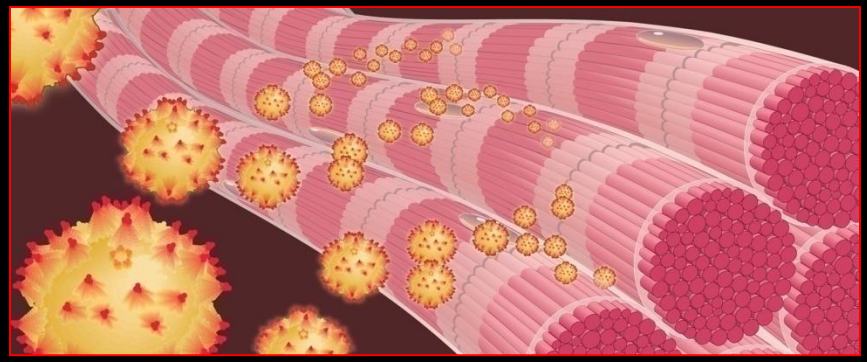


Gene Therapy for Muscular Dystrophy

A Decade of Research and Challenges

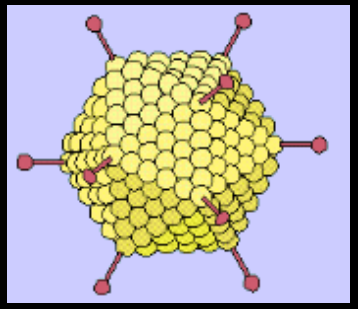
Jerry R. Mendell, M.D.,
Research Institute at Nationwide Children's
Professor of Pediatrics and Neurology

Objectives

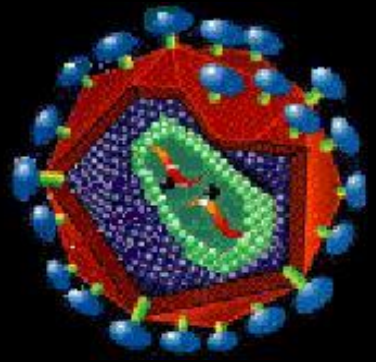
- *Describe current status and future challenges for gene therapy for muscular dystrophy*
  - *Discuss the following points considering the <u>clinical implications</u> of each:*
    1. Choice of virus for gene delivery
    2. Methods of Gene Delivery
    3. The challenges posed by large size of the Dysferlin Gene
    4. Choices of potential genes to deliver

Choice of Virus

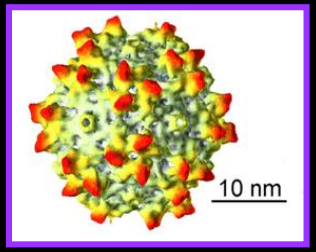
- Adenovirus



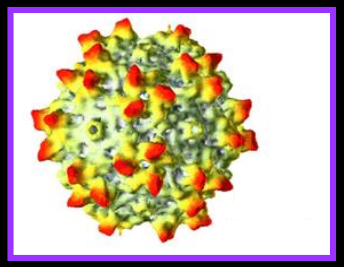
- Lentivirus



- Adeno-Associated virus (AAV)



