



# Phase I Gene Therapy Trial for LGMD2D: Vascular Delivery of AAVrh.74.hSGCA to Lower limbs (RAC#1301-1200)

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# Presentation Objectives

- Describe Clinical Disease and need for treatment
- Review results of prior IM AAV.hSGCA Clinical Gene Therapy Trial
- Summarize the Clinical Protocol for vascular delivery
- Discuss the delivery of AAV.hSGCA using an isolated limb recirculation approach
- Address Questions raised by RAC Reviewers

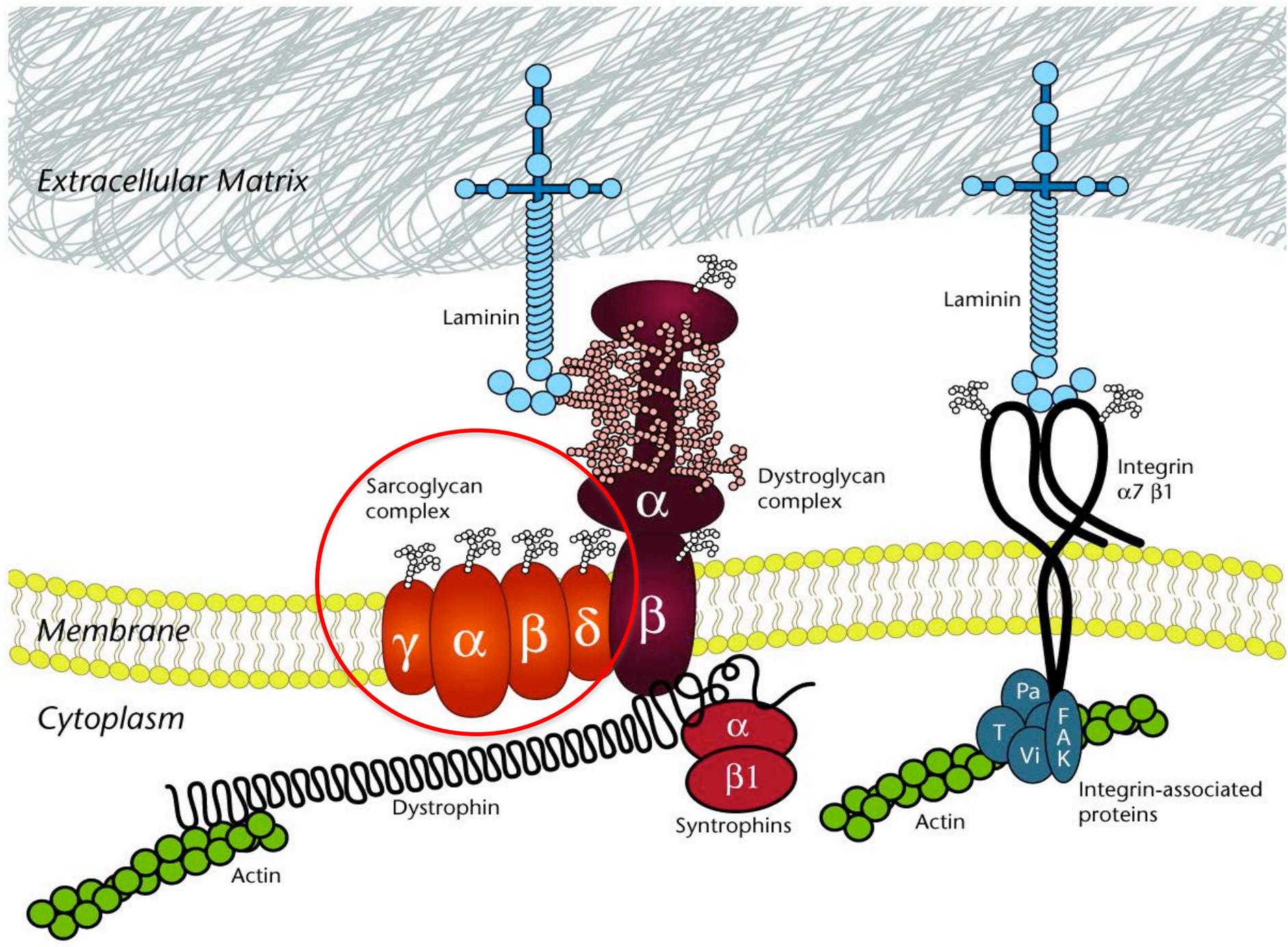
LGMD AR 2A-N

14 Disorders



LGMD AD 1A-G

7 Disorders



Extracellular Matrix

Laminin

Laminin

Sarcoglycan complex

Dystroglycan complex

Integrin  $\alpha 7 \beta 1$

Membrane

Cytoplasm

Dystrophin

Syntrophins

Integrin-associated proteins

Actin

Actin

$\gamma$

$\alpha$

$\beta$

$\delta$

$\alpha$

$\beta$

$\alpha$

$\beta 1$

Pa

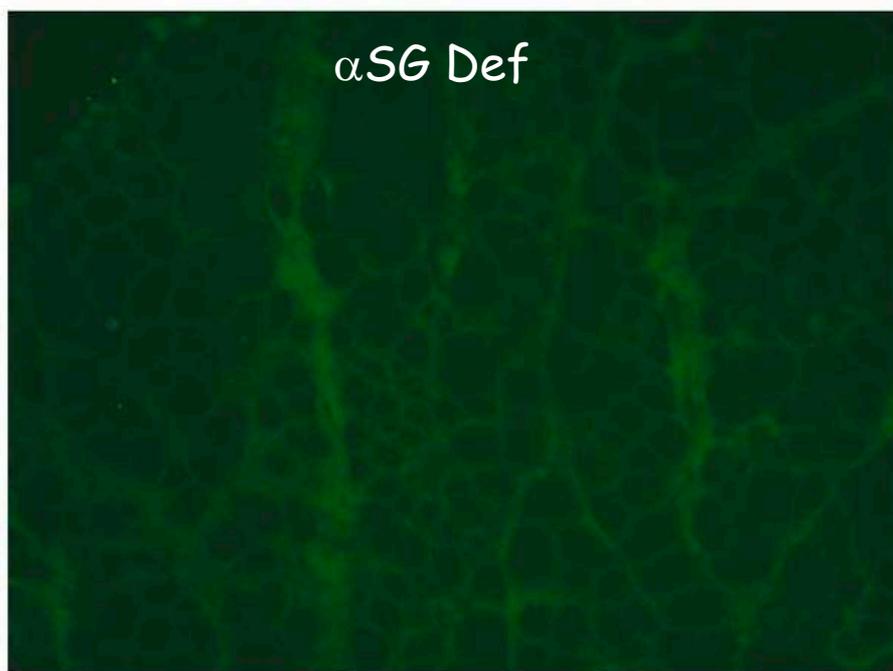
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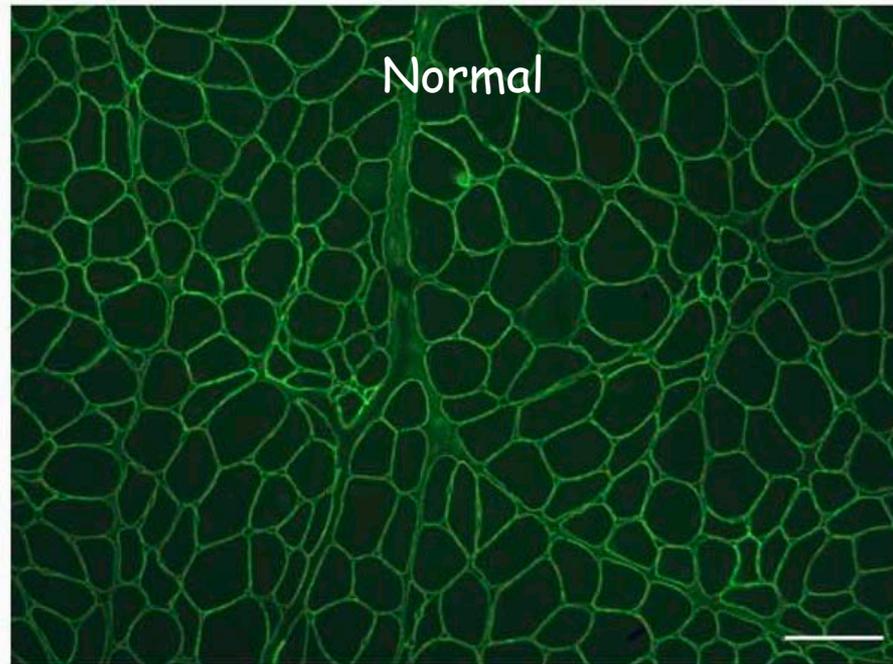
FAK

