The Development of Transgenic Livestock for Biomedical Applications

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Xenotransplantation:

A Therapeutic Solution

- ***Organ Replacement***
- ***Tissue Engineering***
- ***Cell Transplantation***

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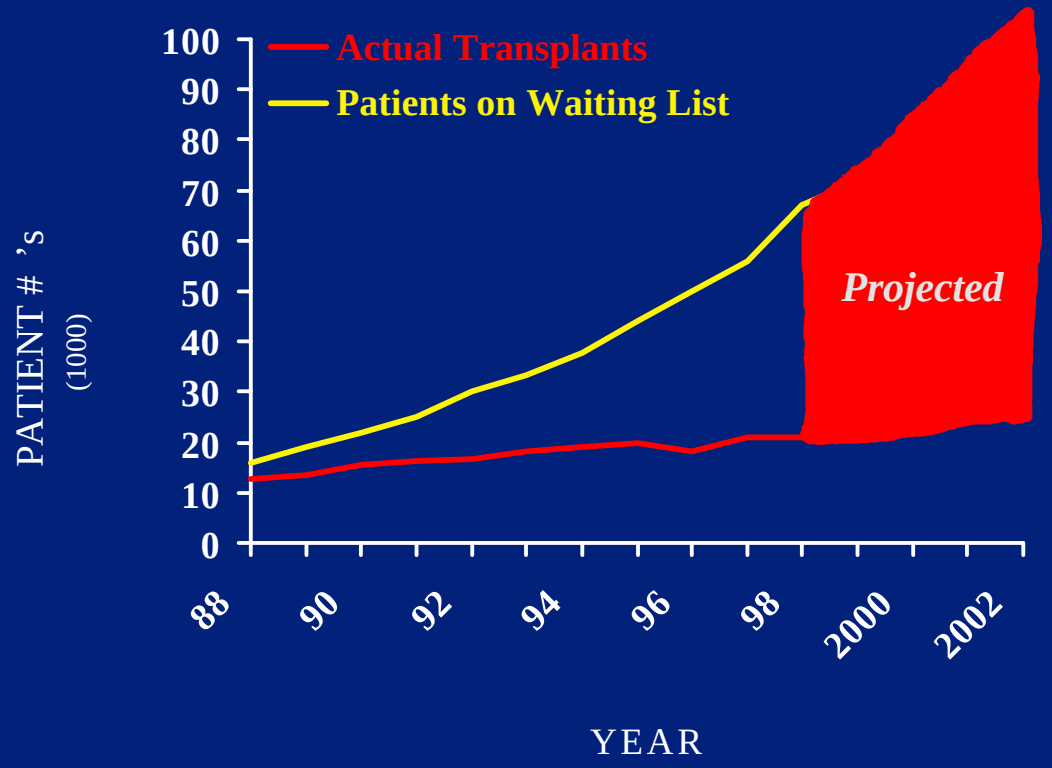
*Unmet Clinical Need: A Severe Shortage of Donor Organs**



* Compiled from UNOS Data December 1998

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Unmet Clinical Need: Cell & Tissue Transplantation

➢Neurologic Indications

Parkinson's Disease	~1,000,000 affected Individuals
Spinal Cord Injury	~200,000 SCI Patients U.S. alone

➢Cartilage Repair

Articular Cartilage Defects	~500,000 cases U.S. alone
Meniscus Repair	~800,000 cases U.S. alone

➢Islets

Type I Diabetes	~800,000 affected Individuals
Type II Diabetes	~15,000,000 affected Individuals

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The Pig as a Donor

- ***Many Agricultural, Domestic & Biomedical Uses***
- ***Herd Maintenance & Husbandry***
- ***Organ Size & Physiology***
- ***Genetic manipulation***

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Immunologic Barriers Discordant Xenotransplantation

- Hyperacute Rejection → ***Immediate Graft Rejection***
  - Natural Antibody Reactivity
  - Activation of Complement
- Acute Vascular Rejection → ***Delayed Graft Rejection***
  - Antibody Mediated
  - Neutrophil & NK Cell Activation
- Acute Cellular Rejection → ***Delayed Graft Rejection***
  - T Cell Activation And Proliferation

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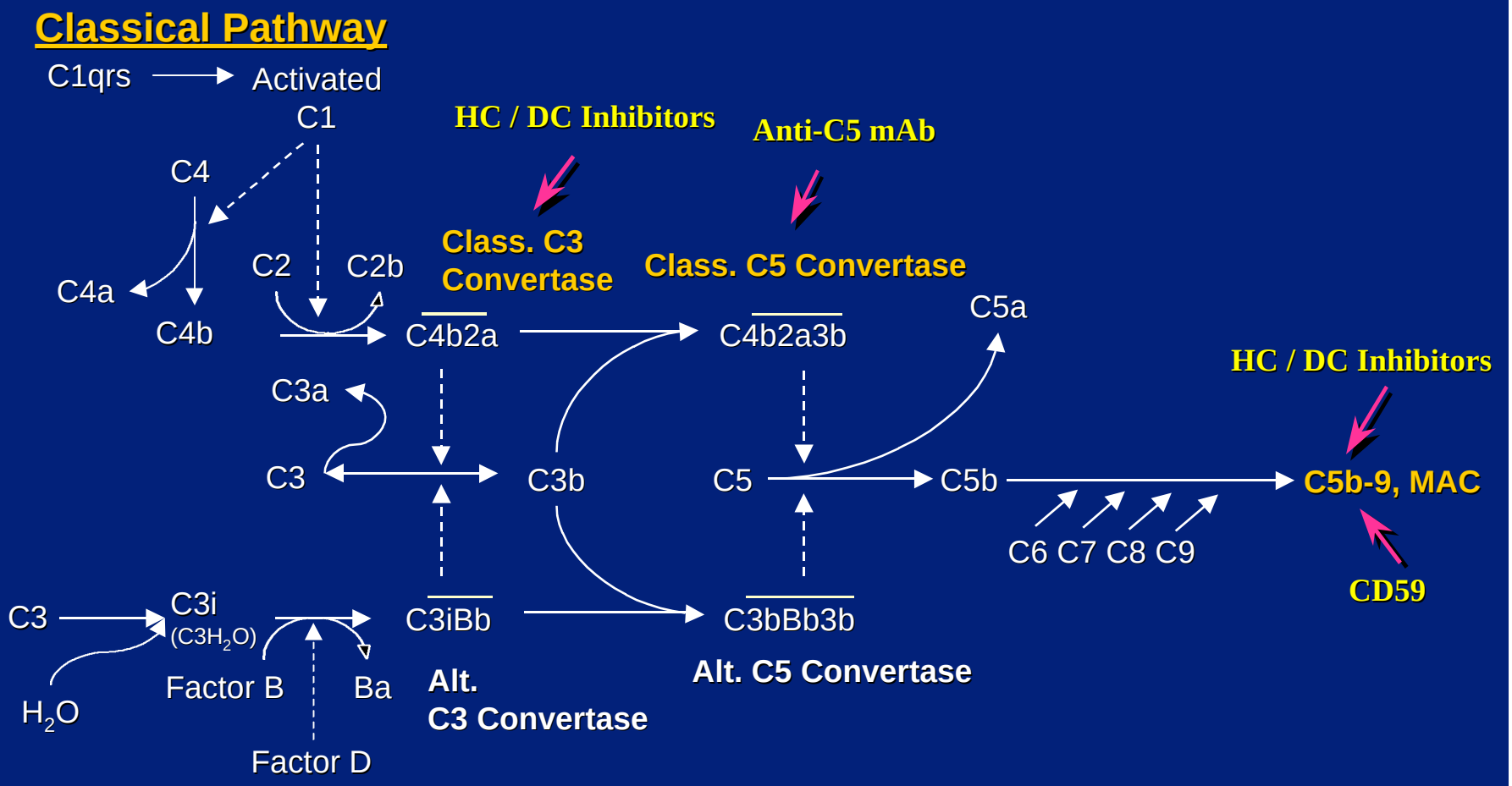
Targeting Complement Inhibitors

