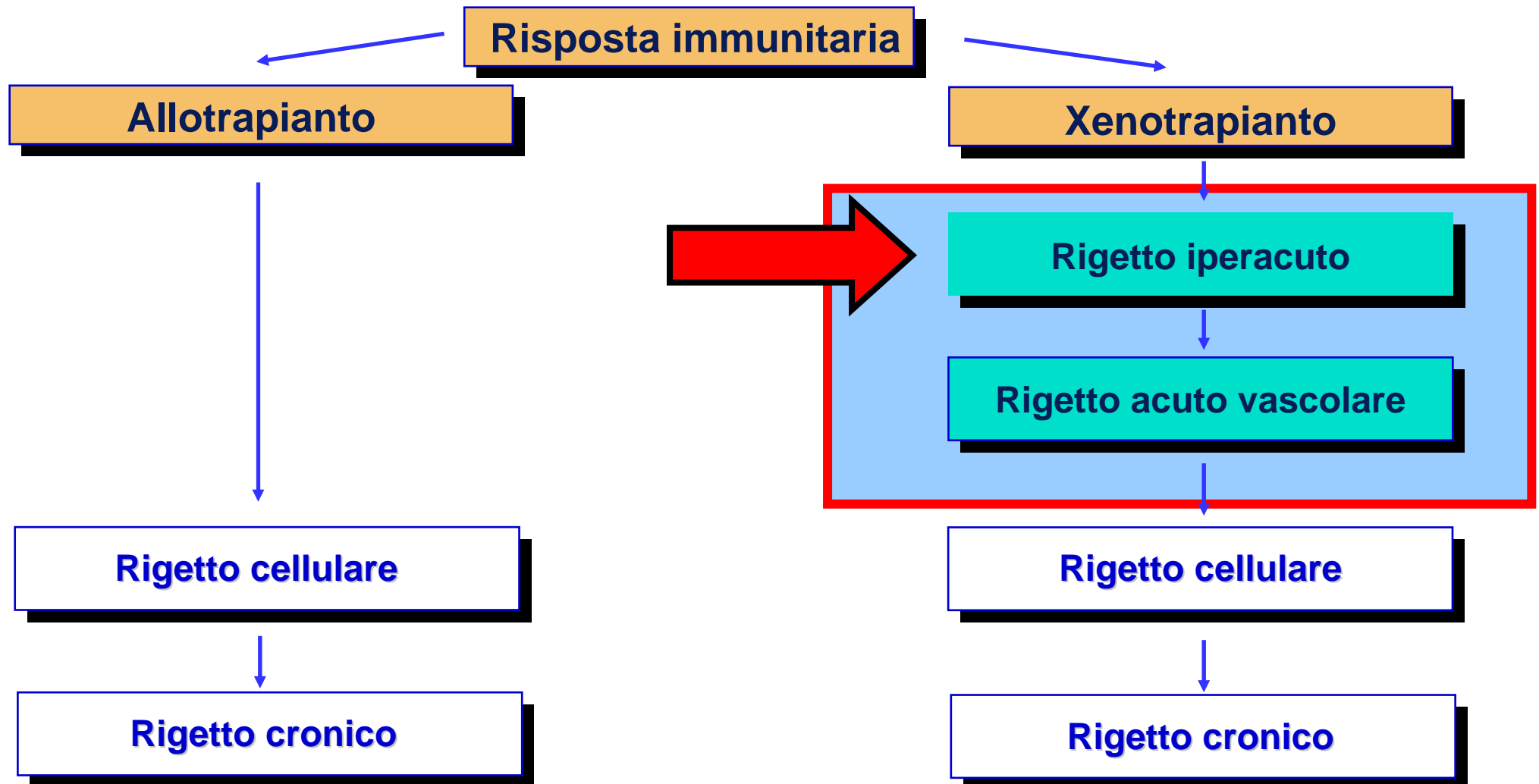
Xenotransplantation and the need for nonhuman primates (I)

- Primates represent an invaluable and indispensable tool for preclinical/biomedical research at large
- The species-restriction of some molecules (such as complement regulators) underline the need for primate models in xenotransplantation
- Furthermore, the limitations presented by models developed in other mammals species (i.e. Gal KO-mice) indicate that primate work **MUST** be pursued.