K

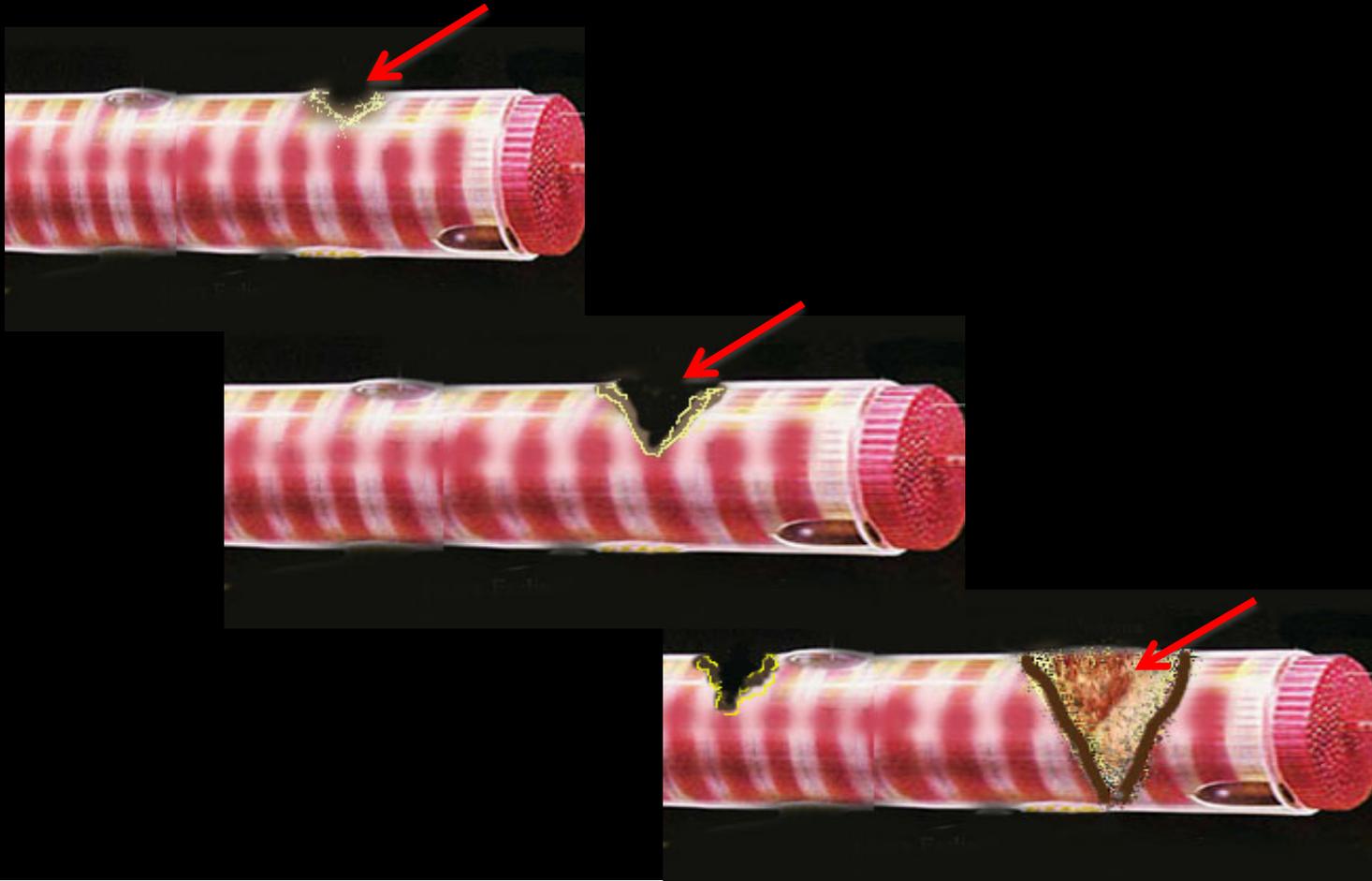
$\alpha$ SG Def



Normal



# Consequences of Absent Sarcoglycans



The process continues with loss of muscle fibers causing muscular dystrophy

# Sarcoglycanopathies

- Phenotypes simulate dystrophinopathies
  - Duchenne-like (SCARMD)
  - Mild, later onset (Becker-like)
- Age of onset 3 to 15 yo
- Present with difficulty running, jumping, climbing stairs about age 3-5
- Serum CK elevated 50-100 X normal
- Wheelchair-dependent by 15
- Develop scoliosis and respiratory insuff in Age 20-30
- SPARES CARDIAC and COGNITIVE FUNCTION\*\*

# No Effective Treatment

Connolly et al Muscle Nerve 1998

## Case History

- 8 yo LGMD2D patient
- Treatment with prednisone (2 mg/kg/d) for 2 months improved prox muscle strength
- At 6 mo pred tapered to 1 mg/kg/qod + azathioprine
- Strength maintained for 2 years and then declined