(Dalmasso, Platt, Bach)



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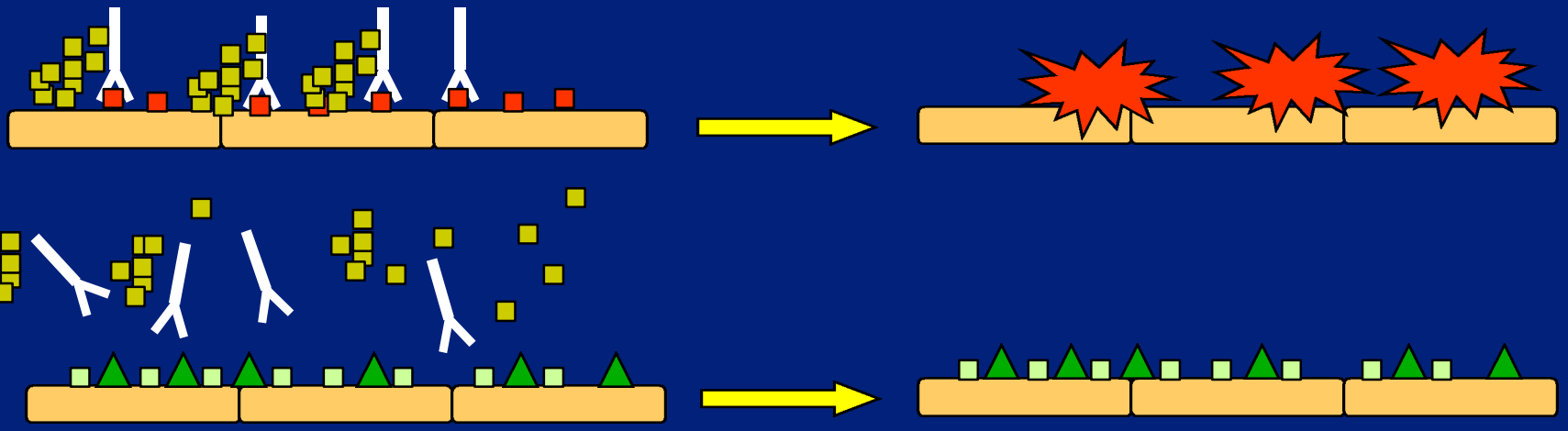
Antibody and Complement Mediated Rejection

Human Serum

Porcine Cells - Non Engineered



Porcine Cells -Engineered

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A Combinatorial Genetic Approach to Prevent Complement Activation

- **Hyperacute Rejection is a Major Barrier to Xenotransplantation**
- **Strategy : Genetically Engineer the Xenogeneic Donor**
  - **Inhibition of Complement Activation: Human CD59**
  - **Inhibition of Complement Activation: Engineered Bi-Functional Complement Inhibitors**
  - **Inhibition of Antibody Reactivity: Expression of Glycosyltransferases**

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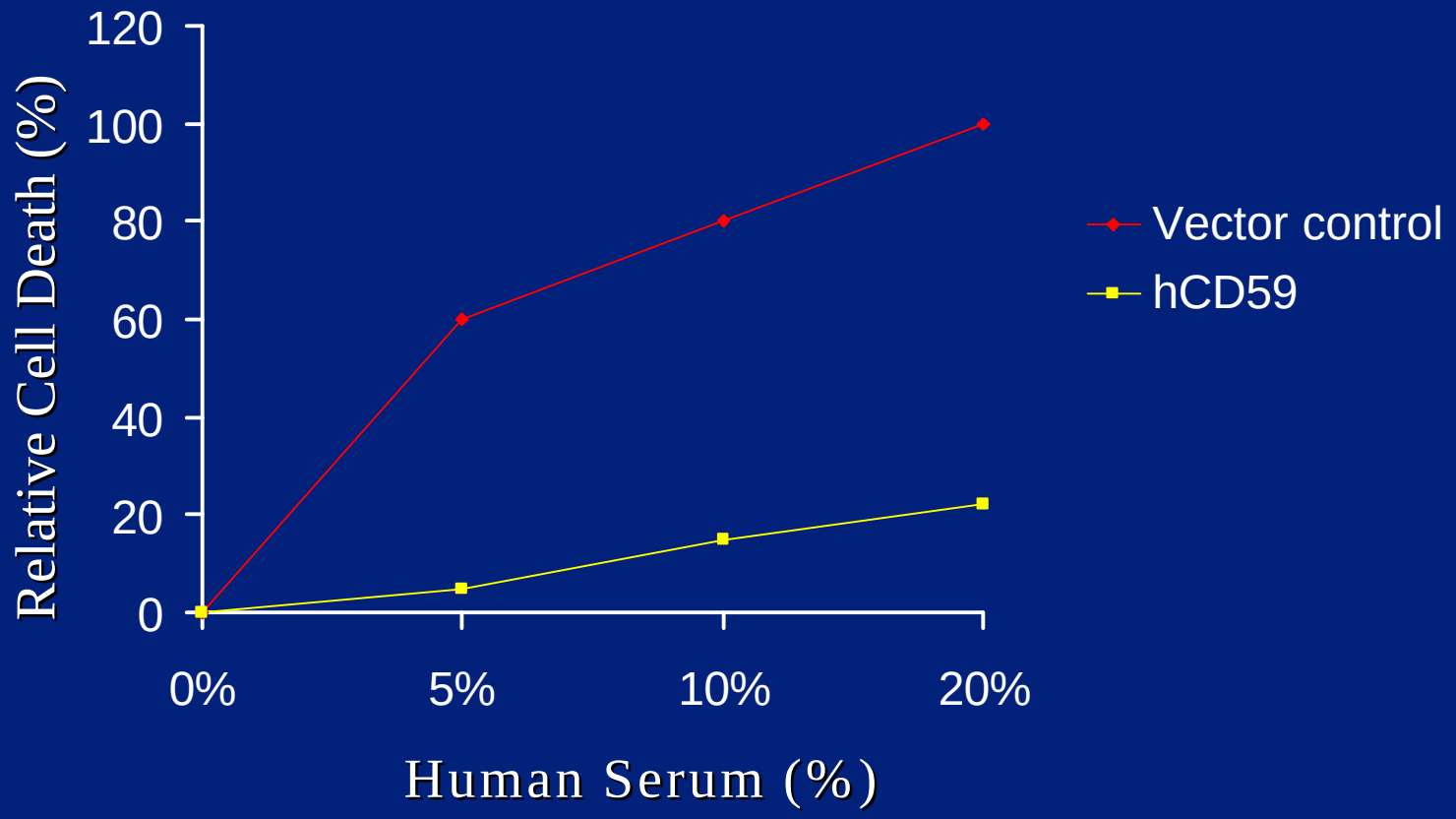
Expression of CD59 Prevents Complement-Mediated Cell Lysis



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Eliminating Natural Antibody Reactivity

A Critical Component for Eliminating the Xenogeneic Immune Response

- **~ 1% of Circulating Ig Molecules React to Xenogeneic Cell Surface Carbohydrate, Galα1,3Gal**
  (Gallili, Lowe, Sandrin)
- **Antibody Reactivity Activates Complement**
- **Antibody Reactivity Induces Acute Vascular Rejection: Antibody Dependant Cellular Cytotoxicity**

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Strategies to Inhibit Xenoreactive Natural Antibody Reactivity