Complexity of a project with primates



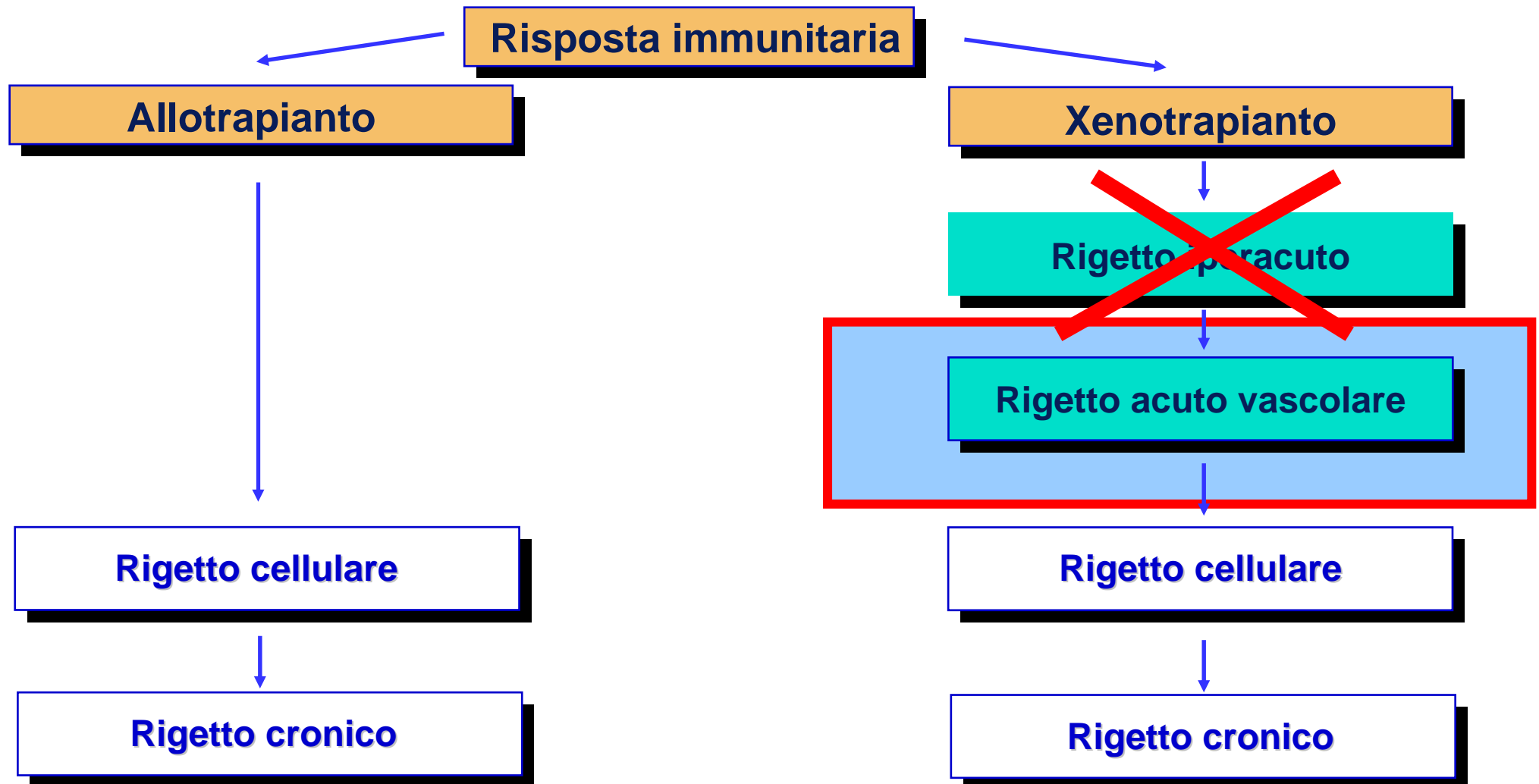
Rigetto di allotrapianti e xenotrapianti



