NC TraCS/NC State RFA Description and Goals

- Functional tissue engineering/regenerative medicine
- Development and utilization of large animal models for translational medicine
- Manufacturing and scale up of biomaterials
- Evaluating the potential of naturally occurring disease models for therapeutic discovery
- Development and testing of medical devices
- Improvement of imaging technologies
- Antimicrobial resistance
- Zoonotic diseases
- Tissue-on-chip model systems

https://tracs.unc.edu/docs/pilotprogram/NC_TraCS-NC_State_Collaborative_RFA.pdf
Large Animal Models in Comparative Medicine

• Induced models. Dr. Anthony Blikslager
• Genetically modified models. Dr. Jorge Piedrahita
• Naturally occurring models. Dr. Duncan Lascelles

Opportunities. Induced Animal Models

Anthony Blikslager, DVM, PhD
Professor, Equine Surgery and Gastroenterology
Director, Large Animal Models Core,
Center for Gastrointestinal Biology and Disease

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Opportunities. Induced Animal Models

Comparative mechanisms of ischemia/reperfusion injury

Availability of facilities, equipment, and know-how for large animal research at the College of Veterinary Medicine
Opportunities. Induced Animal Models

Use of both research and veterinary clinical facilities to research models of human disease

Opportunities. Induced Animal Models

Use of state-of-the-art techniques to strengthen model development
Opportunities. Induced Animal Models

Surgical techniques to model human disease

Pros and Cons of Large Animal Inducible Models

**Advantages**
- Physiological similarities to human beings
- Advantage of size of the animal
- Crossover knowledge for veterinary or agricultural knowledge

**Disadvantages**
- Expense
- Availability of reagents
- Fundability
Opportunities. Transgenic Pig Models

- Dr. Jorge Piedrahita
- MSC, PhD
- Professor of Translational Medicine
- Director, Comparative Medicine Institute

Why a transgenic pig?

- Similar anatomy, physiology, metabolism, and pathology.
- Similar size.
- Large litters and short generation intervals compared to primates.
- Can do complex genetic manipulations.
When to make a pig

• Mouse mutant does not recapitulate human disease (cystic fibrosis).
• Pig size or physiology has a distinct benefit when compared to mice/rats (regenerative medicine, cardiology).
• Additional costs can be justified.

How do we make transgenic pigs?
Making a TG pig using SCNT

Step 2. Making the pig using somatic cell nuclear transfer (SCNT).

- Modify pigs cells with TALENs or CRISPR/Cas
- Select the green cells for expansion
- Expansion to form colonies for DNA analysis and SCNT
- DNA analysis

AS pig

AS cells

Oocytes

Emulsification

Fusion

Embryos with H2O-GFP

Embryo transfer into surrogate mother

Cloned transgenic animals

AS pig

Step 2. Making the pig using somatic cell nuclear transfer (SCNT).
Meganucleases

Zinc Finger Nucleases (ZNF)
Tal Effector Nucleases (TALENs)
CRISPR-Cas9

All same concept. A DNA binding domain linked to a nuclease

Homing Endonucleases (HE)

PRE-HE
- Rates averaging 1 event per million cells
- Need enriching/selectable markers
- Need complex targeting constructs
- In pigs would take 1-2 years
- For double KOs would need breeding or 2 rounds of SCNT

POST-HE
- Rates as high as 1/1 (100%)
- No need for enriching/selectable markers
- No needs for complex targeting constructs
- In pigs takes 6-9 months
- For double KOs no need to breed. Drastically reduces generational intervals
H2B-GFP pigs for cell tracking. “Knock in”

Cell tracker TG pig
Pig model of dwarfism “Knock out”

![Image of pigs](image)

**Growth Curve**

<table>
<thead>
<tr>
<th>HMGA2+/+ SCNT (n=7)</th>
<th>HMGA2+/+ NB (n=5)</th>
<th>HMGA2-/+(n=8)</th>
<th>HMGA2-/- (n=3)</th>
<th>HMGA2-/-Stra8 (n=1)</th>
</tr>
</thead>
</table>

- Birth
- 2 weeks
- 4 weeks
- 6 weeks
- 8 weeks

Weight range: 0 to 30 kg
Humanized pig. “Double Knock out”

First Round. Gene 1 Null

Second Round. Gene 2 Null

Humanized pig. Double Knock out

A

IL2RG<sup>−/−</sup> RAG1<sup>−/−</sup>

non-transplanted

IL2RG<sup>−/−</sup> RAG1<sup>−/−</sup>

transplanted

B

Spleen

IL2RG<sup>−/−</sup> RAG1<sup>−/−</sup>

non-transplanted

IL2RG<sup>−/−</sup> RAG1<sup>−/−</sup>

transplanted

C
When to make a pig

• Mouse mutant does not recapitulate human disease (cystic fibrosis).
• Pig size or physiology has a distinct benefit when compared to mice/rats (regenerative medicine, cardiology).

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Opportunities. Companion Animal Models

• Dr. Duncan Lascelles
• PhD. MRCVS, CertVA, DSAS (ST), DECVS, DACVS
• Professor of Small Animal Surgery and Pain Management
• Associate Director, Comparative Medicine Institute
Diseases in pets that accurately reflect the target disease in humans (*fidelity* or *face validity* with the target disease)
Opportunities. Companion Animal Models

PROOF OF CONCEPT TESTING OF THERAPEUTICS OR DEVICES IN PETS with diseases that accurately reflect the target disease in humans (fidelity or face validity with the target disease)
Opportunities. Companion Animal Models

Cardiac disease
Hypertrophic cardiomyopathy (C); Dilated cardiomyopathy (D)

Musculoskeletal disease
Osteoarthritis (D,C,H); Tendon injuries (H); ACL tear (D)

Neurological disease
Acute spinal cord injury; chronic paralysis (D); Chiari malformation/neuropathic pain (D); Eilepsy (D)

Cancer
Osteosarcoma (D); Melanoma (D); Non-Hodgkin’s lymphoma (D)

Others…………

Concerns:
Lack of validated outcome measures
Lack of access to subjects
Outcome Measures  
*e.g.* osteoarthritis pain: Validated Measures of the Functional Impact of Pain

- Somatosensory Function
- Activity
- Sleep quality
- Pain; Function
- Limb use

Access to subjects:

66% of owners are very willing to participate in *research studies and trials*  
- Sampling; novel therapeutics  
- Clinical trials on pets often performed

NC State CVM/CMI Clinical Studies Core:  
- Facilitates the use of client-owned pets for  
  - Sampling;  
  - Clinical trials of novel therapeutics or devices  
  *All under informed owner consent and IACUC approval*
https://tracs.unc.edu/docs/pilotprogram/NC_TraCS-NC_State_Collaborative_RFA.pdf

Oct 18th Deadline

NC STATE UNIVERSITY
Comparative Medicine Institute

NC STATE UNIVERSITY
College of Veterinary Medicine