Treatment of Recipient

Antibody Removal Therapy

- **Standard Plasmapheresis**
- **Standard Plasmapheresis + Gal Specific Chromatography**

Modify the Donor Phenotype

Genetic Modifications

- **Gene Knock-out**
- **Enzyme Competition**
  Carbohydrate Remodeling

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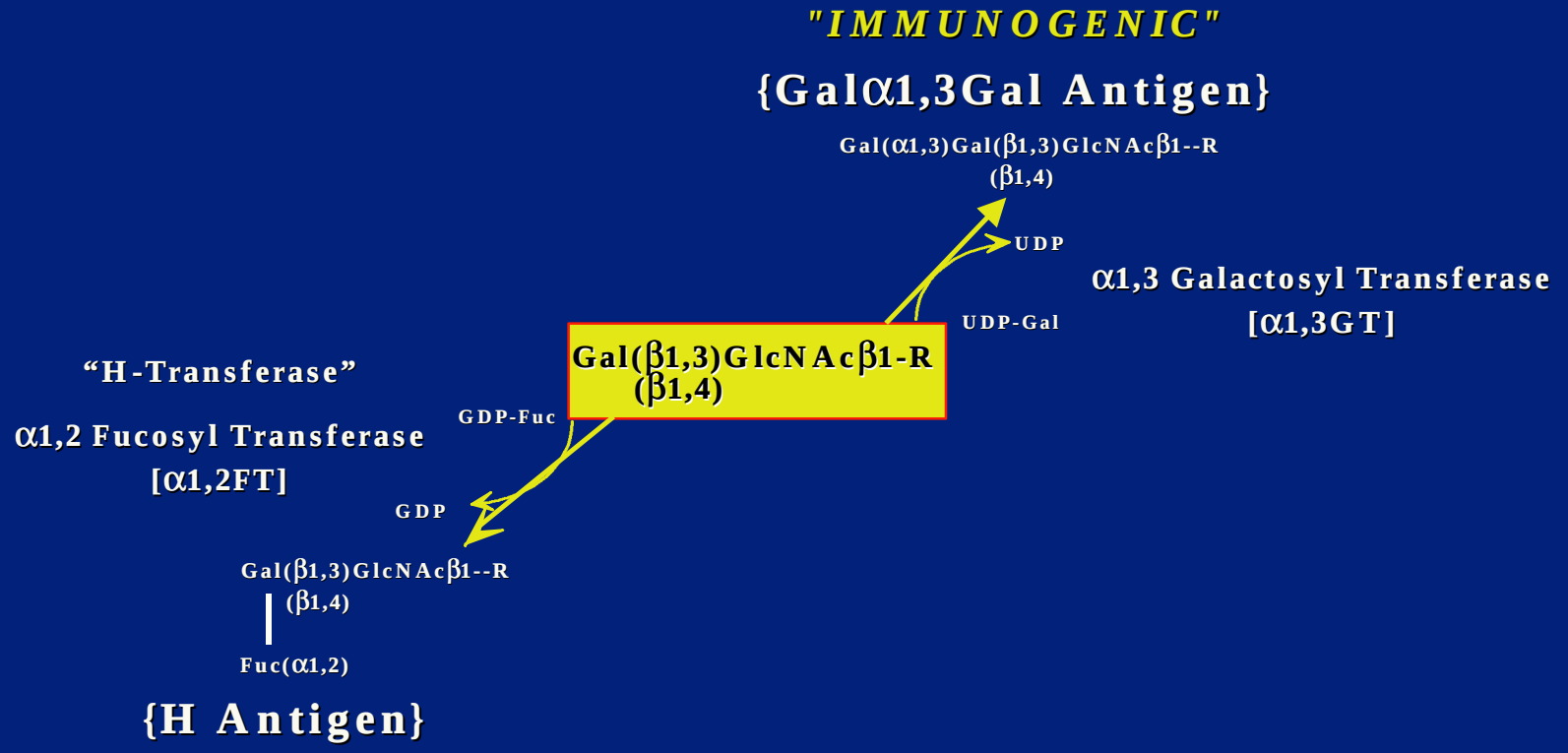
Enzyme Competition & Carbohydrate Remodeling

GLYCOSYLTRANSFERASE ENZYME COMPETITION STRATEGY



"UNIVERSALLY ACCEPTED 'O' PHENOTYPE"

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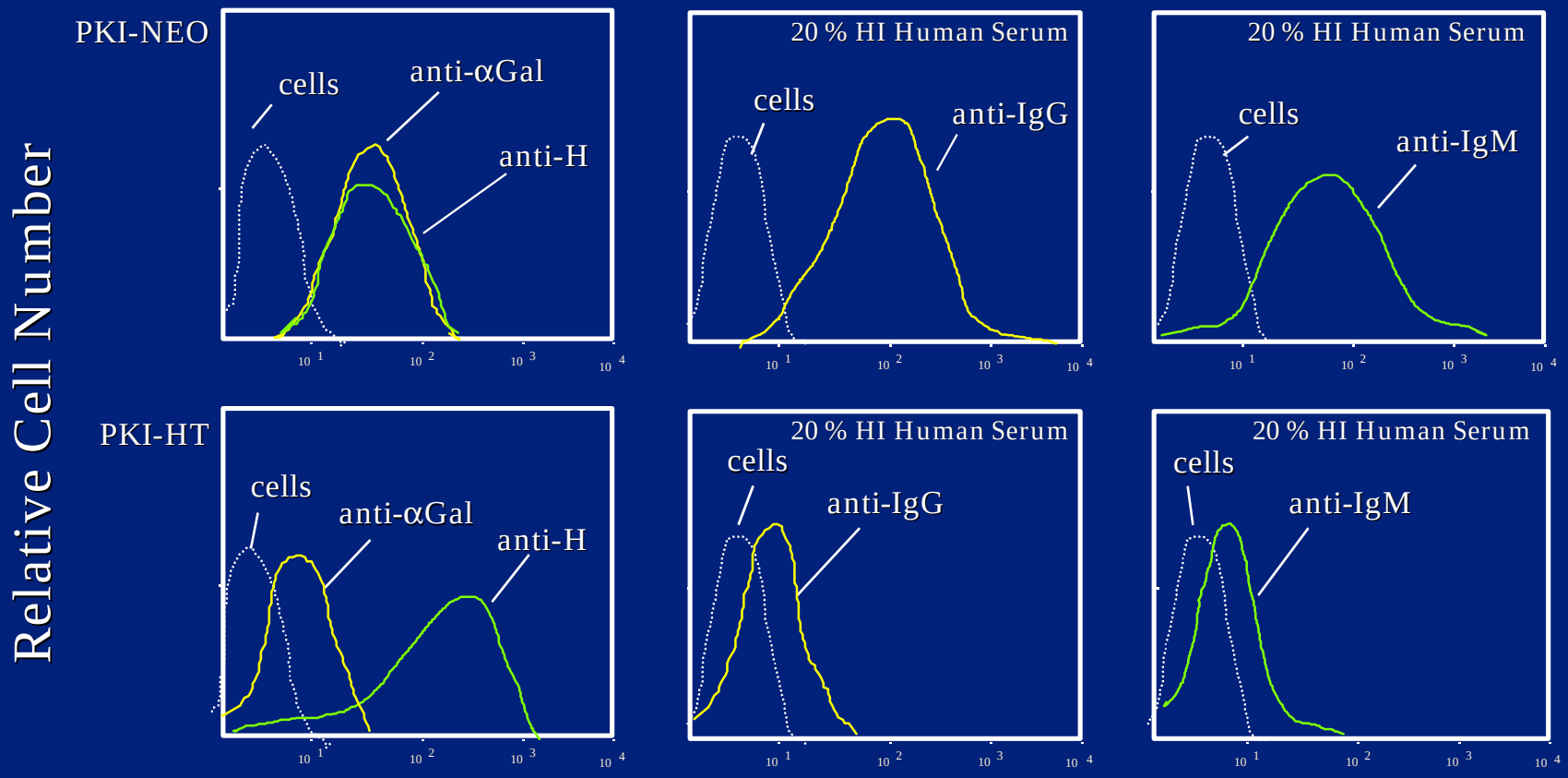
HT Expression Reduces Cell Surface Expression of Gal Epitope