Hyperacute rejection using hDAF transgenic organs

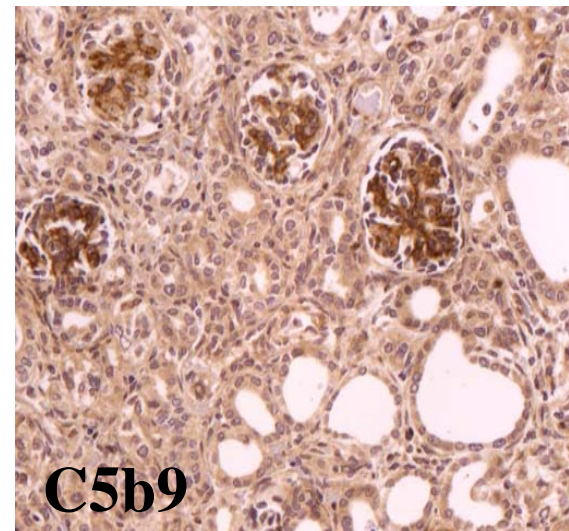
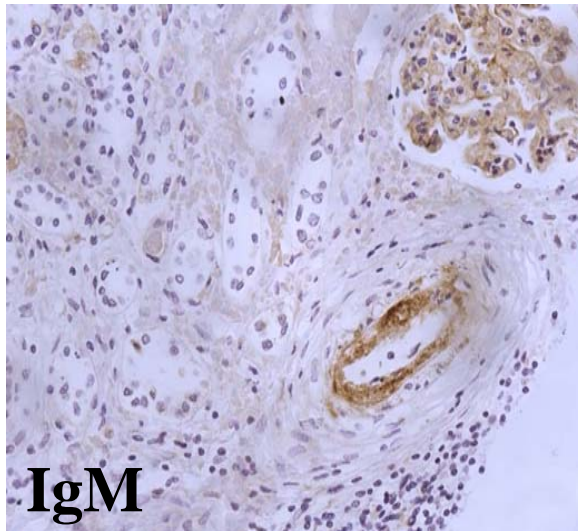
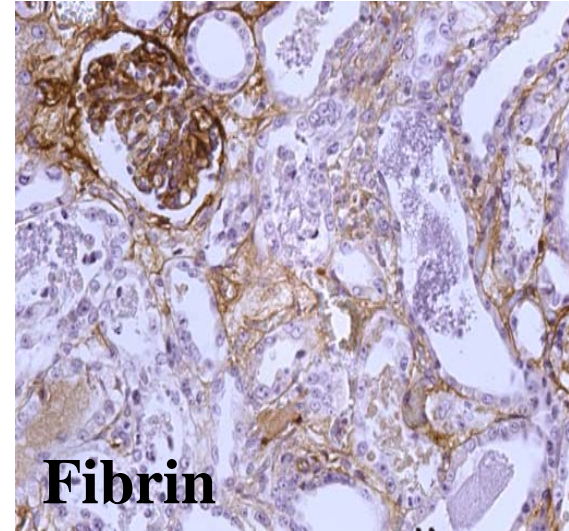
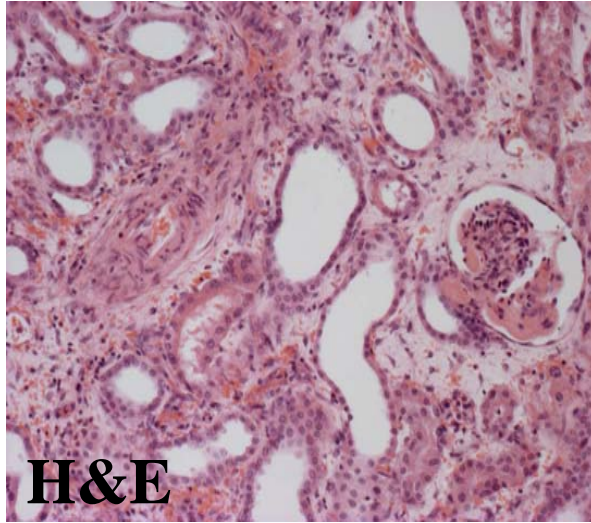
ORGAN	SPECIES	TX	HAR	% HAR
Kidney	Cyno	227	0	0
Hetero. heart	Cyno	55	4	7
Hetero. heart	Baboon	28	3	11
Ortho. heart	Baboon	16	1	6
TOTAL		326	24	7

Rigetto di allotrapianti e xenotrapianti

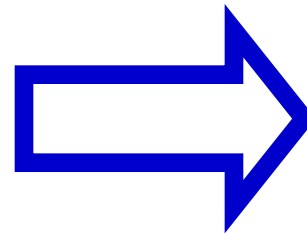
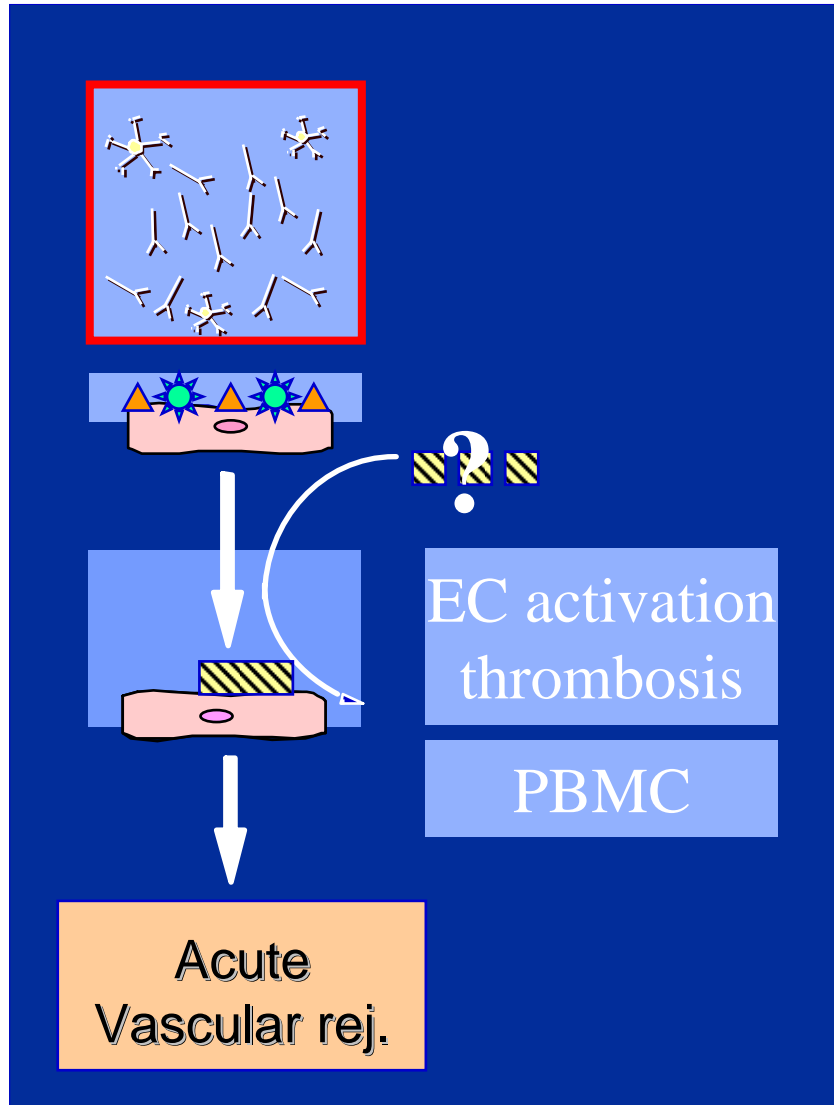


