Large Animal Models

Large size/long life span may be important for certain applications

- Cellular constructs with scaffolds (too big for rodents)
- Mimic clinical conditions of tissue damage as well as tissue repair/regeneration (Surgical model)
  - Cardiac infarct model
  - Subretinal injections
- Long term follow-up of individual animals
  - Multiple biopsies possible
    - Not necessary to sacrifice animals at each time-point for proper assessment
  - Long-term effects of treatment can be determined
    - Toxicity studies
    - Repeat injections of cellular therapy
Large animal models for biomedical research

Non-Human Primate, Sheep, Dog, Pig

- NOT useful for testing immunogenicity of human cells/cellular constructs
  - Xenogeneic barriers – NK cells, MΦ
    - CD47: CD172 (SIRPα) incompatibility
      - Yang YG. CD47 in xenograft rejection and tolerance induction 2010 Xenotransplantation 17(4):267-73

- Need to develop an analog of the human cells/constructs for each species and test in autologous setting or across MHC barriers depending on the clinical application
Miniature Swine

- Size range similar to humans (10-125kg)
  - Omnivore physiology similar to humans
- Extensively used in biomedical research
  - Porcine specific reagents available
    - Numerous phenotypic markers to assess immune response
    - Markers to distinguish donor from host cells available for certain pig strains
      - Pig Allelic Antigen (PAA)
      - CD4 allele specific monoclonal antibody
      - SLA specific monoclonal antibodies
Miniature Swine

- Pig skin similar to human skin
  - Low hairiness, thick stratum corneum, similar lipid composition, dermis structure
    - Useful for trans-dermal vaccine development
    - Useful for assessing skin substitute biologics

- Responses to BMT similar to patients
  - Manifestations of Graft-versus-Host Disease (GvHD) – similar grading
  - Complications of post transplantation lymphoproliferative disorder (PTLD)
    - Porcine $\gamma$ herpesvirus involvement (PLHV-1)
Miniature Swine
Average Weight versus Age
**MGH MHC-Defined Miniature Swine**

<table>
<thead>
<tr>
<th>Haplotype (SLA)</th>
<th>Origin of Regions</th>
<th>Class I</th>
<th>Class II</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>a</td>
<td>a</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>c</td>
<td>c</td>
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<td>a</td>
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</tr>
</tbody>
</table>

**MGH MHC-Defined Miniature Swine**

<table>
<thead>
<tr>
<th>Clinical Situation</th>
<th>Miniature Swine Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA identical sibling transplants</td>
<td>SLA-matched haplotypes</td>
</tr>
<tr>
<td>Cadaveric or non-matched siblings</td>
<td>SLA-disparate haplotypes</td>
</tr>
<tr>
<td>One-haplotype mismatched sibling transplants or parent into offspring transplants</td>
<td>One-haplotype mismatched heterozygotes (haploidentical) ($\text{SLA}^{ac}\rightarrow\text{SLA}^{ad}$)</td>
</tr>
</tbody>
</table>
Currently maintaining G11 animals with coefficient of inbreeding >95%
Recent data confirms skin and lung graft acceptance without immunosuppression
Adoptive transfer studies in large animals now possible
Immunogenicity Studies in MGH MHC-Defined Miniature Swine

Immunogenicity of umbilical cord tissue–derived cells

Patricia S. Cho,1 Darin J. Messina,2 Erica L. Hirsh,1 Nina Chi,2 Stephanie N. Goldman,2 Diana P. Lo,1 Ian R. Harris,2 Sicco H. Popma,2 David H. Sachs,1 and Christene A. Huang1

1Transplantation Biology Research Center, Massachusetts General Hospital, Boston; and 2Centocor R&D, Stem Cell Internal Venture, Radnor, PA

Approaches to Avoid Immune Responses Induced by Repeated Subcutaneous Injections of Allogeneic Umbilical Cord Tissue-Derived Cells

Bram V. Lutton,1 Patricia S. Cho,1 Erica L. Hirsh,1 Kelly K. Ferguson,1 Alexander G. S. Teague,1 John S. Hanekamp,1 Nina Chi,2 Stephanie N. Goldman,2 Darin J. Messina,2 Stuart Houser,3 Beow Y. Yeap,4 Sicco H. Popma,2 David H. Sachs,1 and Christene A. Huang1,5
Immune Response Testing in Miniature Swine

Naïve SLA-matched bleeder pigs are available from each haplotype for assay controls.

Recombinant haplotypes are available to distinguish responses to MHC class I vs class II.