Choice of Virus for Gene Replacement



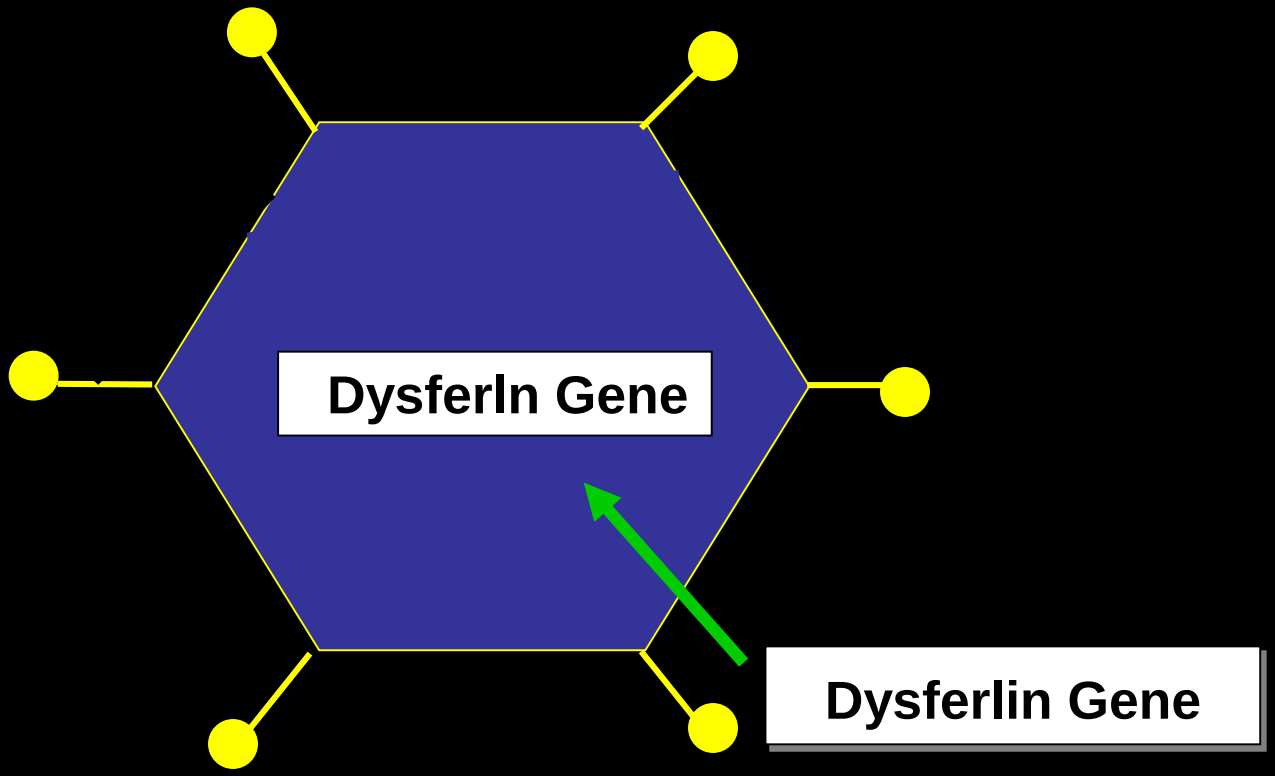
Adeno-associated virus

Small Capacity 4.7 kb

Dysferlin needs 6.2 kb

- No Human disease
  - Reduced immune response
  - Reduced toxicity
  - Delivered to nucleus & no integration to genome
- Limitations
  - Small insert capacity

Preparing AAV as Gene Delivery Vehicle



Methods of Gene Delivery

- Intramuscular
  - Gene delivered directly to muscle
    This method tests the validity of the gene and is the first safety test
  - May be required by the FDA if first time in clinical trial

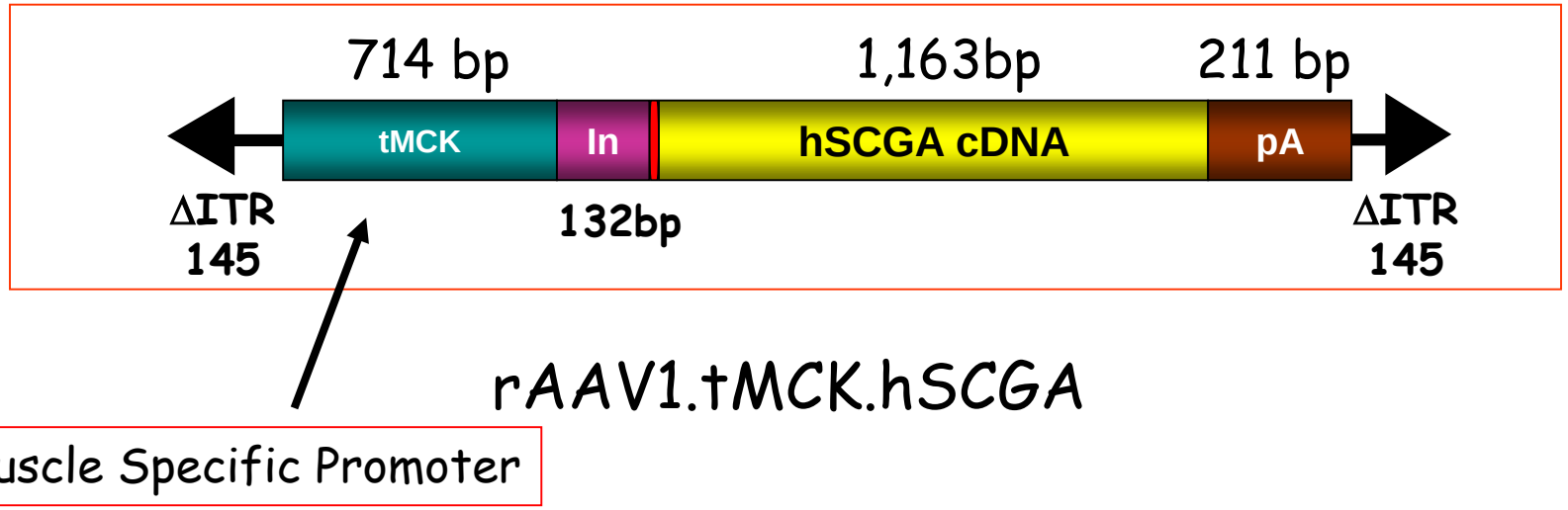
First Gene Therapy Clinical Trial for LGMD type 2D