Acute vascular rejection

Acute vascular rejection

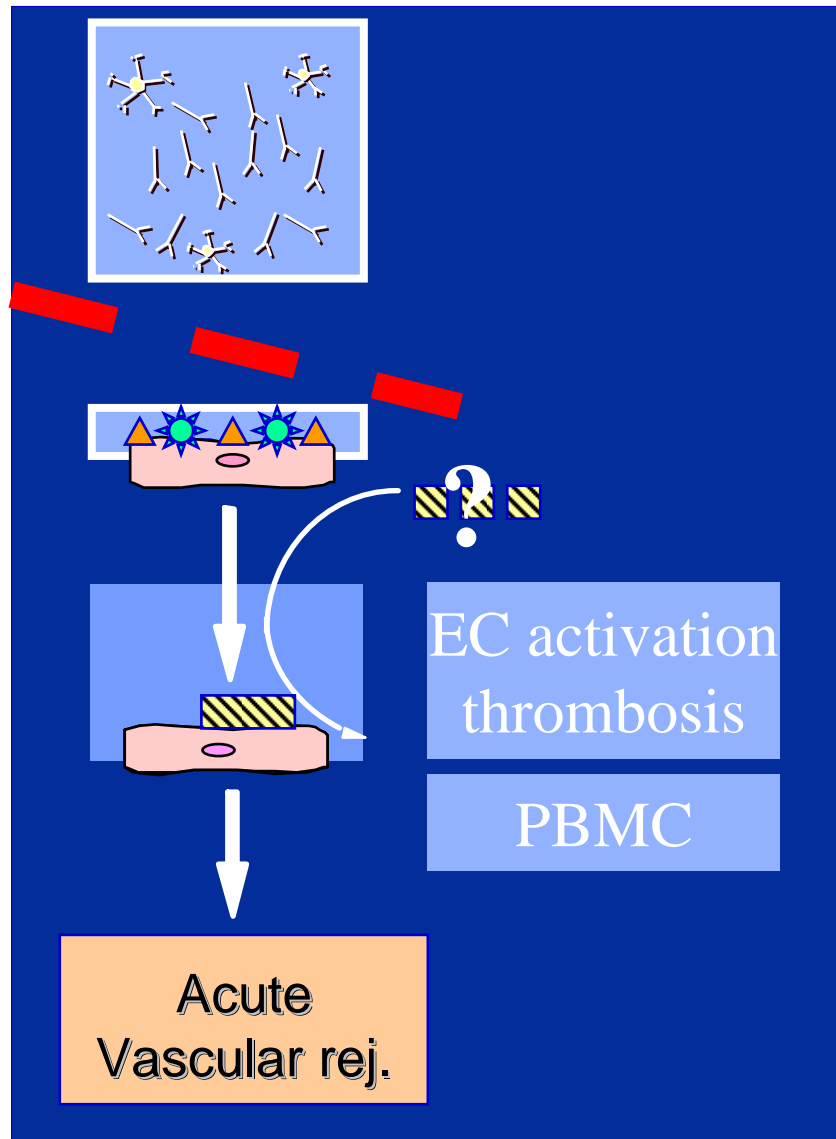


AVR: understanding the rejection mechanisms



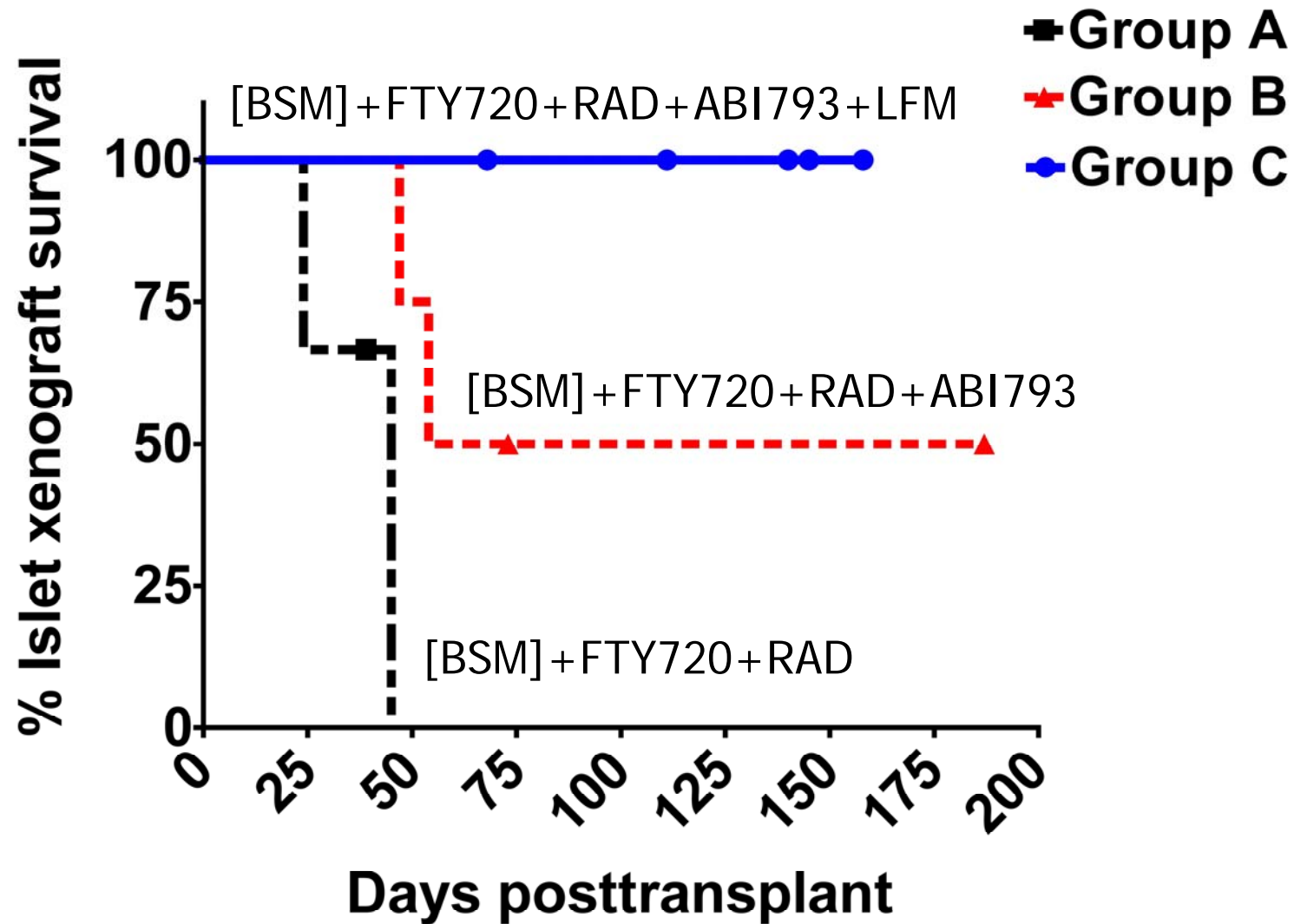
**Identification
of specific
targets**

Proposed immunological strategies to overcome the onset of acute vascular rejection



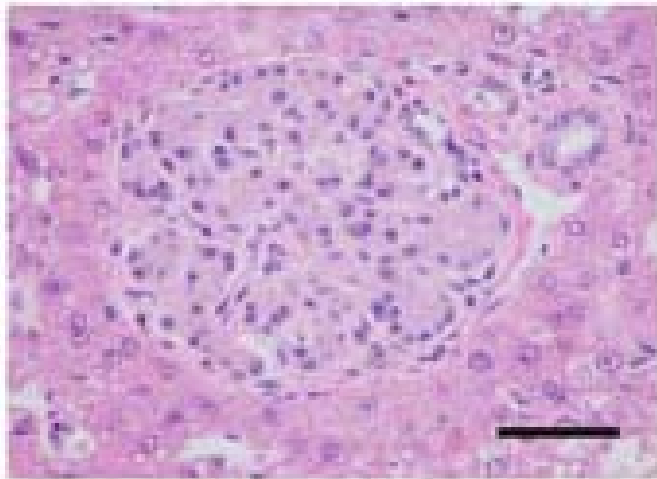
NO antibody
↓
NO rejection!!!
(*Lin et al, JCI 1998*)