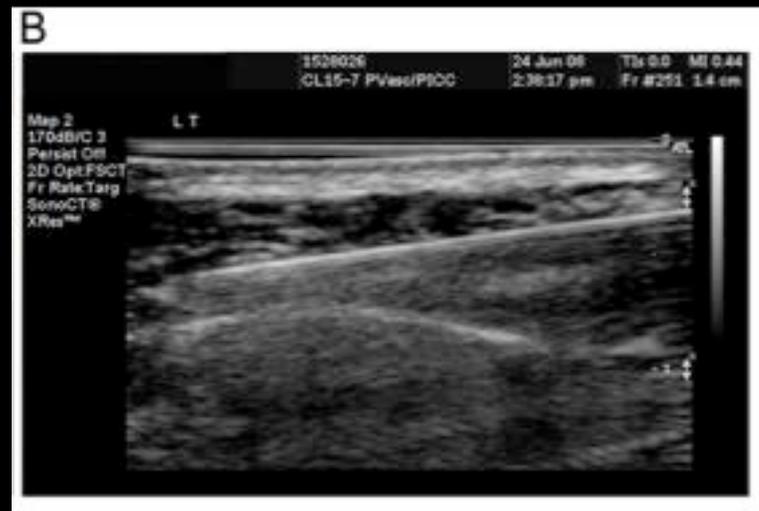
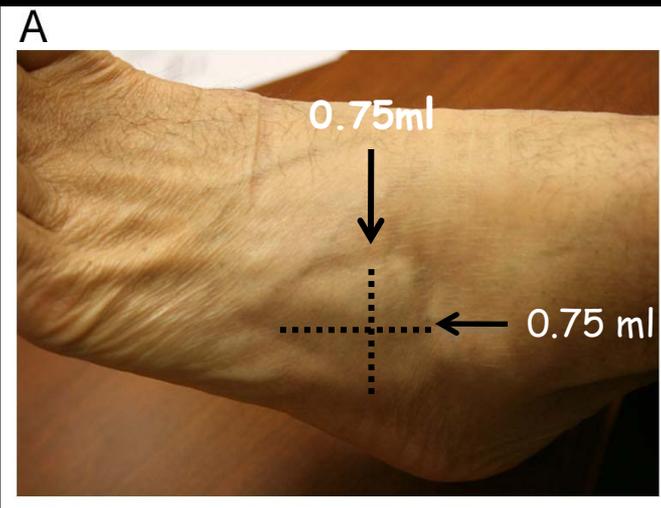
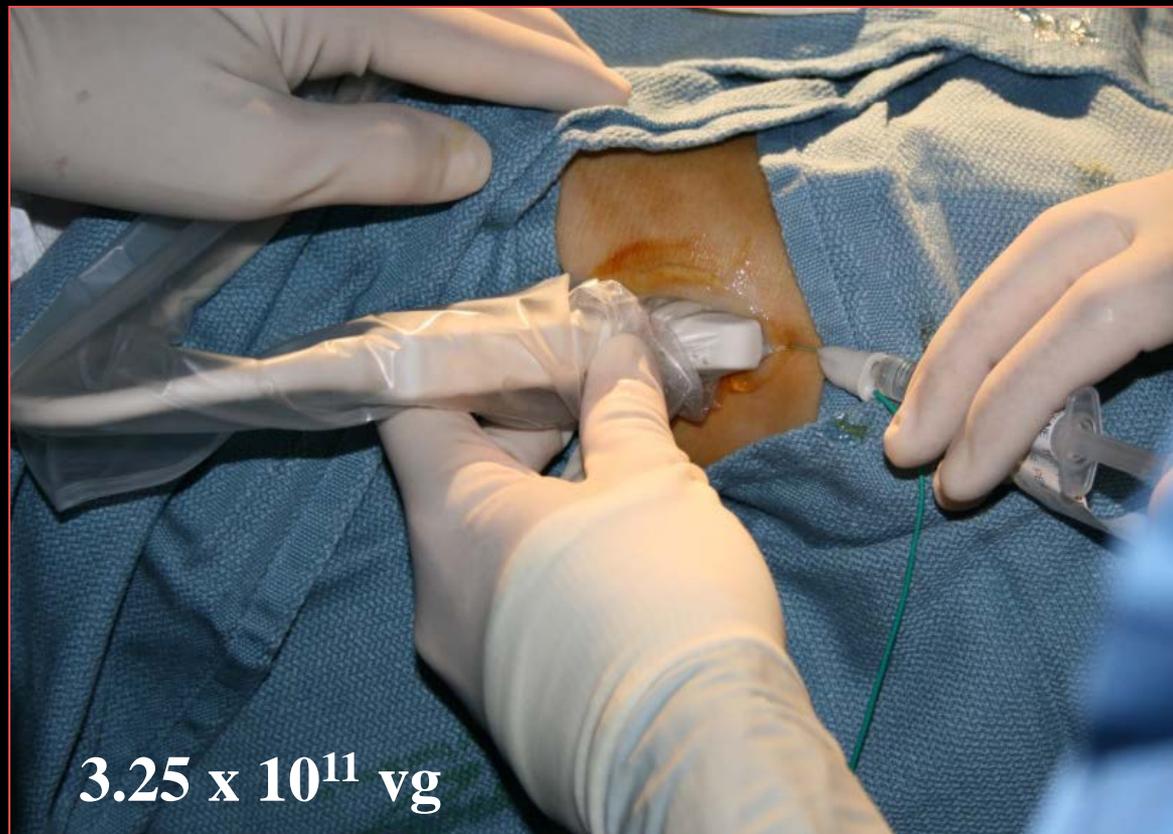
Gene Therapy for LGMD2D  
Introduced by Intramuscular  
Delivery in 2008

# Intramuscular Gene Therapy Trial

- Double-blind, placebo controlled, IM gene transfer to extensor digitorum brevis (EDB) muscle
- AAV1.tMCK.hSGCA vs saline (placebo)
  - Extremities were randomized for treatment
- Code was not broken until all data reported

# Cassette used for Gene Transfer





$3.25 \times 10^{11}$  vg

$3.25 \times 10^{11}$  vg

TREATED

PLACEBO

TREATED

PLACEBO

6 wks

6 mo

3 mo

6 mo

6 wks

6 mo

Subject 1

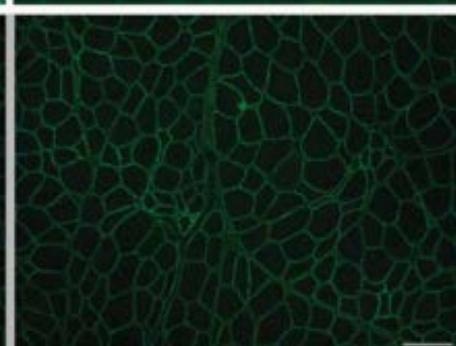
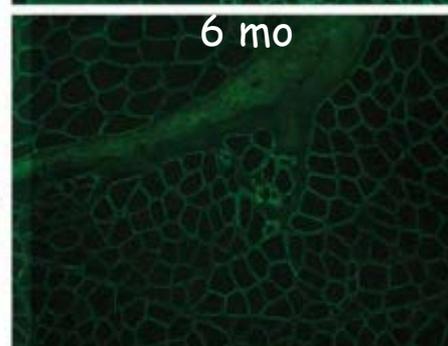
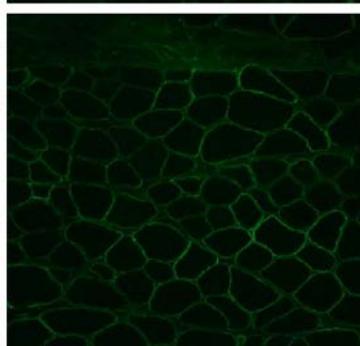
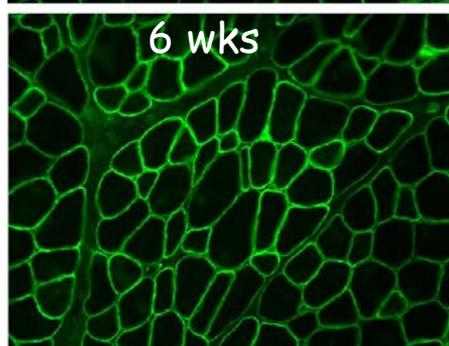
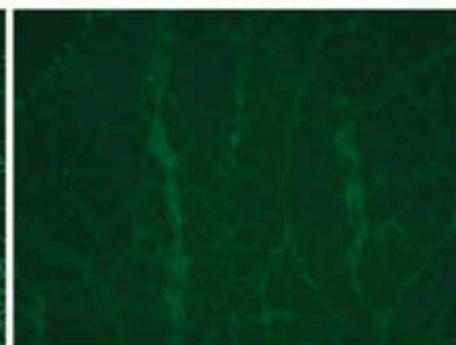
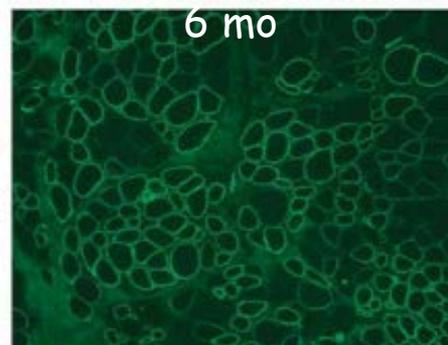
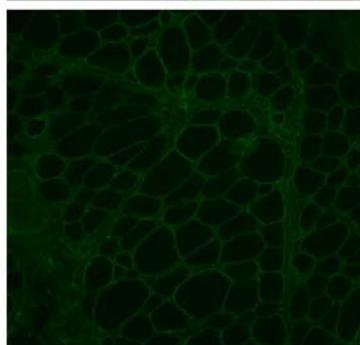
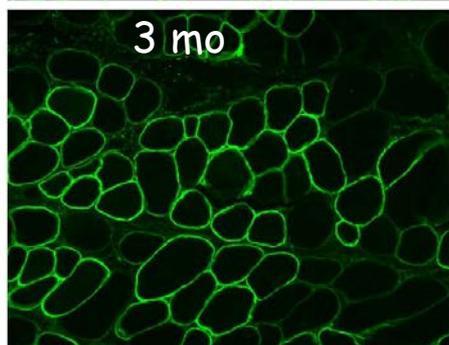
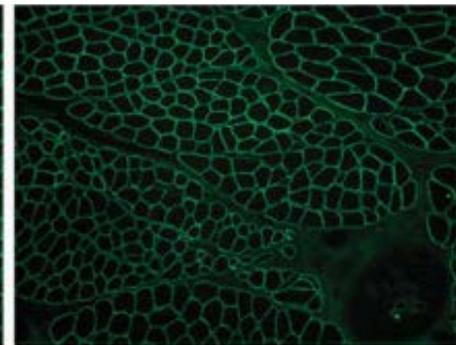
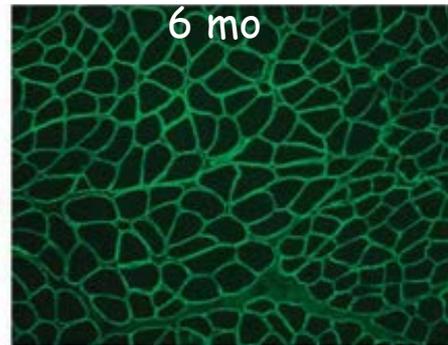
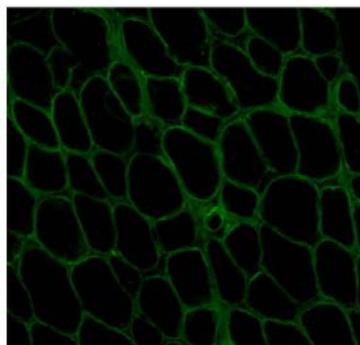
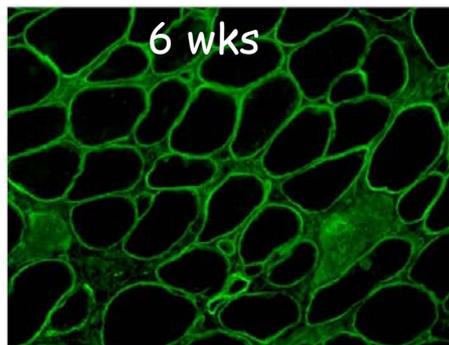
Subject 4