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Development of Transgenic Pigs Expressing Human Complement Inhibitors and H-Transferase

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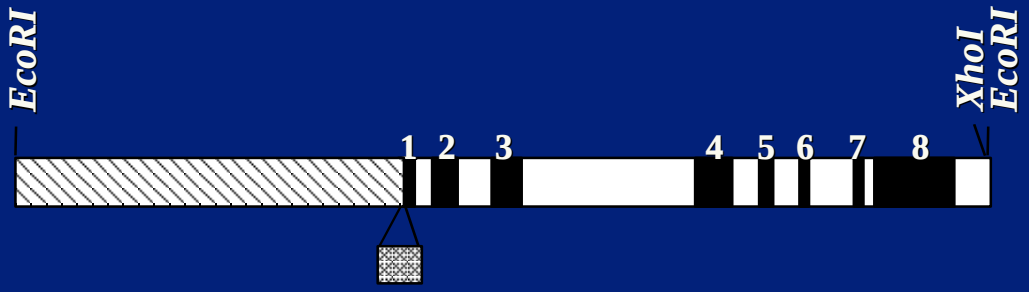
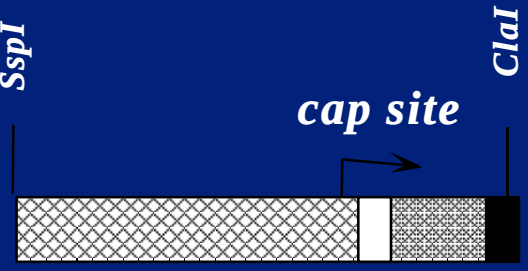
Transgenic Expression Constructs





CMV promoter + Ig Intron
SV40 POLY A+ signal sequence
HT cDNA insert
intron sequence

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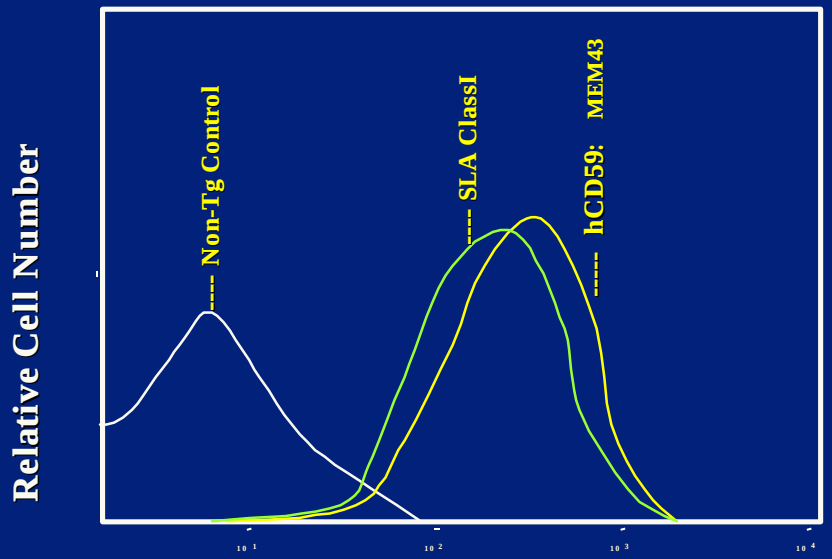
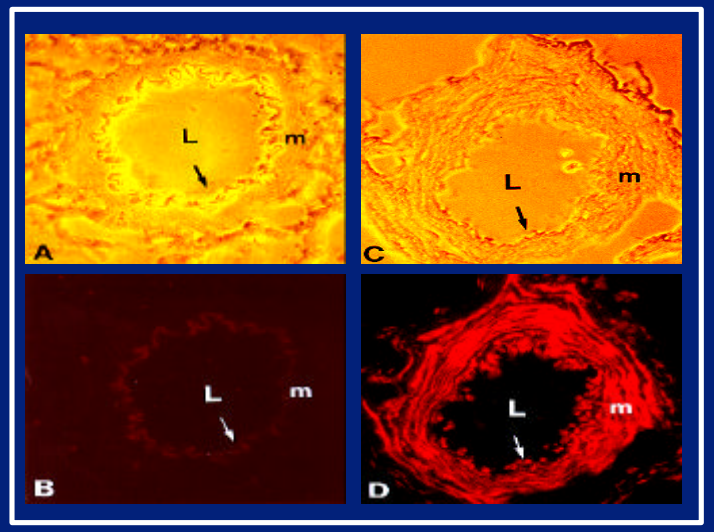
Expression of hCD59 in Transgenic Pig Cells and Tissues

FACS Analysis of PBMCs



Immunohistochemistry



Phase

CD59 mAb MEM43

Non-Tg Control **CD59-Tg**

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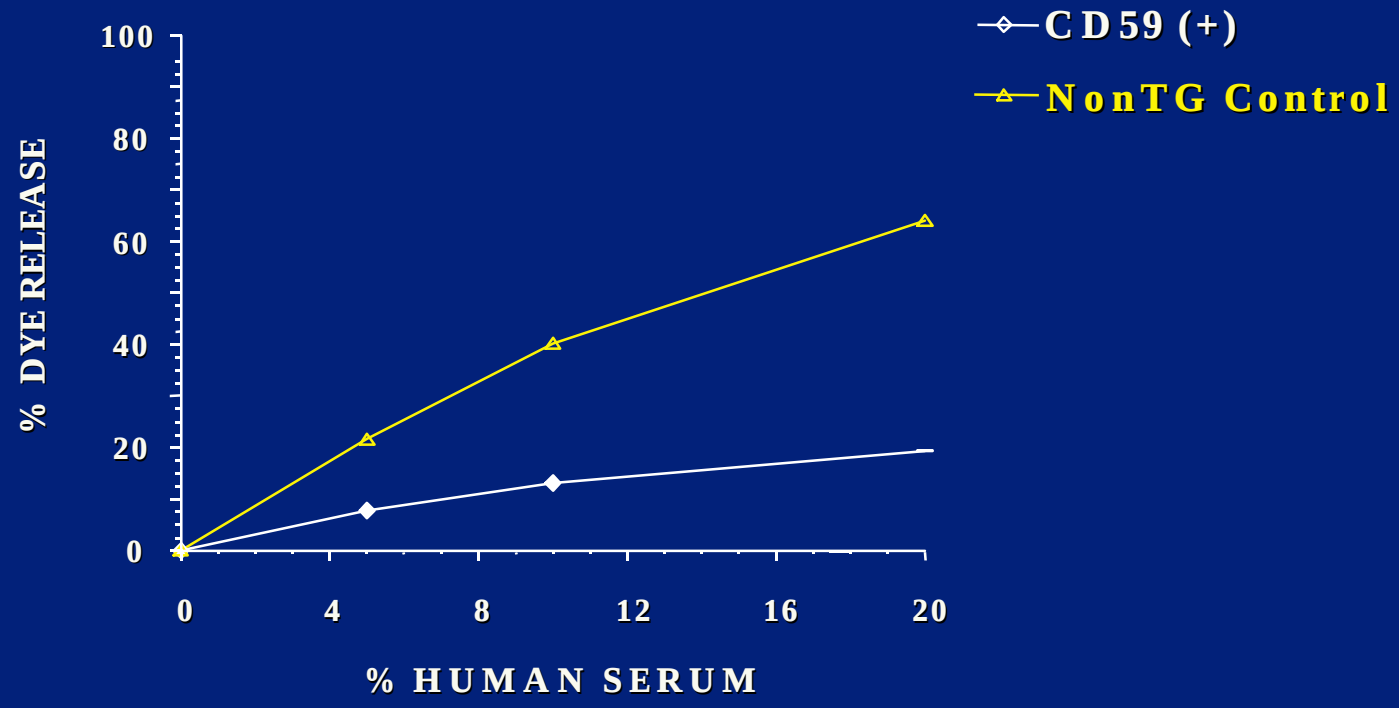
CD59 Transgenic Pigs Cells Resistant to Human Serum Cytolysis