Longest survival of porcine islet xenografts

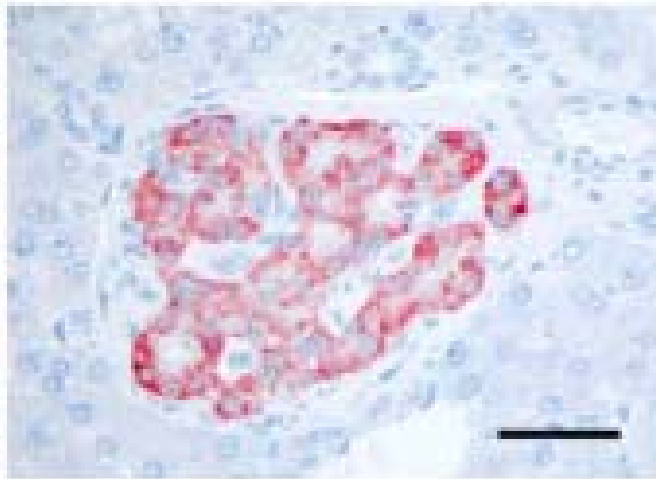


Longest survival of porcine islet xenografts

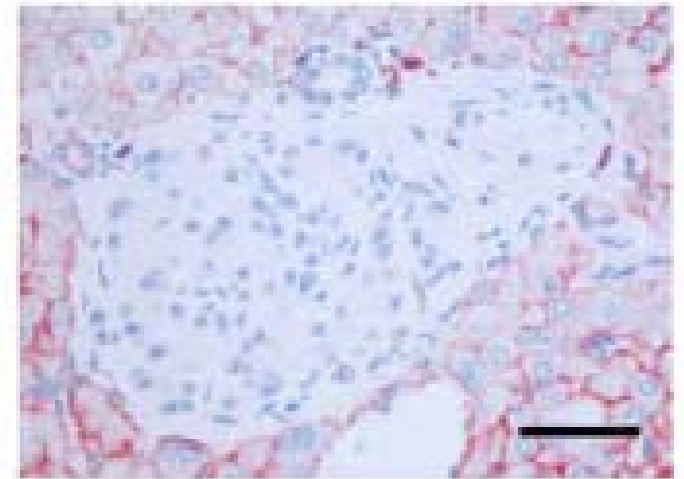
H&E



Insulin



CD3



[BSM]+FTY720+RAD+ABI793+LFM

Longest survival of porcine islet xenografts

- animals become normoglycemic
- porcine c-peptide is measurable
- survival of ≥ 180 days
- **NO REJECTION** (no Ab, no cellular infiltrate) at the time of euthanasia

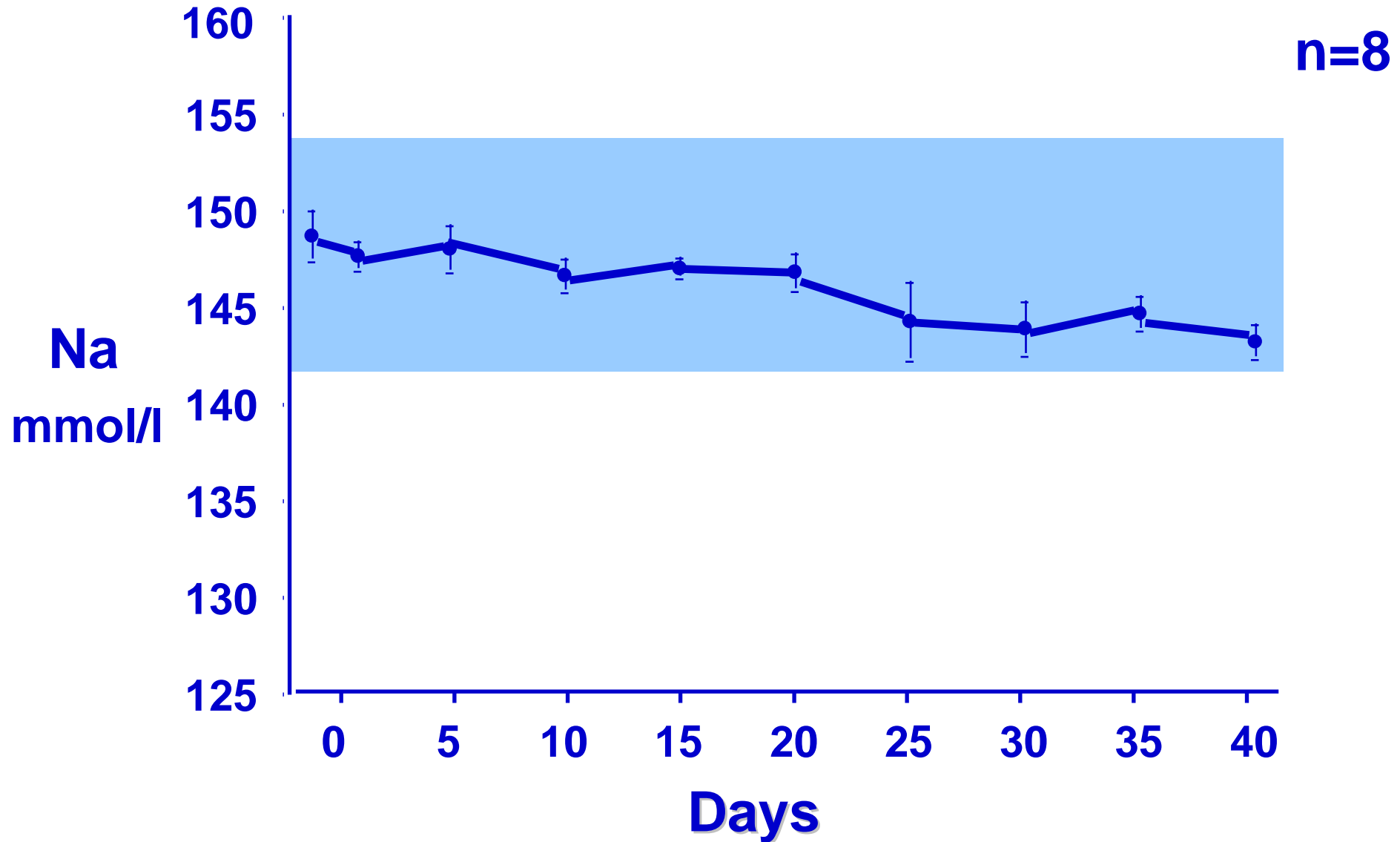
Massima sopravvivenza di primati trapiantati con organi o cellule di maiale