April 23, 2008

LGMD Classification

DISEASE	LINKAGE	GENE	GENE PRODUCT
LGMD1A	5q22.3-31.3	MYOT	Myotilin
LGMD1B	1q21.2	LMNA	Lamin A/C
LGMD1C	3p25	CAV3	Caveolin-3
LGMD1D	6q23	X	Unknown
LGMD1E	7q	X	Unknown
LGMD1F	7q32.1	X	Unknown
LGMD2A	15q15.1-21.1	CAPN3	Calpain-3
LGMD2B	2p13	DYSF	Dysferlin
LGMD2C	13q12	SGCG	γ-sarcoglycan
LGMD2D	17q12-21.33	SGCA	α-sarcoglycan
LGMD2E	4q12	SGCB	β-sarcoglycan
LGMD2F	5q33-34	SGCD	δ-sarcoglycan
LGMD2G	17q11-12	TCAP	Telethonin
LGMD2H	9q31-33	TRIM32	E3-ubiquitin-ligase
LGMD2I	19q13	FKRP	Fukutin related protein
LGMD2J	2q31	TTN	Titin
LGMD2K	9q34	POMT1	Protein O-Mannosyltransferase 1
LGMD2L	11p13	ANO5	Anoctamin
LGMD2M	9q31	FCMD	Fukutin
LGMD2N	19q13	POMT2	Protein O-Mannosyltransferase 2

LGMD2D Gene Therapy Trial (n = 6)
Alpha-Sarcoglycan Deficiency



Nationwide Children's
When your child needs a hospital, everything matters.™

Sustained Alpha-Sarcoglycan Gene Expression after Gene Transfer in Limb-Girdle Muscular Dystrophy, Type 2D

Jerry R. Mendell, MD,[1,2,3] Louise R. Rodino-Klapac, MD,[1,3] Xiomara Q. Rosales, MD,[3] Brian D. Coley, MD,[4] Gloria Galloway, MD,[1,2,3] Sarah Lewis, MD,[3] Vinod Malik, MD,[3] Chris Shilling, MD,[3] Barry J. Byrne, MD,[5,6] Thomas Conlon, MD,[5,6] Katherine J. Campbell, MD,[7] William G. Bremer, MD,[7] Laura E. Taylor, MD,[3] Kevin M. Flanigan, MD,[1,3] Julie M. Gastier-Foster, PhD,[8] Caroline Astbury, PhD,[8] Janaiah Kota, MD,[3] Zarife Sahenk, MD,[1,2,3] Christopher M. Walker, MD,[1,7] and K. Reed Clark, MD[1,3]

Objective: The aim of this study was to attain long-lasting alpha-sarcoglycan gene expression in limb-girdle muscular dystrophy, type 2D (LGMD2D) subjects mediated by adeno-associated virus (AAV) gene transfer under control of a muscle specific promoter (tMCK).