Subject 2

Subject 5

Subject 3

Subject 6

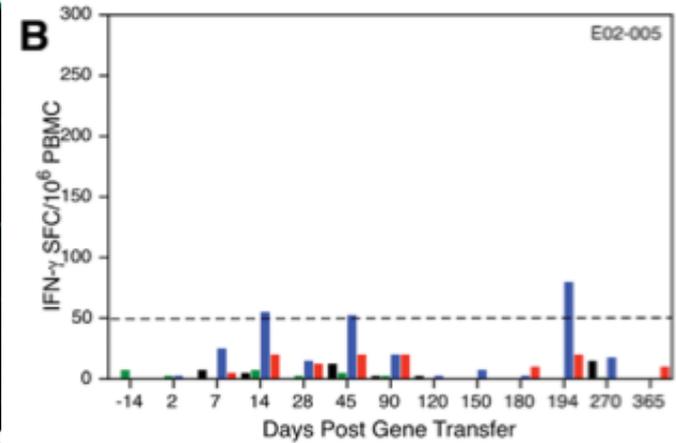
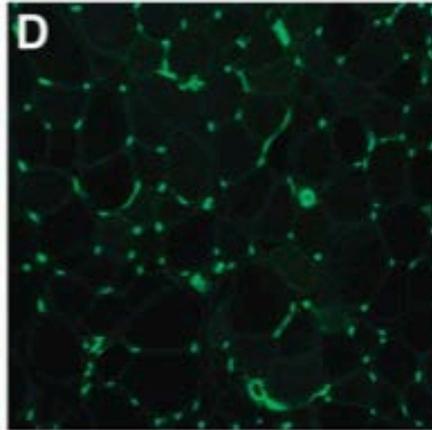
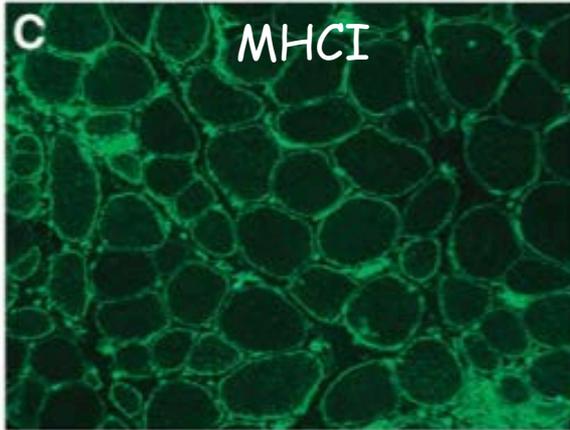


Treated

Placebo

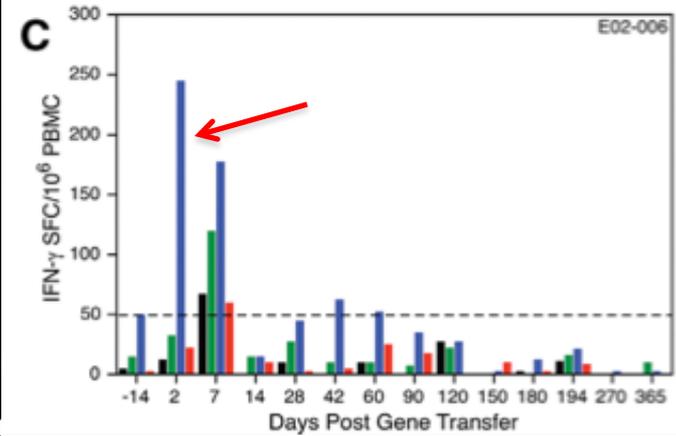
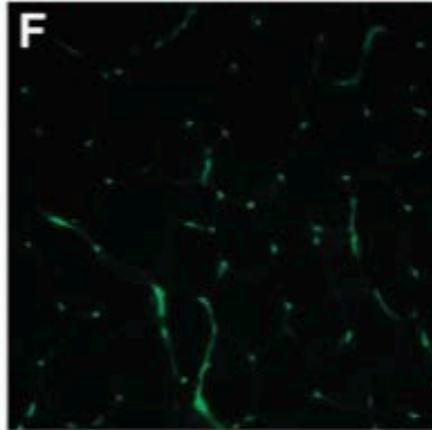
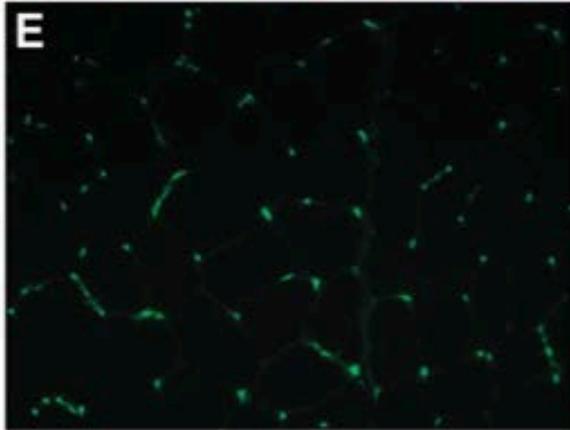
INF- $\gamma$  ELISpot

Subject 5



Subject 5

Subject 6



Subject 6

# Pre-existing Antibody Levels Influence Gene Expression

**TABLE 2: Enzyme-Linked Immunoassay for Neutralizing Antibody Titers against Adeno-Associated Virus 1 before and after Gene Transfer**

Time after Injection	Patient 4 (E02-004)	Patient 5 (E02-005)	Patient 6 (E02-006)
Pretreatment	1:800	<1:50	1:1,600
Day 7	1:1,600	1:3,200	>1:102,400 
2 weeks	1:25,600	1:6,400	>1:102,400
6 weeks	1:25,600	1:3,200	>1:102,400
12 weeks	1:51,200	1:12,800	>1:102,400
26 weeks (6 months)	>1:102,400	1:12,800	>1:102,400

The results for Subject 6 are 1 dilution higher before treatment compared to Subject 4. However, by day 7 the neutralizing antibody titers rose >30-fold compared to other subjects.

# Summary of LGMD IM Trial

- Intramuscular gene transfer with sustained gene expression demonstrated for 6 weeks to 6 months
- No Adverse effects
- First use of muscle specific promoter in clinical trial (tMCK)

Taking this to clinical trial  
by vascular delivery

# Clinical Protocol For Vascular Delivery

- Six LGMD2D patients ages 7 and older
- Confirmed SGCA mutations
- Clinically established muscle weakness
- Ability to ambulate and cooperate for muscle testing
- AAVrh.74 antibody titers <1:1600 by ELISA
- Protocol for Vascular Delivery (Louis Chicoine) of rAAVrh.74.tMCK.SGCA

# Protocol for Vascular Delivery

- Developed by:
  - Louis Chicoine, MD
    - Neonatologist, Director of Neonatal-ECMO Services
  - Thomas Preston, CCT, CCP, FPP
    - Chief, Mechanical Cardiopulmonary Assist Programs
- We have been deeply involved with ECMO and perfusion over the past 10 years.