- 90 giorni per un trapianto di rene
- 179 giorni per un trapianto di cuore eterotopico
- 56 giorni per un trapianto di cuore ortotopico
- >180 giorni per una serie di trapianti di insule

Xenotrapianto: questioni fondamentali

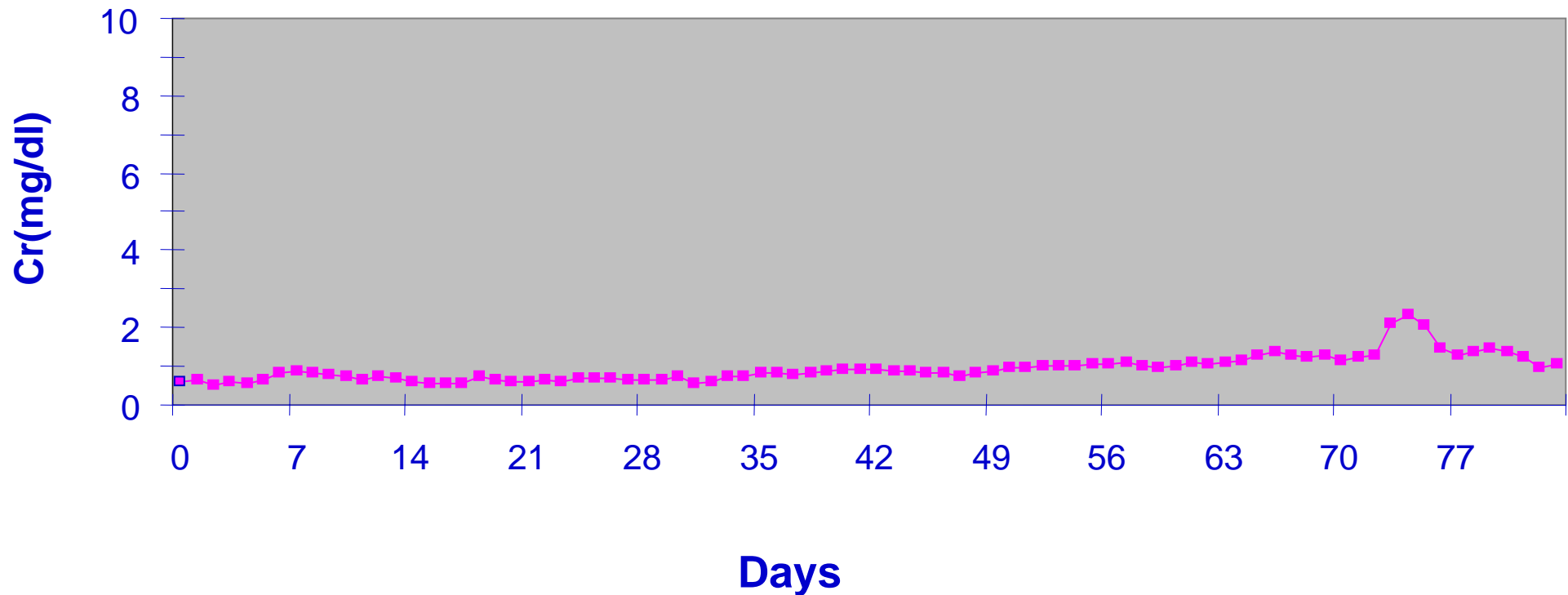
- Immunologia
- **Fisiologia**
- Biosicurezza
- Aspetti etici

Plasma sodium post renal xenotransplantation



Creatinine levels in animal B134 (GalT-KO thymokidney – steroid free regimen)

Life-supporting, orthotopic renal Tx



Pig-to-primate physiological compatibility

- Some molecular incompatibilities have been demonstrated between pig and man (coagulation, liver)
- However, to date no insurmountable physiological or anatomical incompatibilities have been reported (**life-supporting renal/cardiac/islets models**)

Furthermore, even the coagulopathy observed in AVR is avoidable (**Lin et al, JCI 1998**)!

Xenotrapianto: questioni fondamentali

- Immunologia
- Fisiologia
- **Biosicurezza**
- Aspetti etici

Zoonoses: the facts (I)

- More than 60 infectious agents have been indentified in pig which can potentially be responsible for zoonoses in man.
- However, it is possible to obtain pig lines which are free of all **known** pathogenic agents but one.
- Currently, the major remaining risks are those related to the presence of porcine endogenous retroviruses (PERV) and that of potential unknown viruses.