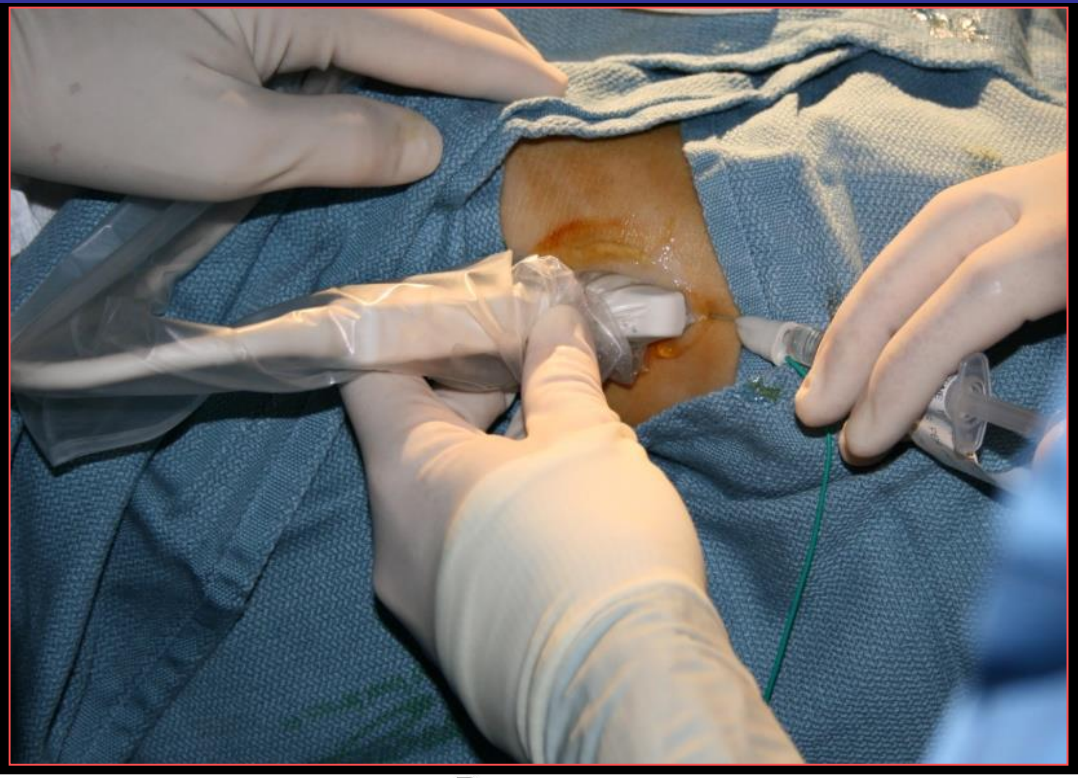
Methods: rAAV1.tMCK.hSGCA (3.25×10^{11} vector genomes) was delivered to the extensor digitorum brevis muscle of 3 subjects with documented *SGCA* mutations via a double-blind, randomized, placebo controlled trial. Control sides received saline. The blind was not broken until the study was completed at 6 months and all results were reported to the oversight committee.

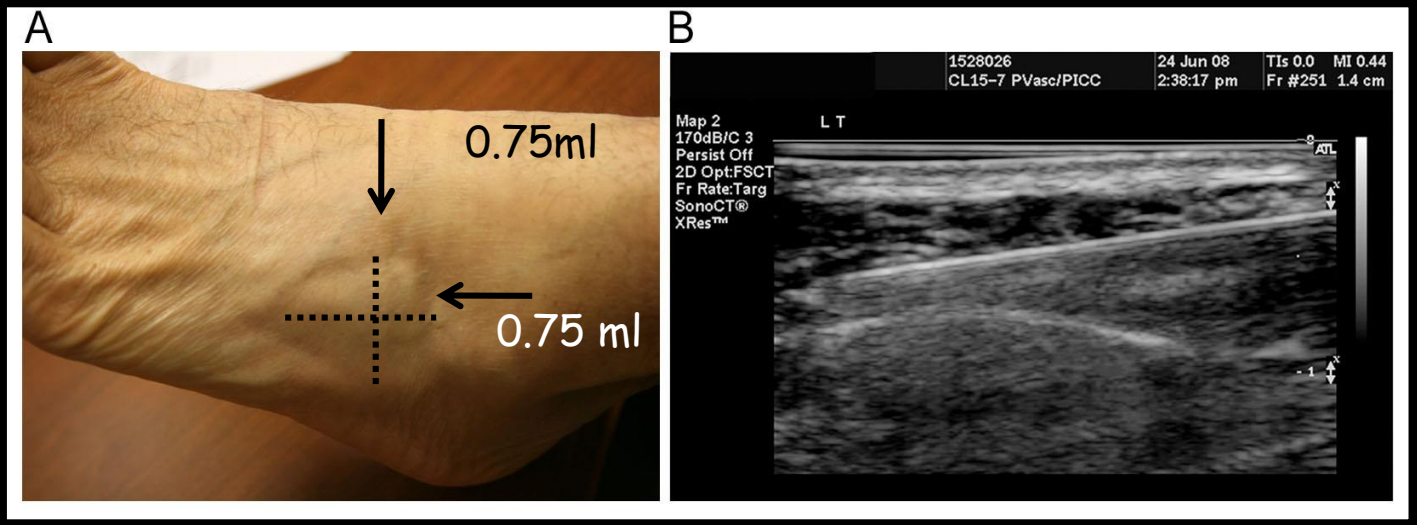
Results: Persistent alpha-sarcoglycan gene expression was achieved for 6 months in 2 of 3 LGMD2D subjects. Markers for muscle fiber transduction other than alpha-sarcoglycan included expression of major histocompatibility complex I, increase in muscle fiber size, and restoration of the full sarcoglycan complex. Mononuclear inflammatory cells recruited to the site of gene transfer appeared to undergo programmed cell death, demonstrated by terminal deoxynucleotide transferase–mediated deoxyuridine triphosphate nick-end labeling and caspase-3 staining. A patient failing gene transfer demonstrated an early rise in neutralizing antibody titers and T-cell immunity to AAV, validated by enzyme-linked immunospot on the second day after gene injection. This was in clear distinction to other participants with satisfactory gene expression.