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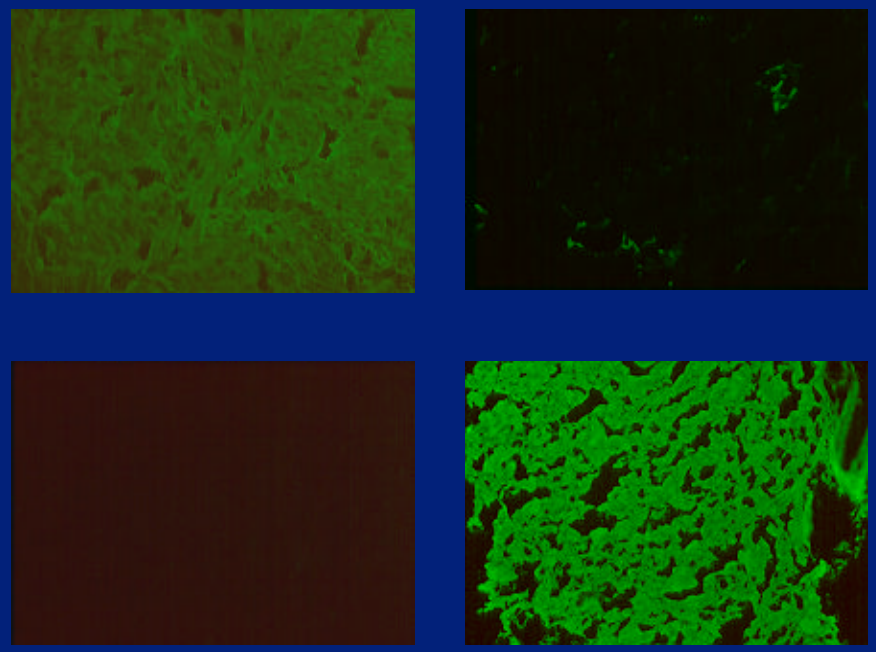
Immunohistochemical Analyses of HT Transgenic Founder Pig



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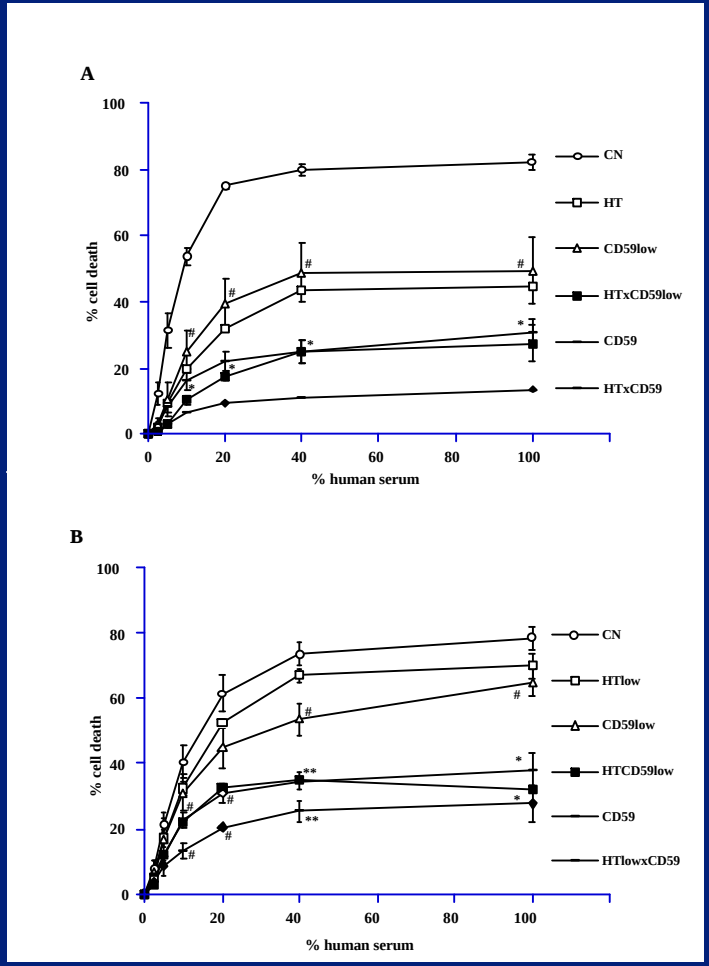
Maximum Resistance to Human Serum Lysis



Relative Cell Death (%)

Human Serum (%)

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Pre-Clinical Studies

1. Organ Perfusion Experiments
2. Organ Transplant Experiments
3. Cell Transplant Experiments
4. Tissue Transplant Experiments

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Ex Vivo Perfusion Studies

Human Blood *Ex Vivo* Perfusion
Transgenic Organs vs. Control

- Hearts
- Kidneys
- Lungs

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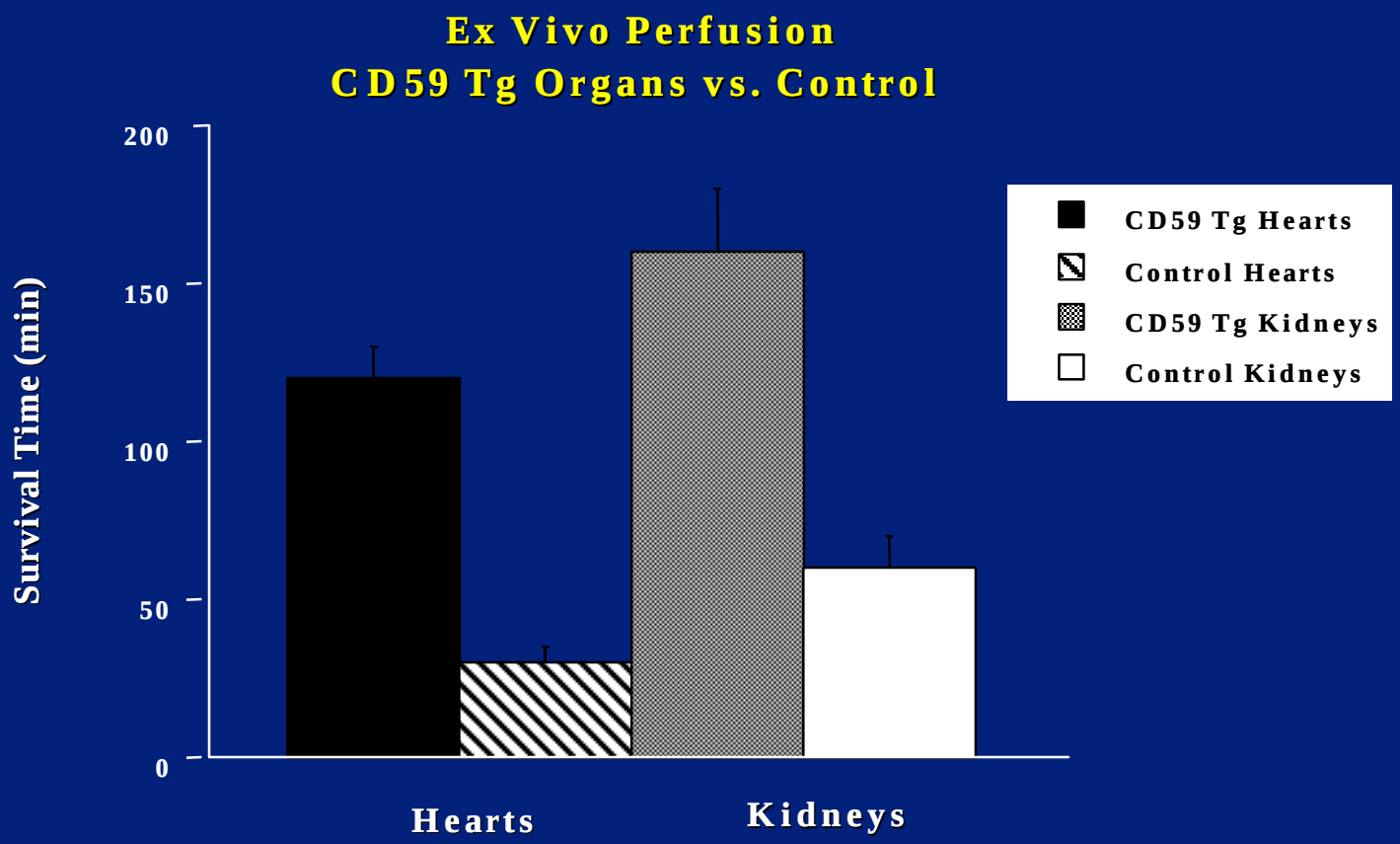
Enhanced Survival of CD59 Transgenic Pig Organs