# Protocol for Vascular Delivery

## Background

- Isolated limb perfusion and isolated limb infusion (ILP/ILI) protocols have been in clinical use since '50s and applied to thousands of patients
- We combined aspects of ILP and ILI in developing our protocol for isolated limb recirculation for gene therapy (ILR-GT) with the emphasis placed on patient safety

# (ILR-GT) Isolated Limb Recirculation for Gene Therapy:

- Current vascular gene transfer protocols provide a single passage of vector through the targeted tissue
- Vascular beds not available at the time of vector administration do not see vector and are not treated

# (ILR-GT) Isolated Limb Recirculation for Gene Therapy:

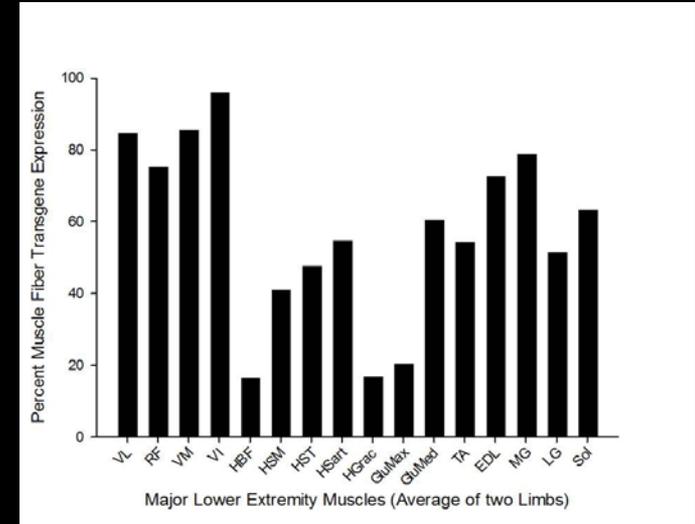
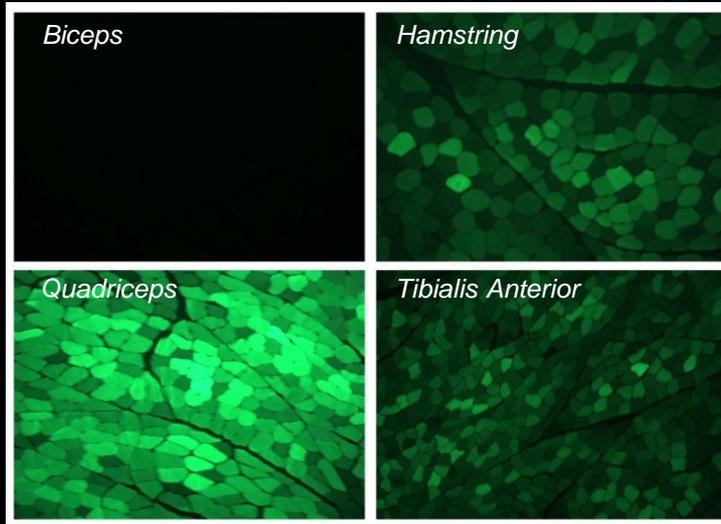
- ILR-GT allows multiple passes of vector during a single treatment by re-circulation
- It provides:
  - Hypoxic and acidemic-induced vasodilatation allowing maximal exposure to vascular bed
  - Minimizes systemic vector exposure by allowing removal of unbound re-circulated vector following the procedure

# Preclinical ILR-GT Studies:

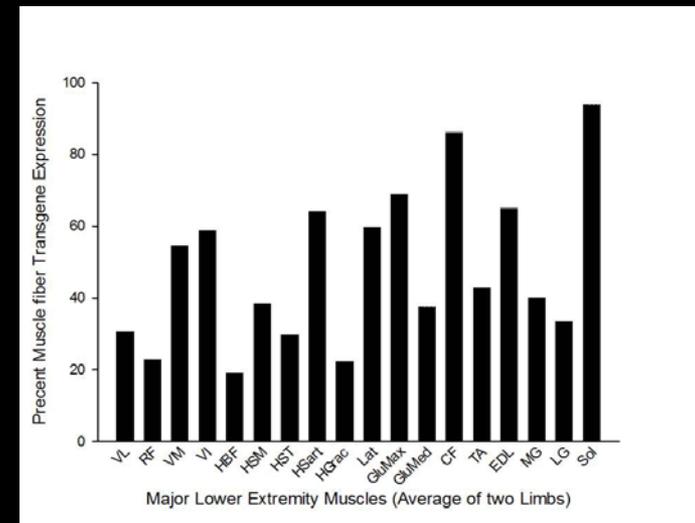
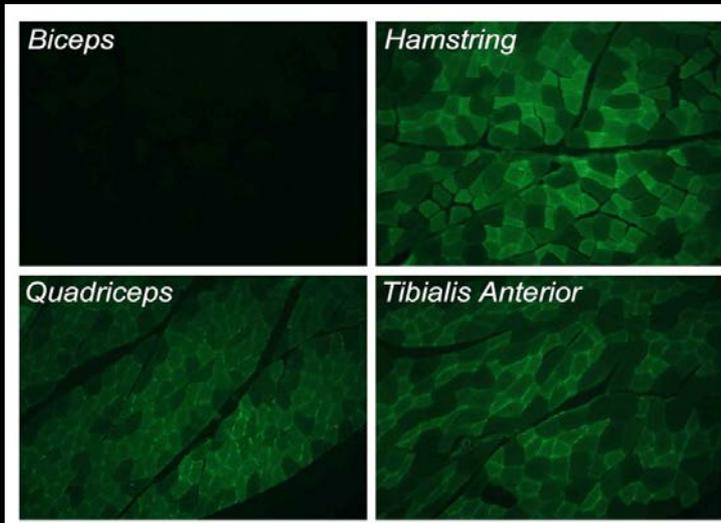
- We have performed ILR-GT on NHPs and have not experienced any untoward effect
- We have seen broad distribution of the transgene throughout the lower extremities of these study animals

# Preclinical ILR-GT Studies: eGFP

AAVrh.74.eGFP  
6e12 vg/kg/limb

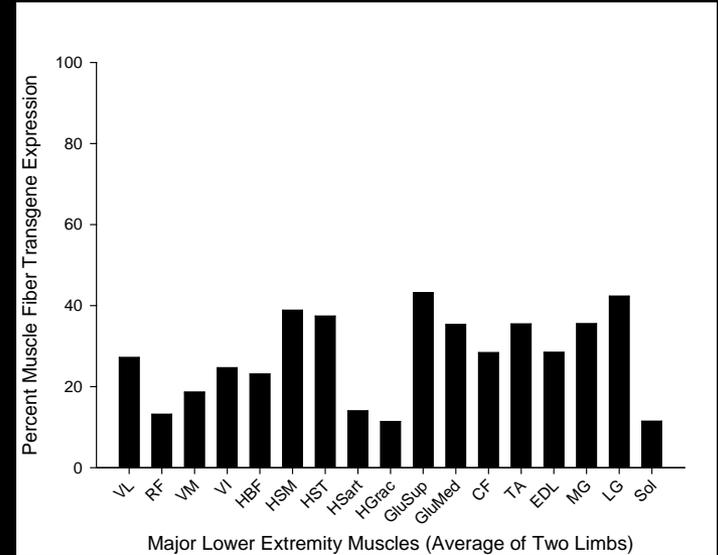
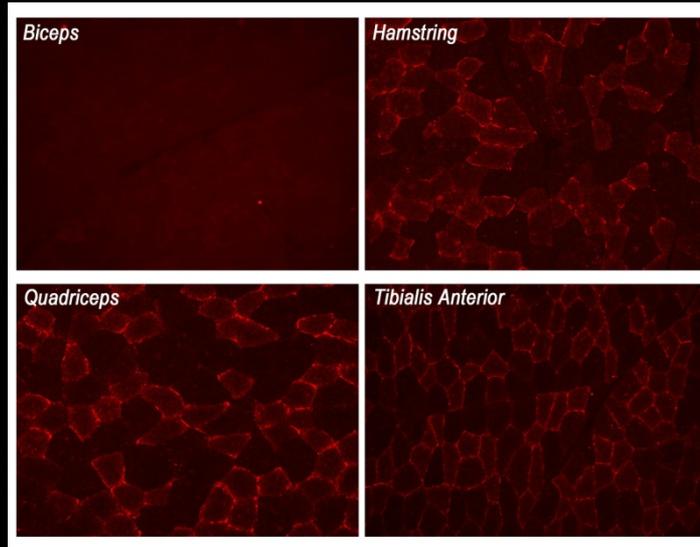