Interpretation: The findings of this gene replacement study in LGMD2D subjects have important implications not previously demonstrated in muscular dystrophy. Long-term, sustainable gene expression of alpha-sarcoglycan was observed following gene transfer mediated by AAV. The merit of a muscle-specific tMCK promoter, not previously used in a clinical trial, was evident, and the potential for reversal of disease was displayed.

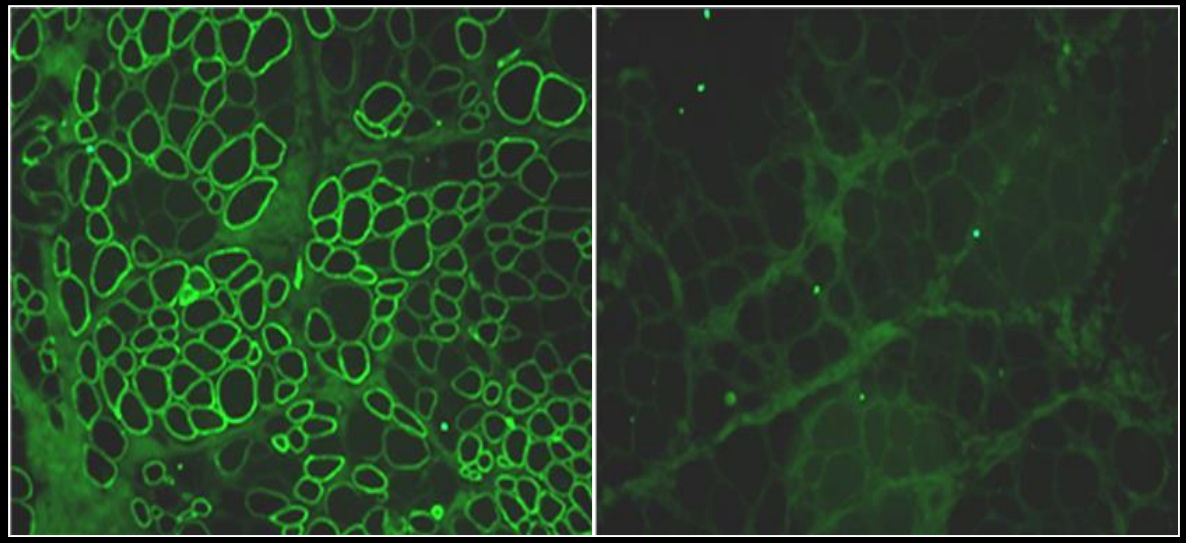
ANN NEUROL 2010;68:629–638

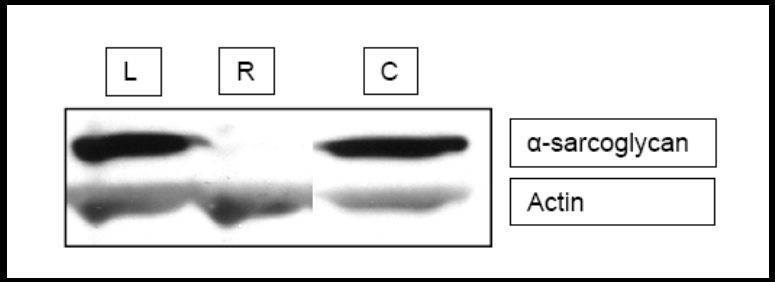
LGMD2D Alpha-Sarcoglycan Gene 06/08





Six LGMD2D Patients
Muscle biopsies at 3 and 6 months
Gene Expression levels reached near normal levels