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Cell & Tissue Transplantation

➢Neurologic Indications

Neuron Replacement Therapy for Parkinson's Disease
Transplantation of Porcine Fetal Ventral Mesencephalic Neurons

Remyelination of Damaged Spinal Cord
Transplantation of Porcine Schwann Cells/ OECs

➢Metabolic Indications

Islet Transplantation Therapy for Insulin Dependant Diabetes

➢Tissue Engineering

Cartilage Repair Therapy for Articular Cartilage Defects

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Spinal Cord Injury

1. Rodent EBX & Transection Models

- **a. Transplantation of Tg SCs & OECs**
- **b. Cell Engraftment & Remyelination**
- **c. Restore Conduction Across the Defect**

2. Primate LPC Detergent Demyelination Model

- **a. Studies are on-going**

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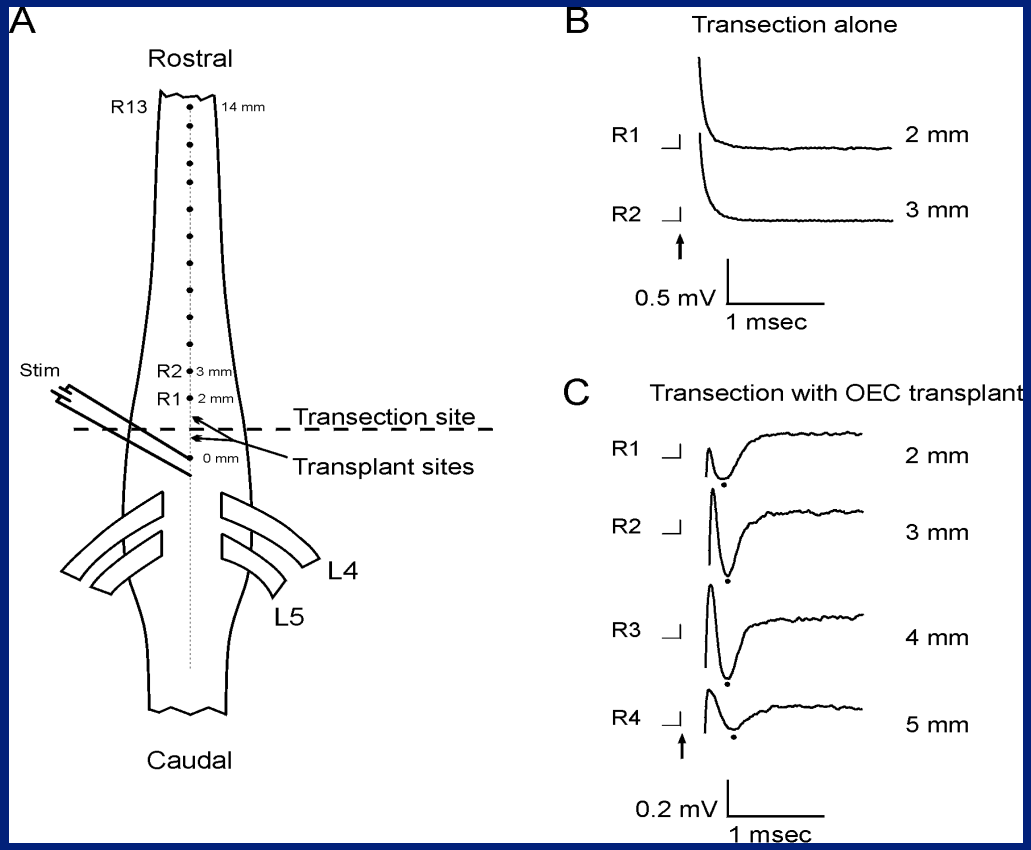
Transection Model & Transplantation



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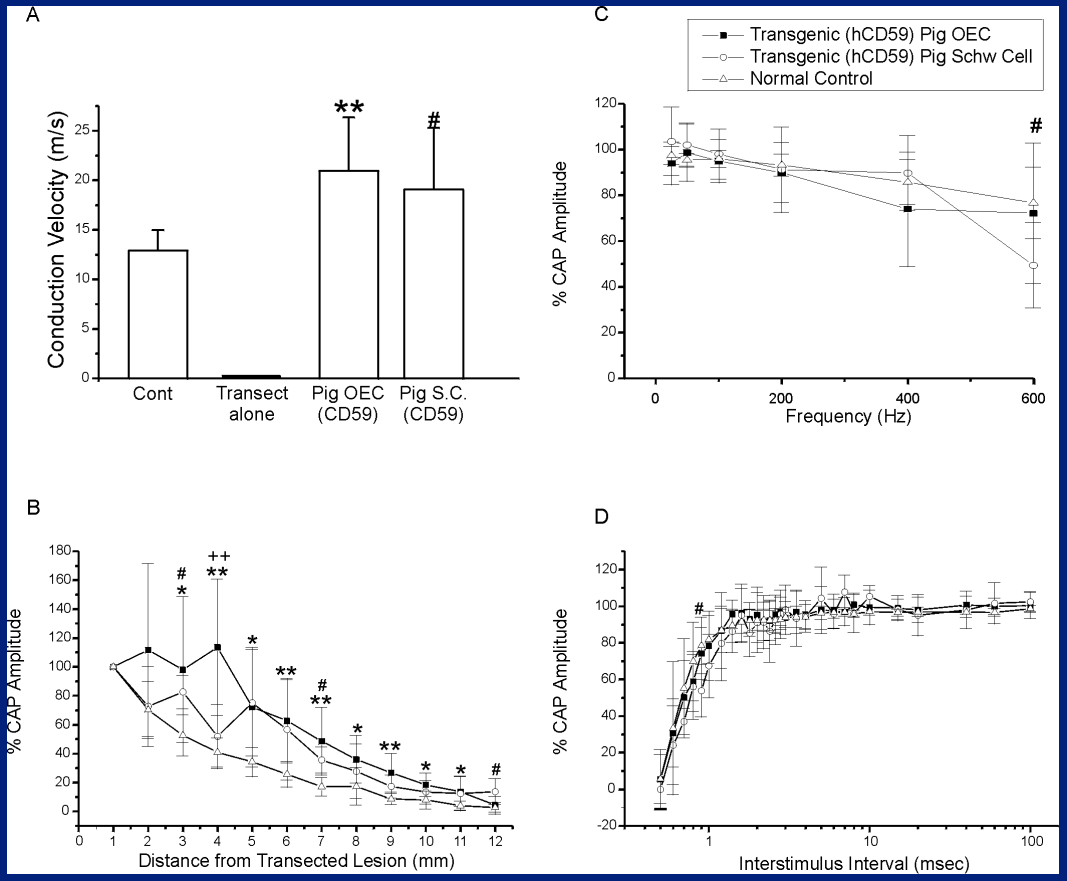
Transplantation of Pig Ensheathing Cells Restores Conduction