AAVrh.74.eGFP  
2e12 vg/kg/limb

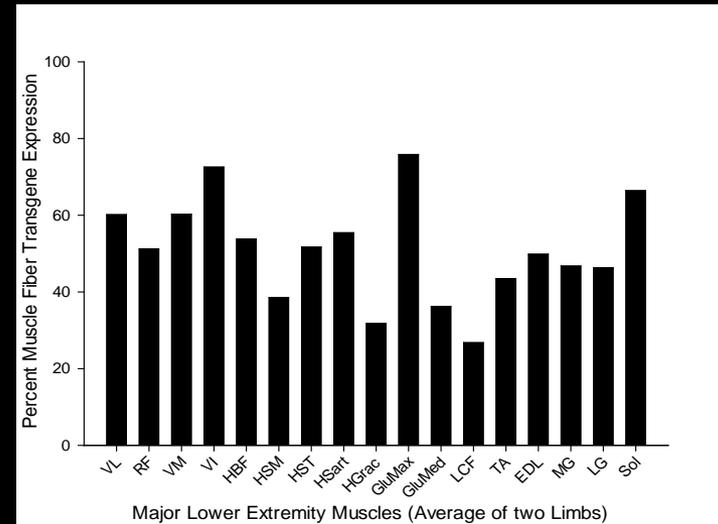
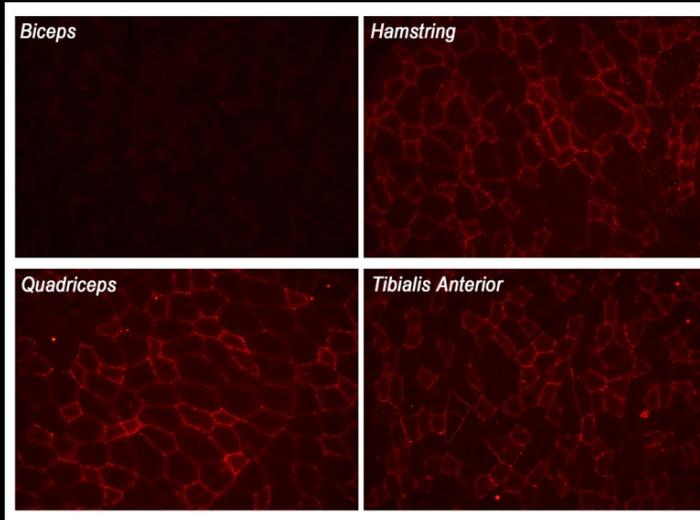