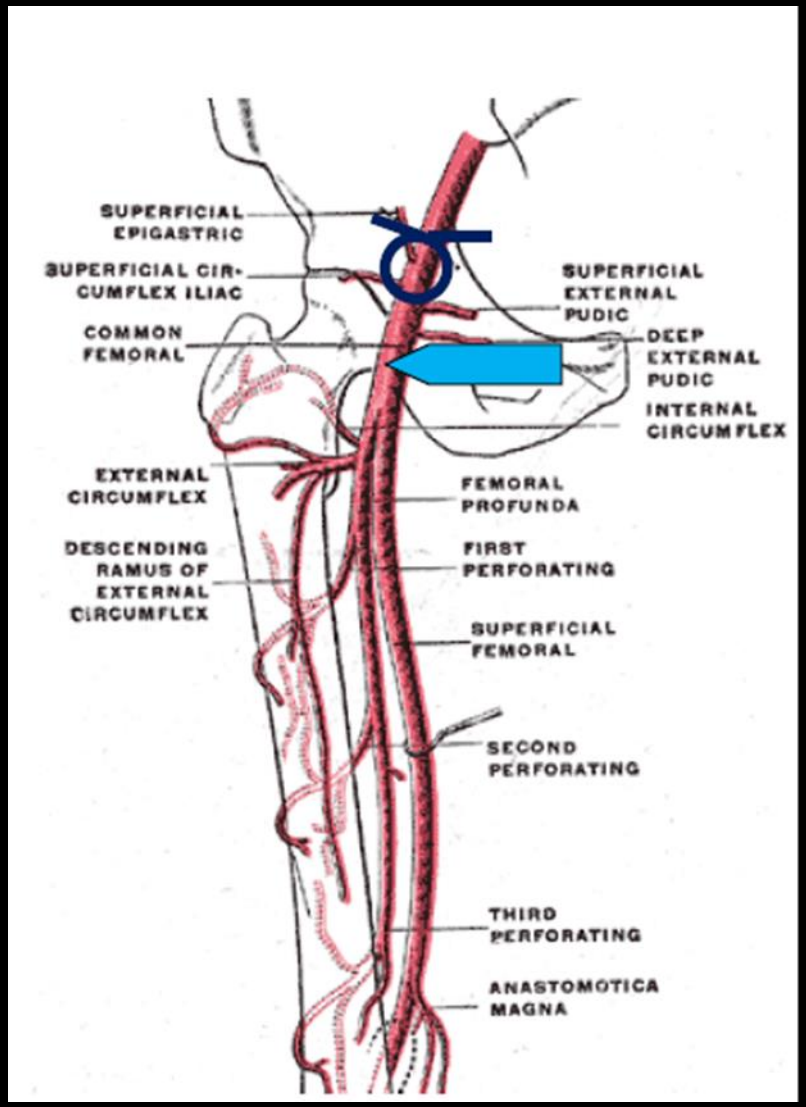


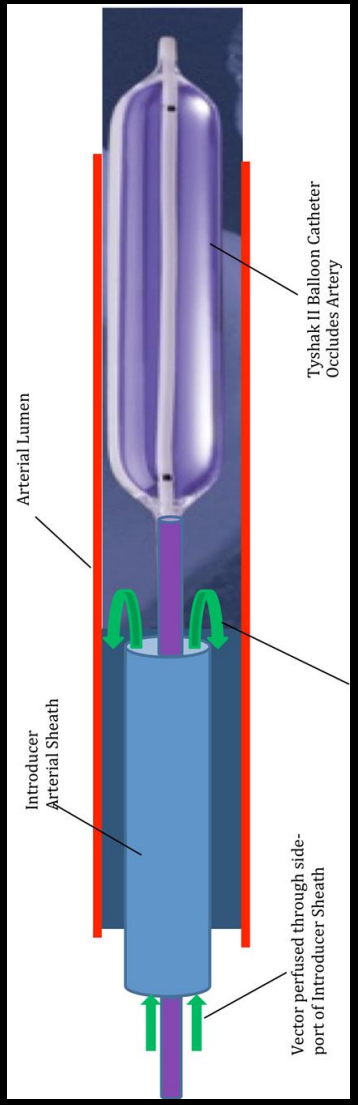


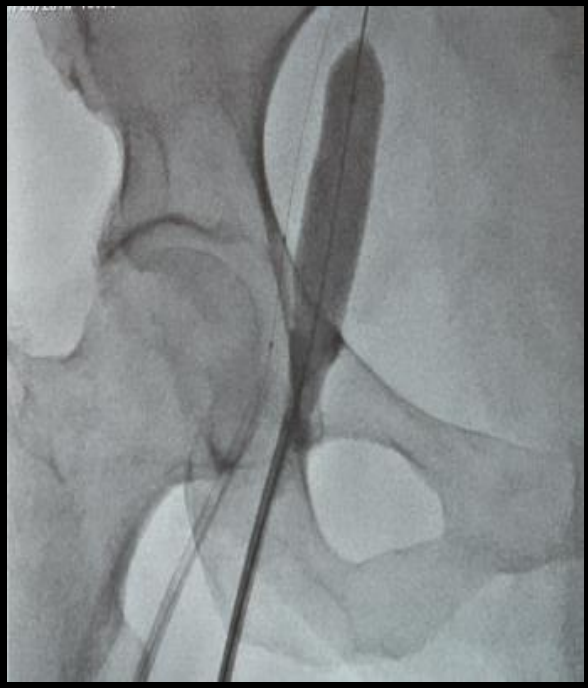
Methods of Gene Delivery

- Intravascular
  Gene delivered directly to circulation
  - Currently using a method called ISOLATED LIMB PERFUSION
  - Will deliver virus to all muscles of the extremity
  - Currently Using this approach for LGMD2D