Zoonoses: the facts (II)

- PERV have been shown to be able to infect human cells *in vitro* and SCID mice *in vivo*.
- However, there is no evidence that PERV are pathogenic in pigs and most PERV sequences in the pig genome are incomplete
- In a retrospective study in 160 patients exposed to living porcine tissues (28 of which were exposed to BAL for 2-30 hours), no signs of PERV-related infection (current, past or latent) or disease were observed.
- There was no evidence of PERV infection in 88 patients with hemofilia exposed, often repeatedly, to porcine factor VIII preparations which were retrospectively shown to contain PERV.

Approaches aimed at reducing the infectious risks related to PERV

- A line of pigs carrying PERV that do not have the capacity of replicating *in vitro* in human cells
- PERV replication inhibited by antiviral agents (including AZT)
- Anti-PERV vaccine prior to exposure to porcine tissues
- Genetically engineering of the donor
 - Transgenics expressing intracellular scFv antibodies
 - PERV- *knock out* pigs

Xenotrapianti e zoonosi

- La ricerca di potenziali agenti patogeni ignoti rimane di primaria importanza
- tecnologie genomiche d'avanguardia si stanno dimostrando molto promettenti nella identificazione tempestiva di microorganismi sconosciuti eventualmente presenti in un tessuto umano.
- Tra queste vanno ricordate
 - la reazione di polimerasi a catena (PCR)
 - tecnologie di ibridazione sottrattiva
 - la tecnologia del microarray.

Zoonoses control procedures

As there is a **theoretical** and **not quantifiable** risk of occurrence of new zoonoses, measures must be put in place to allow clinical xenotransplantation to be performed with minimal and acceptable risks:

- QPF pigs should be the only source donor
- Screening of the colonies should be performed on a regular basis
- well-defined and rigorous public international health guidelines should be in place
- clinical trials only in a restricted number of centres of excellence; enrolment of a limited number of patients with close and prolonged monitoring of the patients and their nearest relatives
- National registries of xenografted patients should be established

Xenotrapianto: questioni fondamentali

- Immunologia
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Ethical issues

- Prevention of disease (although not always possible)
- Use of animals
- Transgenesis
- Resource allocation
- Commercial pressure even for public funding (!)
- Create expectations
- Which patient? (life-supporting organ?)
- Informed consent
- Zoonoses
- Follow up for the rest of your life
- Public engagement
- Failure
- UNKNOWN

The Mexican trial...

⇒ **RISK / BENEFIT RATIO**

A worrying rumor.....

- At least 400 islet transplants have been done in China so far
- In Russia hundreds of rabbit islet transplants have already been done (possibly more than 800....)
- In China at least 2000 patients have undergone bovine cells transplantation used as pain killers....
- In Mexico...

Xenome

The EU FP6 research programme

Thematic priority area 1: “**Life sciences, genomics and biotechnology for health**”

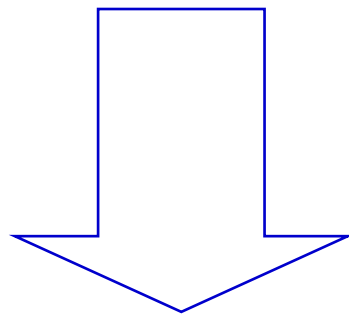
[focuses on integrating post-genomic research into the more established biomedical and biotechnological approaches (**TRANSLATIONAL APPROACH**)]

Application of post-genomics to xenotransplantation research

[

EU Integrated Projects

- Ideally: 10-15 partners
(includes Ethical, social and regulatory experts)
- 5-10M € for the whole project
- support spread over up to 5 yrs



Very low success rate (5/23 for the last call!)

Xenome and the xenotransplantation research

Xenome is:

- is a EU funded Consortium
- It includes 22 academic/privates institutions (SMEs) in 11 countries
- Its key objective is to bring xenotransplantation closer to the clinical application (“translational approach”)
- The Padua Medical Center is the coordinator for this EU research effort

Xenome: the partners

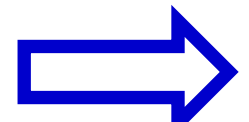
- Italy (5 centers)
- United Kingdom (4 centers)
- Germany (3 centers)
- Portugal (2 centers)
- France (1 centers)
- Spain (1 center)
- The Netherlands (1 center)
- Belgium (1 center)
- Sweden (1 center)
- Czech Republic (1 center)
- Austria (1 center)

Xenome: the key areas of research

- Immunology
- Safety
- Genetic engineering
- Physiology
- Ethics
- Social and regulatory aspects

Conclusions

- A better understanding of the mechanisms underlying rejection of and advances in genetic engineering have allowed **prolonged survival** of pig organs transplanted into primates.
- **To date, NO insurmountable physiological incompatibilities** between pig and man has been demonstrated.
- Also from a **biosafety** standpoint, encouraging results are being obtained. **This research MUST continue!**
- **Reborn world-wide interest** in xenotransplantation (XENOME)
- It is critical that we initiate xenotransplantation clinical trials only in the presence of a **favorable risk-benefit ratio.**
- **For the time-being, whilst tangible results are also eagerly expected from the research on stem cells, the human organ remain the only real clinical option**



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