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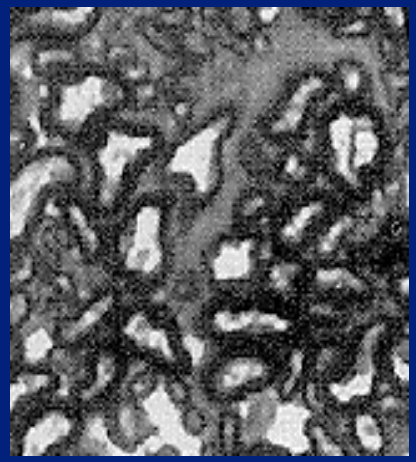
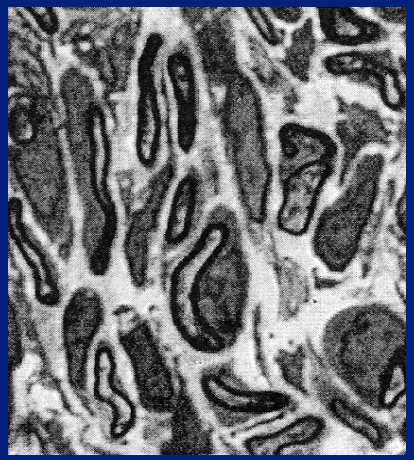
Spinal Cord Injury: Transgenic Pig Cells Repair Injured Spinal Cord



Normal Spinal Cord

Injured Spinal Cord



Injured Spinal Cord With Transgenic Pig Cells

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Insulin Dependent Diabetes Mellitis

1. Isolation of Porcine Islets

a. Initial procedures on Non-Tg control Neonates

b. Transplantation into SCID Mice

c. Demonstrate Pig Insulin Production

2. Isolation of Transgenic Porcine Islets

a. Isolation from Tg Neonates

b. Transplantation into SCID Mice

c. Assess Pig Insulin Production

d. Adoptive Transfer Human T-cells

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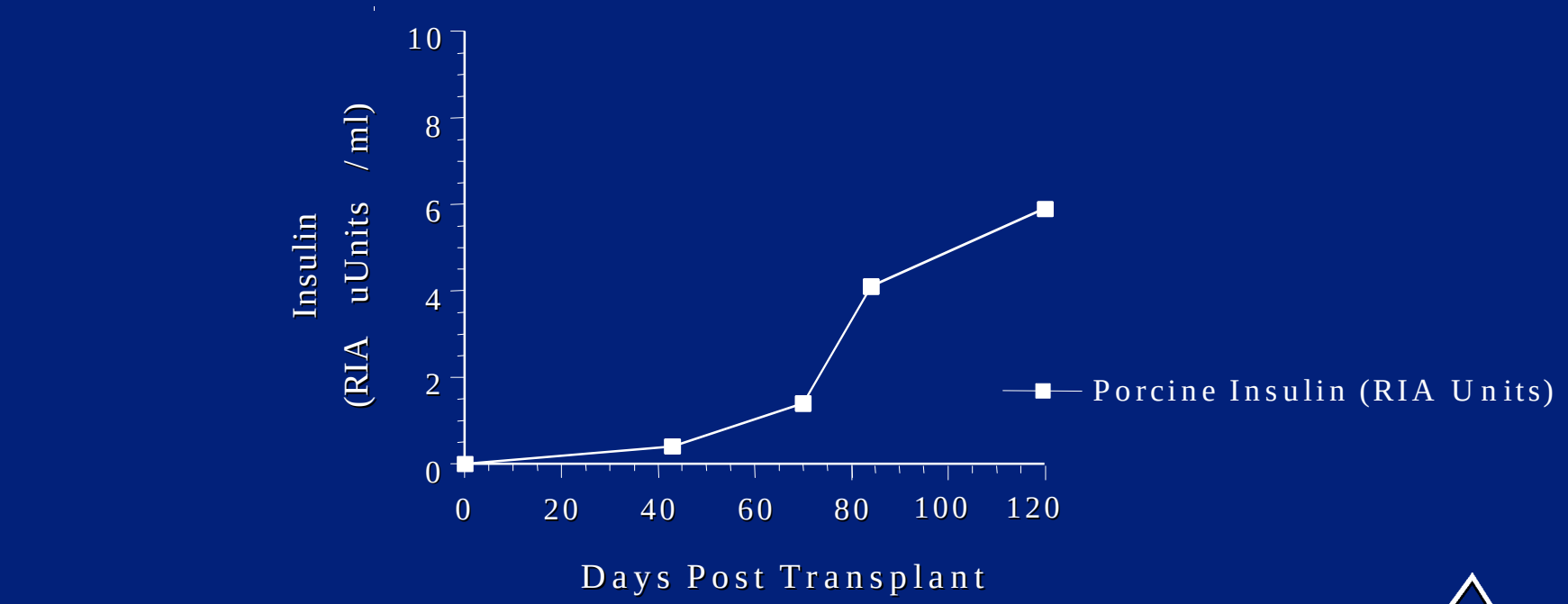
Transplanted Porcine Islets Produce Porcine Insulin

Radio-Immune Assay Analysis for Insulin



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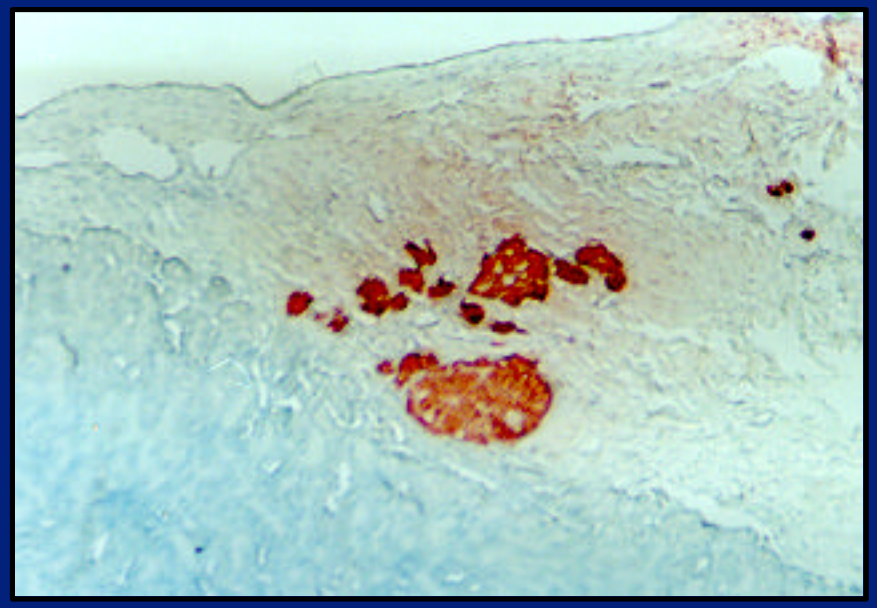
Transplanted Porcine Islets Produce Insulin