Study Initiated March 23, 2015

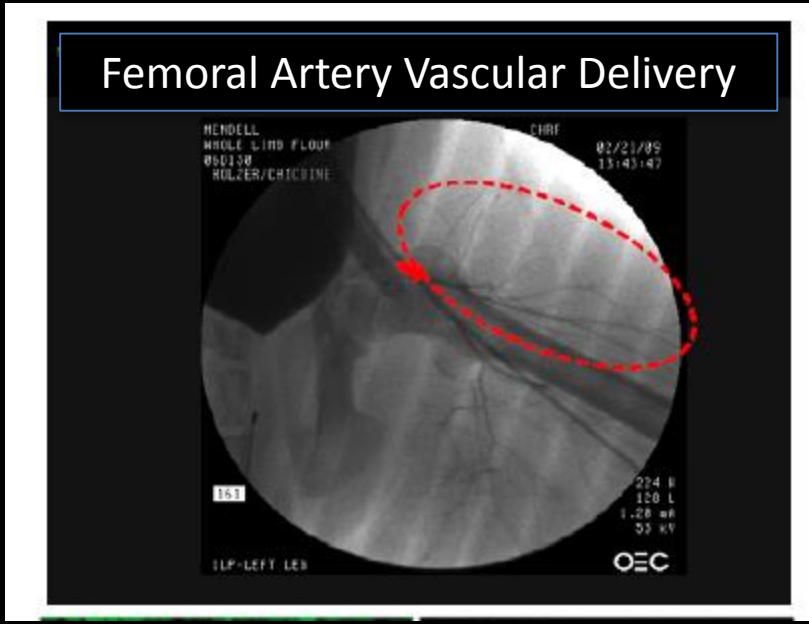


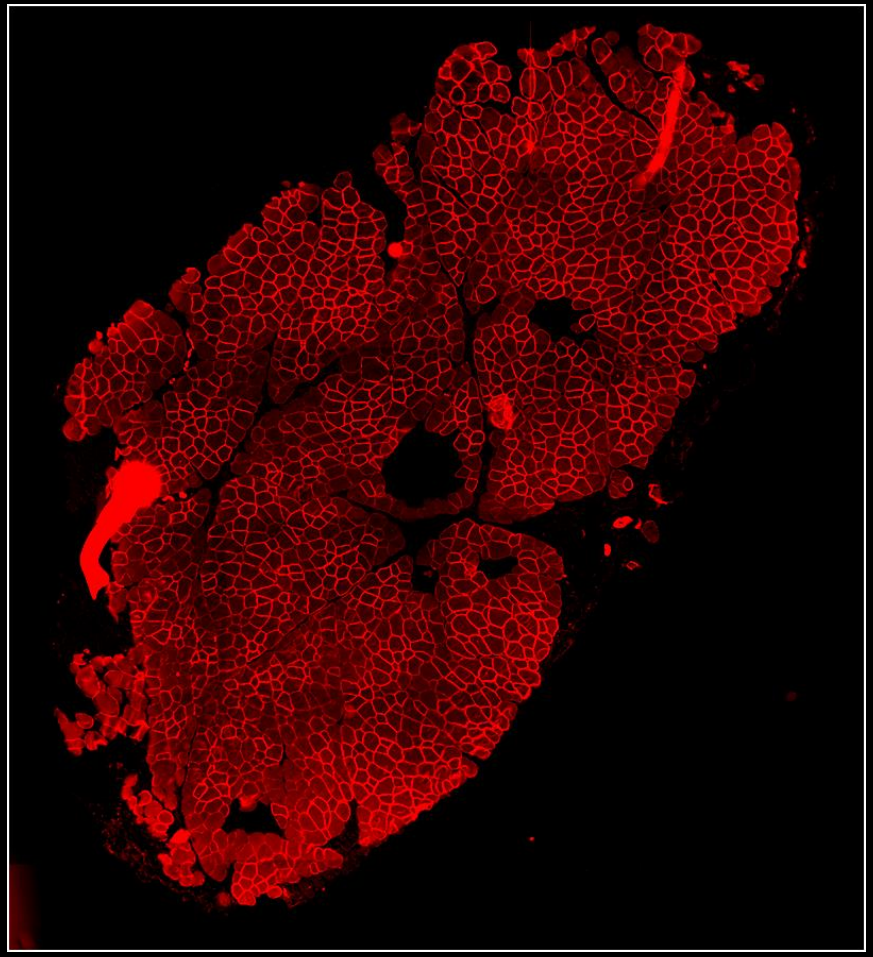