# Preclinical ILR-GT Studies:

AAVrh.74.SGCA  
6e12 vg/kg/limb



AAVrh.74microdys  
6e12 vg/kg/limb

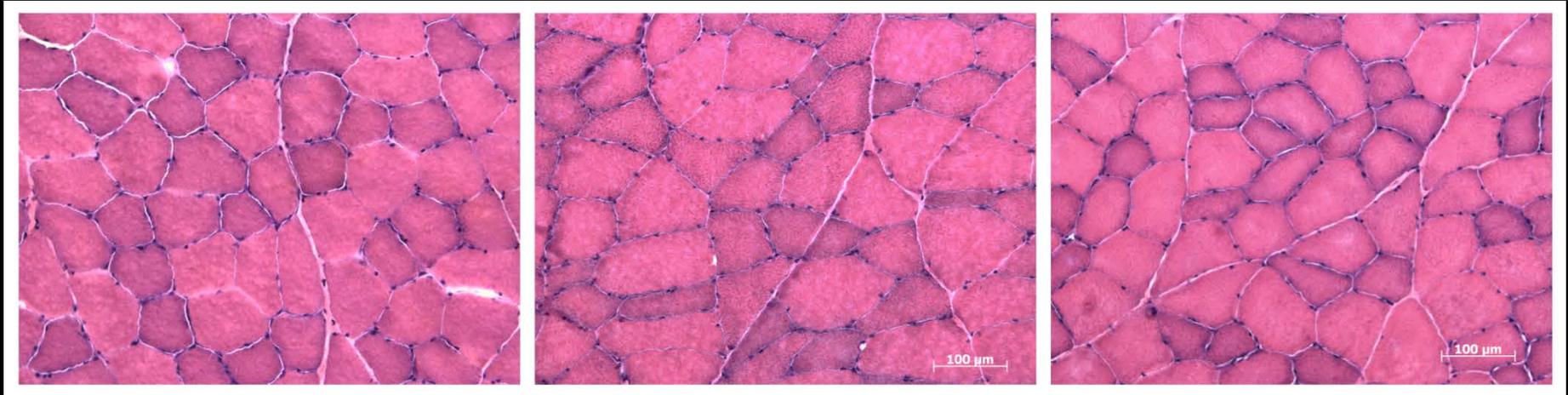


# Preclinical ILR-GT Studies

Untreated  
Biceps

Treated  
Quad

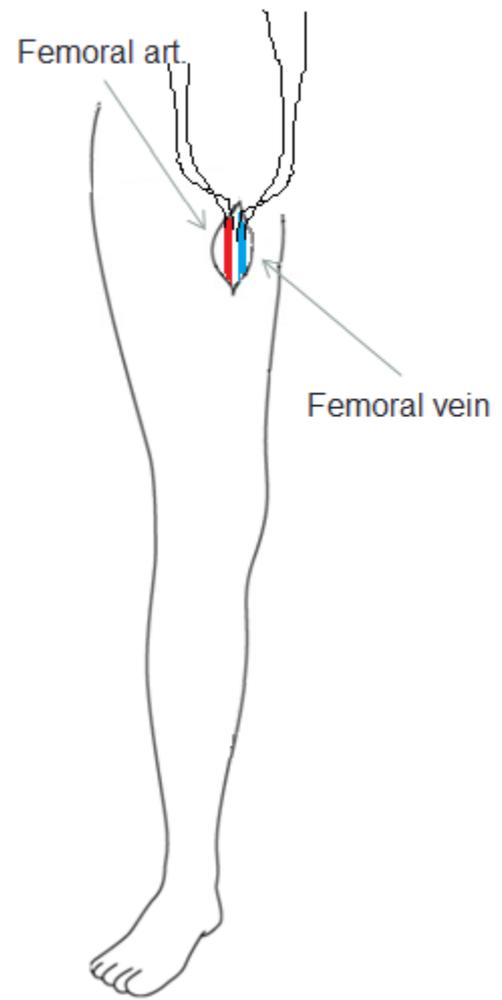
Treated  
Gastroc



No Muscle Fiber damage following gene delivery

# Clinical Protocol:

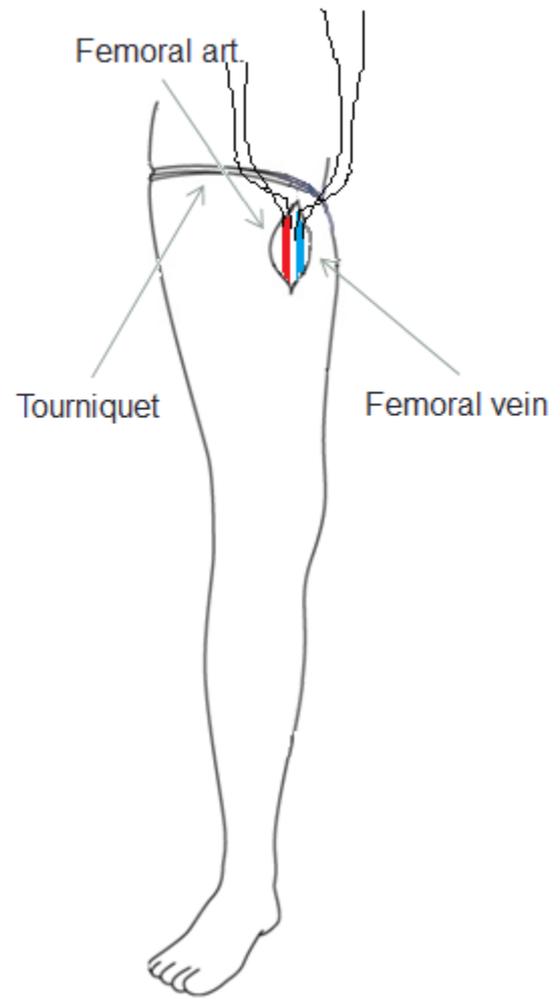
- Vessels snared and occluded



# Clinical ILR-GT Protocol:

## Clinical Protocol:

- Tourniquet is placed loosely in proximal position

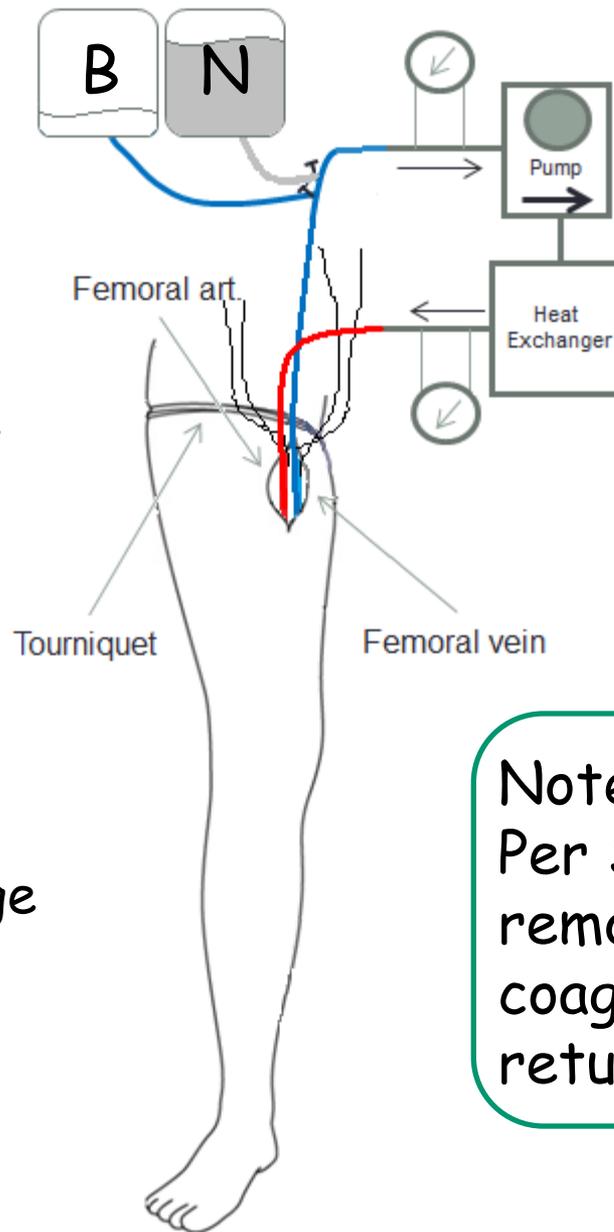


## Clinical ILR-GT Protocol:

## Clinical Protocol:

- Snared fem. art and vein are cannulated and connected
- Circuit comprised of pump, heater
- Tighten Tourniquet

- (B) Bag for blood collection and storage
- (N) Bag of normosol



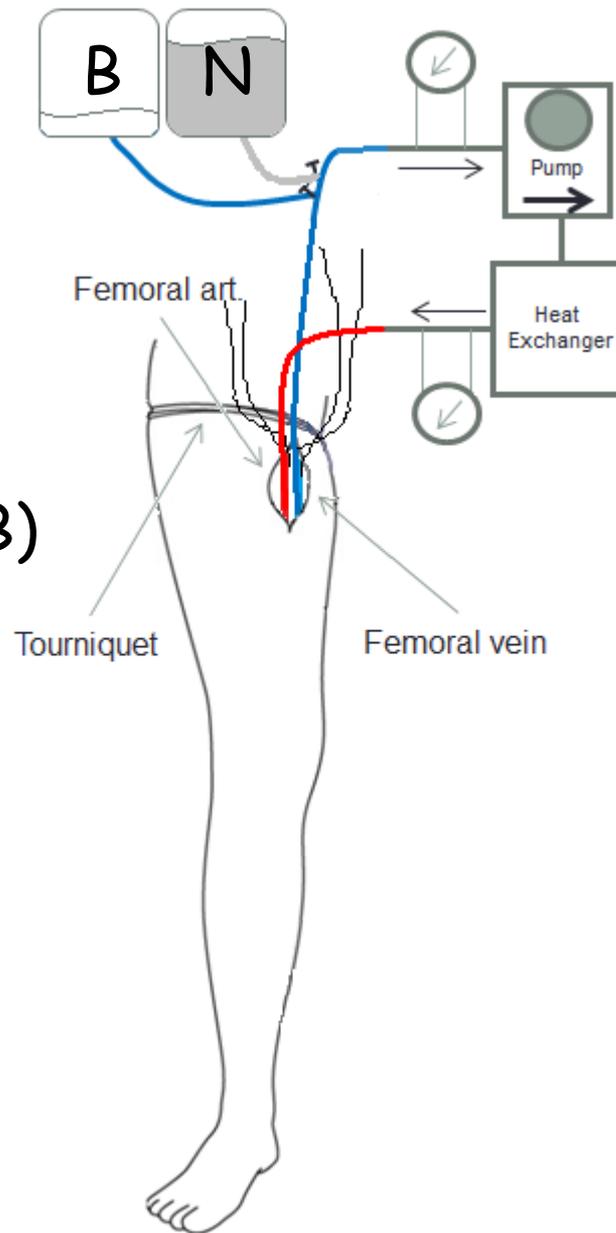
### Notes:

Per SOP NCH: Blood removed and anti-coagulated (ACD) may be returned up to 8 hrs.

## Clinical ILR-GT Protocol:

## Clinical Protocol:

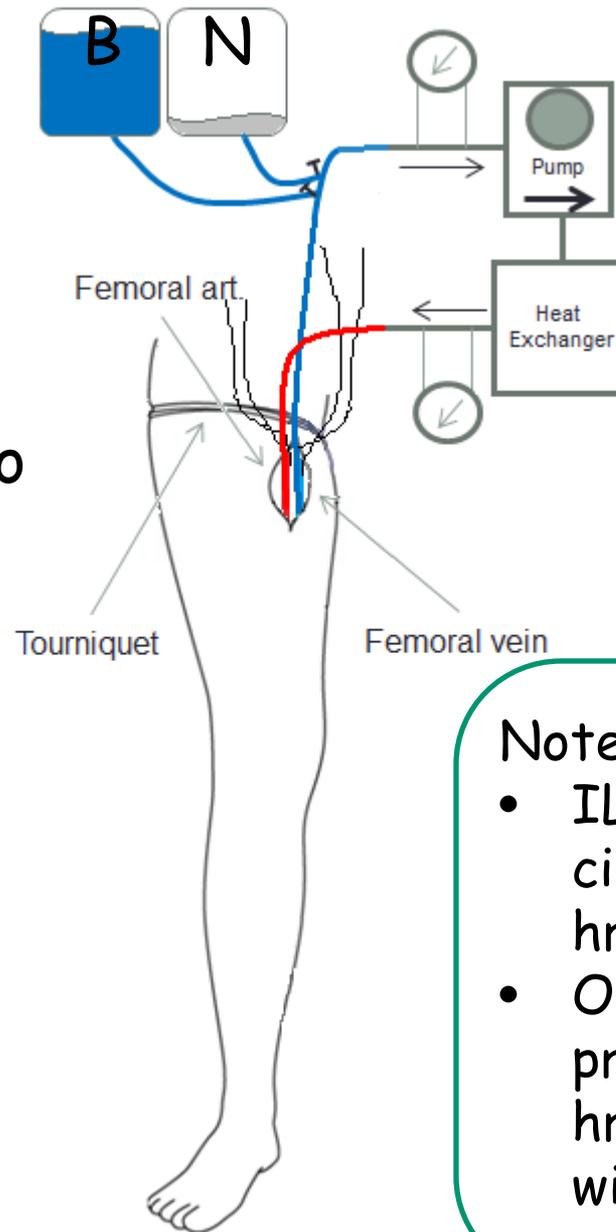
- Assess stasis with Fluoroscopy
- Start normosol infusion (N) while collecting blood in (B)