Immunohistochemical Analysis



- **Porcine Islets Transplanted Kidney Capsule of SCID Mouse**
- **Tissue Harvested at 120d Post Transplant**
- **Immunohistochemistry**

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Cartilage Repair:Tissue Engineering

Development of Transgenic Chondrocytes

- **Resistant to Humoral-Mediated Cytolysis**
- **Resistant to Cell-Mediated Immune Responses**

Establish Culture Conditions with ECM

Utilize In Vivo Rodent Models to Establish Efficacy

- **Murine Heterotopic Model**
- **Rabbit Ear & Knee Model**

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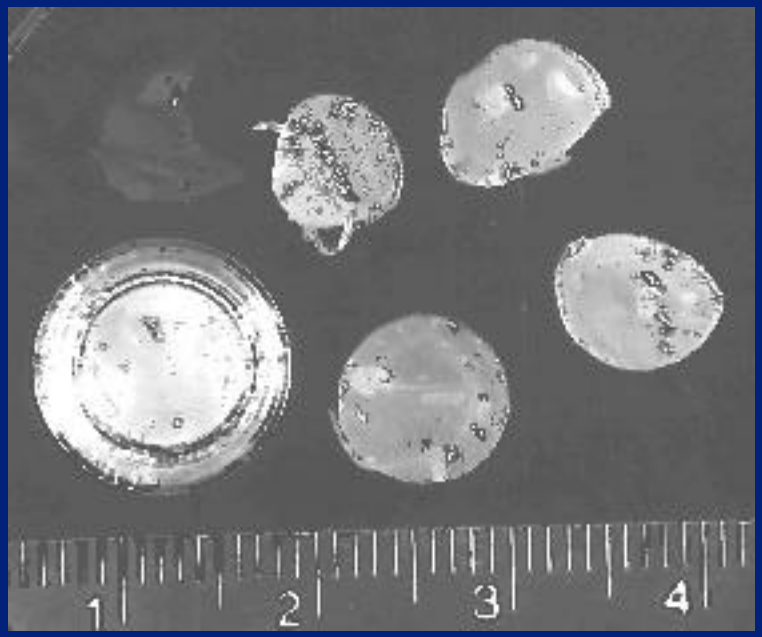
In Vitro Derived Cartilage from Transgenic Chondrocytes



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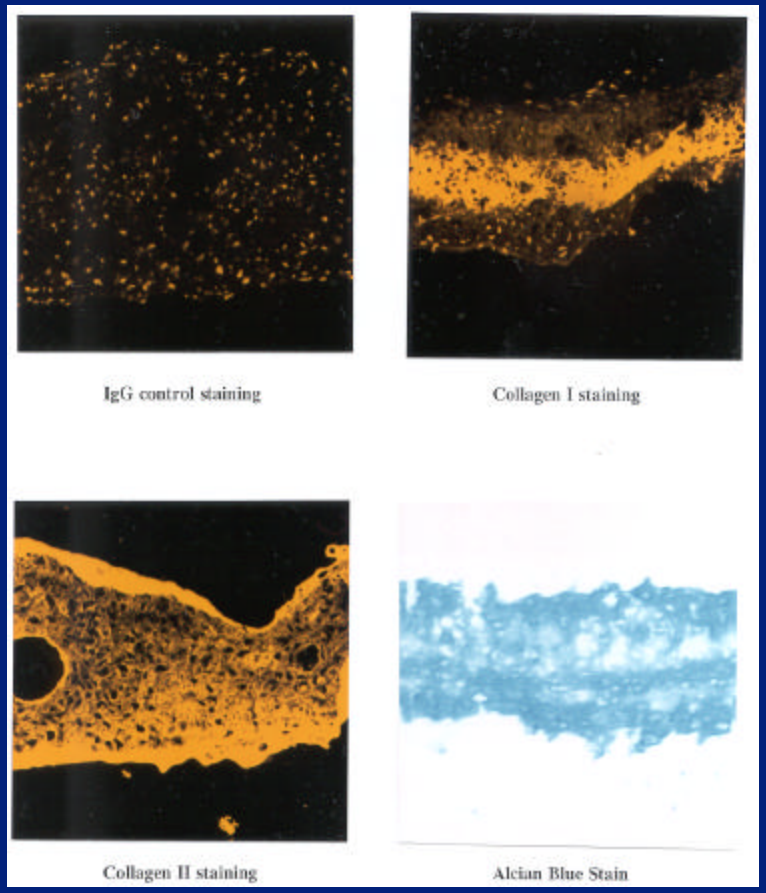
Collagen Expression in In Vitro Engineered Cartilage



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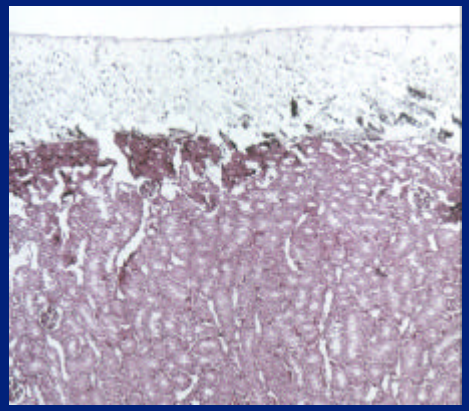
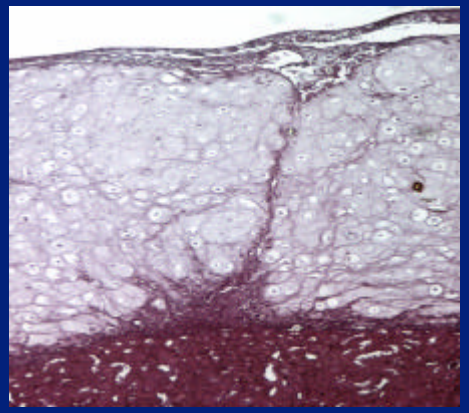
Transgenic Cartilage Heterotopic Transplants

SCID Model **Balb/C Model**

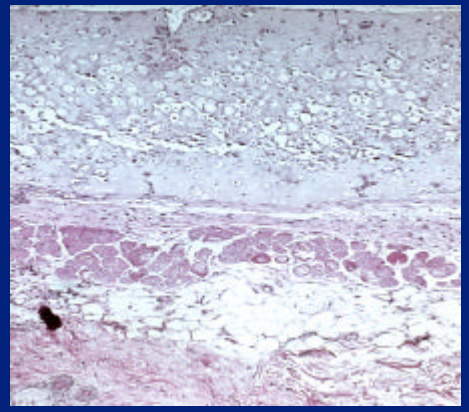
10X

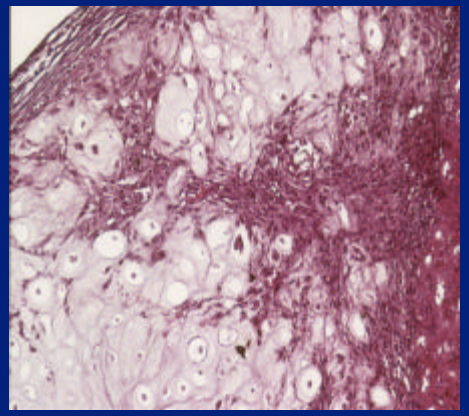




20X

20X





40X

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Summary

1. Developed Novel Genetic Approaches to Create Universal Donor Cells, Tissues and Organs

Inhibition of Natural Antibody Reactivity: ***High Expression of HT***
Inhibition of Complement Activation: ***High Expression of a Human Complement Inhibitor Proteins, Native & Chimeric Bifunctional Inhibitors***

- ***The Combinatorial Approach Prevents Hyperacute Rejection***

2. Established Several Pre-Clinical Transplant Models to Test the Efficacy of Transgenic Cells, Tissues and Organs for Restoring Function

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