- Mixed Lymphocyte Reactivity (MLR)
  - CFSE and H³ Thymidine incorporation
- Cell Mediated Cytotoxicity (CML)
- Donor specific antibody responses
  - Serum antibody binding to PBMC of different haplotypes
  - Complement dependent antibody mediated cellular cytotoxicity assay
Cellular Responses

Mixed Lymphocyte Reactivity (MLR)

Naive SLA\textsuperscript{ad} Responders

Sensitized SLA\textsuperscript{ad} Responders (following SLA\textsuperscript{ac} skin graft rejection)
Cellular Responses

Cell Mediated Lympholysis (CML)

<table>
<thead>
<tr>
<th>Effector:Target ratio</th>
<th>% Specific Lysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>100:1</td>
<td>100</td>
</tr>
<tr>
<td>50:1</td>
<td>50</td>
</tr>
<tr>
<td>25:1</td>
<td>25</td>
</tr>
<tr>
<td>12.5:1</td>
<td>12.5</td>
</tr>
</tbody>
</table>

Legend:

- Naïve SLA<sup>ad</sup>
  - nAD@CC:CC
  - nAD@YORK:YORK
  - nAD@YORK:AD

- Sensitized SLA<sup>ad</sup>
  - 20312@CC:CC
  - 20312@YORK:YORK
  - 20312@YORK:AD
Antibody Responses

Sera from SLA\textsuperscript{ad} animal pre and post SLA\textsuperscript{ac} skin rejection

Control allo-antisera (αSLA\textsuperscript{cc})

Pre SKTx

1:2 1:8 1:128 1:1024

Serum Dilution

% Cytotoxicity

SLA\textsuperscript{ac} target cells

4 weeks post SKTx

2 weeks post SKTx
Hematopoietic cell transplantation and immune tolerance studies in miniature swine

Stable Multilineage Chimerism without Graft versus Host Disease Following Nonmyeloablative Haploidetical Hematopoietic Cell Transplantation

Robert A. Cina,1,2 Krzysztof J. Wikiel,1 Patricia W. Lee,1,3 Andrew M. Cameron,1,4 Shehan Hettiarachy,1,5 Haley Rowland,1 Jennifer Goodrich,1 Christine Colby,6 Thomas R. Spitzer,6 David M. Neville, Jr.,7 and Christene A. Huang1,8

Predictors of Organ Allograft Tolerance Following Hematopoietic Cell Transplantation

Miniature Swine
Assessing hematopoietic stem cell function

- Cobblestone Area Forming Cell Assay (CAFC)
- Colony Forming Cell Assay (CFU) (porcine IL3, SCF, GM-CSF)

Amount of functional stem cell activity infused correlates with engraftment outcome
Tumor studies in miniature swine

Establishment of transplantable porcine tumor cell lines derived from MHC-inbred miniature swine

Patricia S. Cho,¹,² Diana P. Lo,¹ Krzysztof J. Wikiel,¹,² Haley C. Rowland,¹ Rebecca C. Coburn,¹ Isabel M. Mc Morrow,¹,² Jennifer G. Goodrich,¹ J. Scott Arn,¹ Robert A. Billiter,¹ Stuart L. Houser,¹ Akira Shimizu,¹ Yong-Guang Yang,¹,² David H. Sachs,¹,² and Christene A. Huang¹,²

¹Transplantation Biology Research Center, Massachusetts General Hospital (MGH), and ²Harvard Medical School, Boston, MA

Myelogenous leukemia in adult inbred MHC-defined miniature swine: A model for human myeloid leukemias

Raimon Duran-Struuck a,*, Patricia S. Cho a, Alexander G.S. Teague a, Brian Fishman a, Aaron S. Fishman a, John S. Hanekamp a, Shannon G. Moran a, Krzysztof J. Wikiel a, Kelly K. Ferguson a, Diana P. Lo a, Michael Duggan a, J. Scott Arn a, Bob Billiter a, Ben Horner a, Stuart Houser a, Beow Yong Yeap b, Susan V. Westmoreland c, Thomas R. Spitzer d, Isabel M. Mc Morrow a, David H. Sachs a, Roderick T. Bronson e, Christene A. Huang a

¹Transplantation Biology Research Center, Massachusetts General Hospital, United States
²Biostatistics Center, Massachusetts General Hospital, United States
³New England Primate Research Center, Harvard Medical School, United States
⁴Cancer Center, Massachusetts General Hospital, United States
⁵Histopathology Core, Harvard Medical School, United States
Limitations of Large Animal Models

- **Expense**
  - Need for large animal facility
    - OR capability
  - Limited animal numbers per group

- **Cannot assess immune response of human cells/constructs directly**
  - Xeno-responses differ from allo-responses
  - Need to generate analog of human cells
The MGH Miniature Swine Colony, developed in the mid-70s by David H. Sachs, MD, originated from a cross of two separate miniature swine lineages (Fig 1). The colony moved to MGH from NIH in 1990 and have been used extensively by many investigators for immunologic, physiologic and imaging studies.