## Clinical ILR-GT Protocol:

## Clinical Protocol:

- Stop normosol infusion when Hgb/Hct (<6/<18)
- Administer vector to circuit and begin 30 minute recirculation



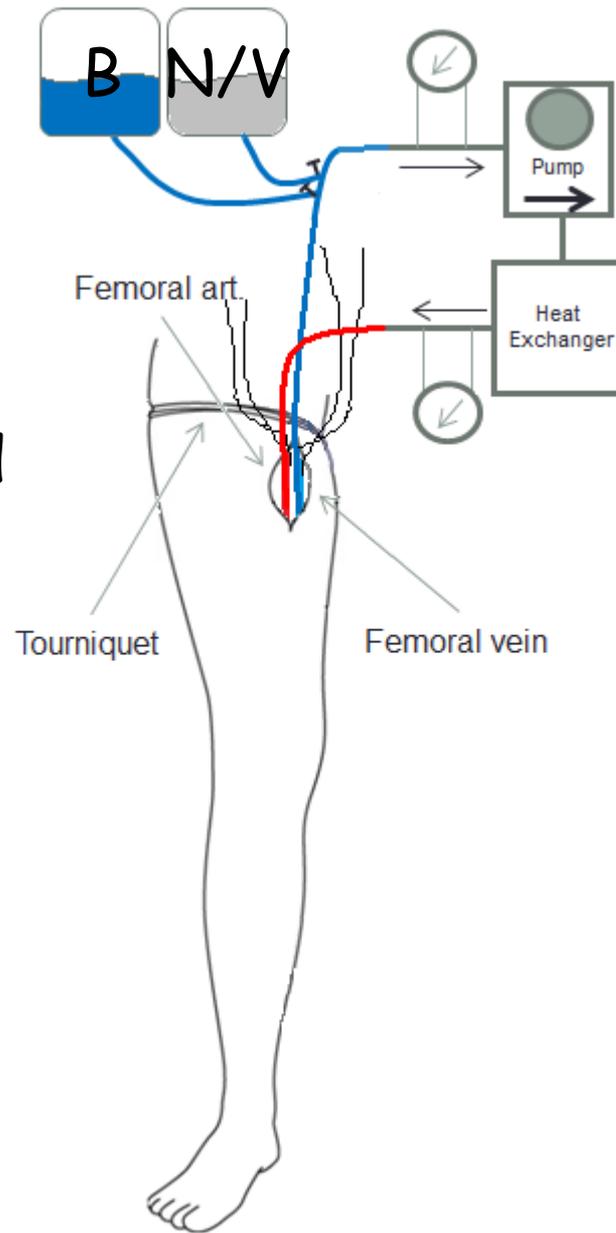
### Notes:

- ILP/ILI protocols are circulated for up to 1.5 hrs.
- Orthopedic limb protocols require up to 2 hrs of tourniquet time without complications

## Clinical ILR-GT Protocol:

## Clinical Protocol:

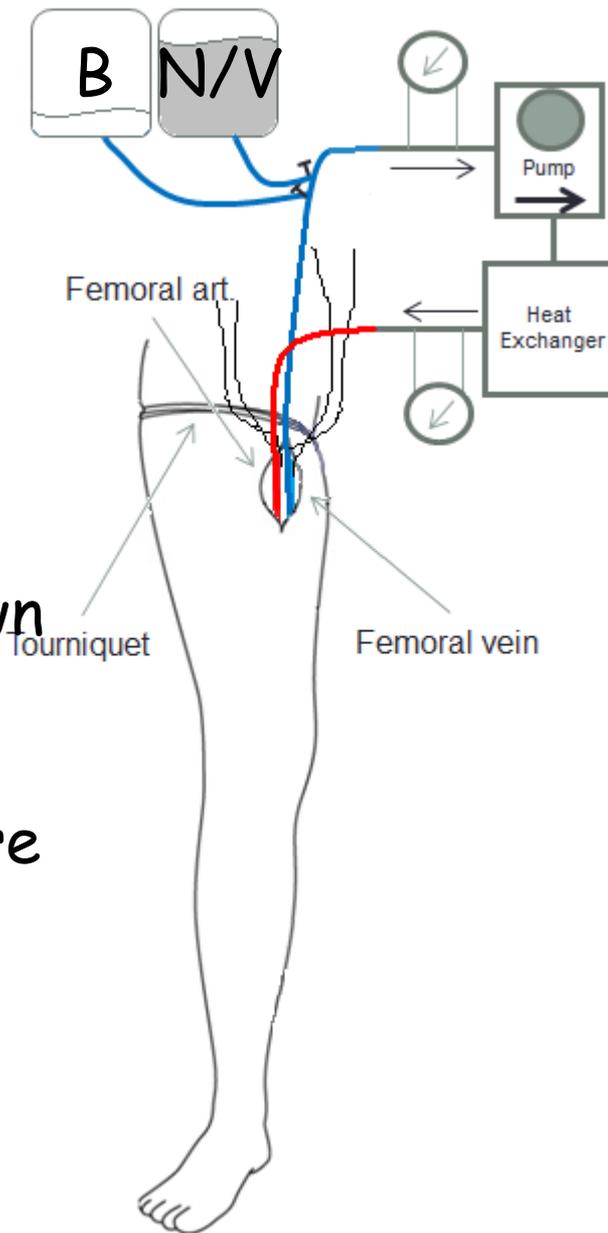
- Recirculation complete
- Return blood (B), collect normosol and vector (N/V)



## Clinical ILR-GT Protocol:

## Clinical Protocol:

- Once blood (B) returned
- Stop pump
- Remove cannulae, snares, and take down tourniquets
- Apply direct pressure to control bleeding



## Clinical ILR-GT Protocol:

# Clinical Protocol

- Dose escalation study
  - Cohort 1 (Low Dose)  $1.0 \times 10^{12}$  vg/kg per leg (n=3)
  - Cohort 2 (High Dose)  $3.0 \times 10^{12}$  vg/kg per leg (n=3)
- Outcome measures:
  - Distance walked on 6MWT
  - Force generated by Knee extension/knee flexion
- Efficacy: Significant improvement pre vs post treatment in distance on 6MWT and comparing high and low dose cohorts using ANOVA ( $p \leq 0.05$ )

## Participants/Research Team

- K. Reed Clark, PhD
  - Virologist/Director of Vector Manufacturing Laboratory NCH
- Louise Rodino-Klapac, PhD
  - Research Scientist
- Mark Hogan, MD
  - Radiologist, ultrasonographer
- Louis Chicoine, MD
  - Neonatology/Director Neonatal ECMO
- Thomas Preston, CCT, CCP, FPP
  - Chief, Mechanical Cardiopulmonary Assist Programs

# Safety Issues Addressed

- Cannulation
  - Surgeon
  - OR/IR facilities
  - Anesthesiology with protocols
- Indwelling catheters/circuit
  - Heparin anticoagulation, monitoring ACT
  - Recirculation with transducer regulated CV pump
  - Hypoxia/Acidosis duration limited to 30 minutes
  - Closed system
  - Fluoro for isolation/stasis
- Biodistribution
  - Final flush to remove unbound vector