In 2007, a 3 million-dollar renovation of the Swine 2 Research facility, located on the Tufts Cummings School of Veterinary Medicine campus, was completed. This state-of-the-art facility, funded by NCRR, includes multiple farrowing and nursery rooms, OR, wetlabs and AI lab (Fig 2), as well as the main housing area (Fig 3).

The MGH Miniature Colony is a closed colony for biosecurity reasons and once animals leave the facility, they may not re-enter. Animal care personnel are required to shower within the facility prior to entry and the operation conforms to the USDA Research guidelines. Figure 4 shows a complete growth profile of the herd.

While the primary focus of this herd is to facilitate preclinical immunologic studies ongoing at Massachusetts General Hospital, surplus animals are available to area institutions for scientific research purposes. Appendix 1 outlines the routine herd health program, while Appendix 2 details the biosurveillance program.

Please call (617)298 0511 for further inquiries
• MGH Miniature Swine

This herd is located in the state of Massachusetts which is USDA-recognized Brucellosis and Pseudorabies (Aujeszky’s Disease) free. In addition, quarterly testing confirms that this colony is free of Transmissible Gastroenteritis (Elisa) and Porcine Reproductive and Respiratory Syndrome (Elisa).

**Vaccination:** All piglets are vaccinated against Mycoplasma Hyopneumoniae, Hemophilus Parasuis, Streptococcus Suis, Pasteurella Multocida, Bordatella Bronchiseptica and Erysipelothrix Rhusiopathiae at day 7, with a booster vaccination at 28 days of age. All swine six months or older are vaccinated against Erysipelothrix Rhusiopathiae, Leptospira (Canicola-Grippotyphosa-Hardjo-Icterohaemorrhagiae-Pomona), Influenza and Parvovirus. Repeat vaccination is performed every six months.

**Parasite Control:** A herdwide deworming program with ivermectin is performed every six months.
• **MGH Miniature swine**

• **Biosecurity/Biosurveillance:** Quarterly bleeds are performed on 10-15% of the breeding animals and submitted to Iowa State Veterinary Diagnostic Laboratory for a complete serology panel (see below). In addition, quarterly nasal and rectal swabs cultures are performed. All animal care personnel are required to shower prior to entering the facility.

<table>
<thead>
<tr>
<th>Organism or Disease</th>
<th>Iowa State Testing Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Encephalomyocarditis Virus</td>
<td>VN</td>
</tr>
<tr>
<td>Leptospira</td>
<td>MAT</td>
</tr>
<tr>
<td>Mycoplasma hyopneumoniae</td>
<td>CF or ELISA</td>
</tr>
<tr>
<td>Porcine Respiratory &amp; Reproductive Syndrome</td>
<td>ELISA or IFA</td>
</tr>
<tr>
<td>Porcine Parvovirus</td>
<td>HI</td>
</tr>
<tr>
<td>Pseudorabies</td>
<td>G1 ELISA, VN, ELISA Screen ALA</td>
</tr>
<tr>
<td>Swine Influenza Virus</td>
<td>HI (H1N1 and H3N2), ELISA (H1N1 only), PFIZER (H1N1 only)</td>
</tr>
<tr>
<td>Transmissible Gastroenteritis Virus</td>
<td>VN</td>
</tr>
<tr>
<td>Vesicular Stomatitis Virus (Indiana)</td>
<td>VN</td>
</tr>
<tr>
<td>Vesicular Stomatitis Virus (New Jersey)</td>
<td>VN</td>
</tr>
<tr>
<td>Bovine Viral Diarrhea type 1</td>
<td>VN</td>
</tr>
<tr>
<td>Bovine Viral Diarrhea type 2</td>
<td>VN</td>
</tr>
<tr>
<td>Brucellosis</td>
<td>Card test (B. abortus/suis)</td>
</tr>
<tr>
<td>Toxoplasmosis</td>
<td>LAT</td>
</tr>
</tbody>
</table>

CF – Complement Fixation
ELISA – Enzyme-Linked Immunosorbent Assay
HI – Hemagglutination inhibition
LAT – Latex agglutination
MAT – Microscopic agglutination test
VN – Virus Neutralization