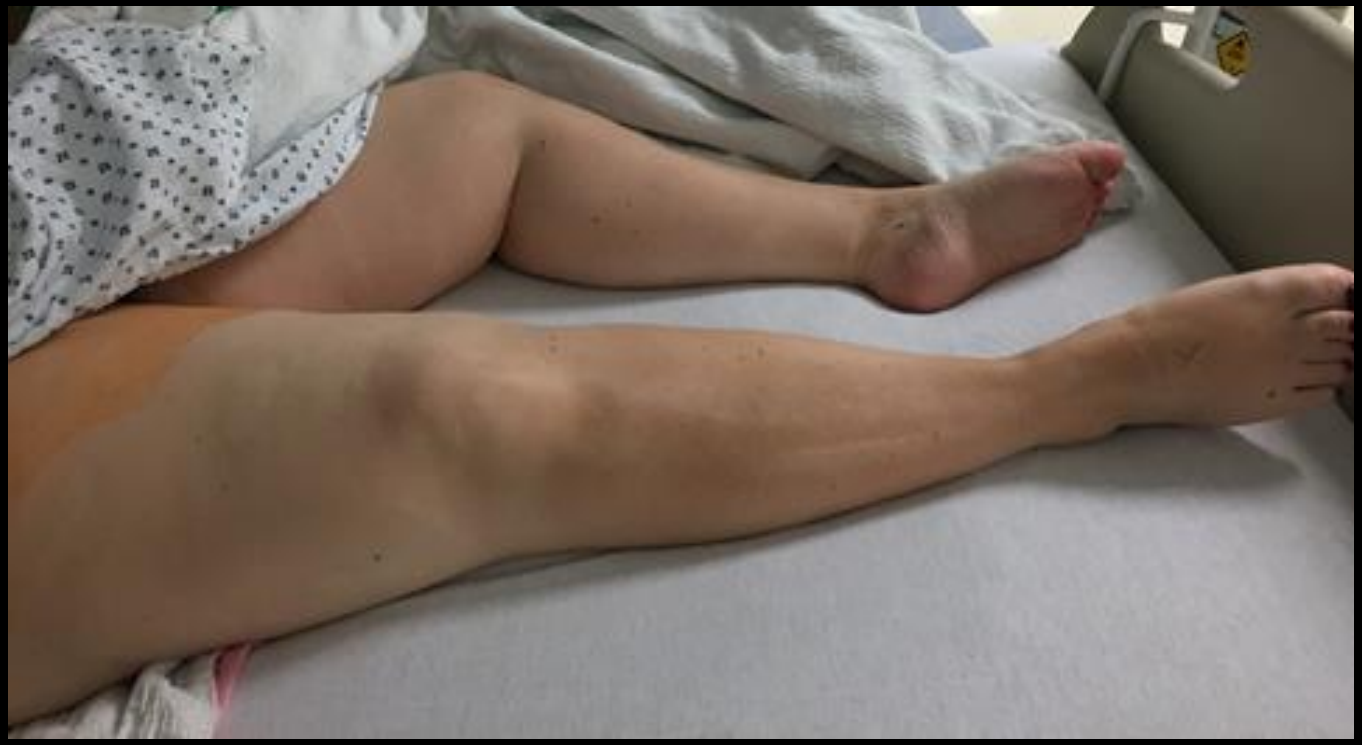

NAME
KG
ALLERGIES
BAIL

Efficient Gene Delivery to Muscle

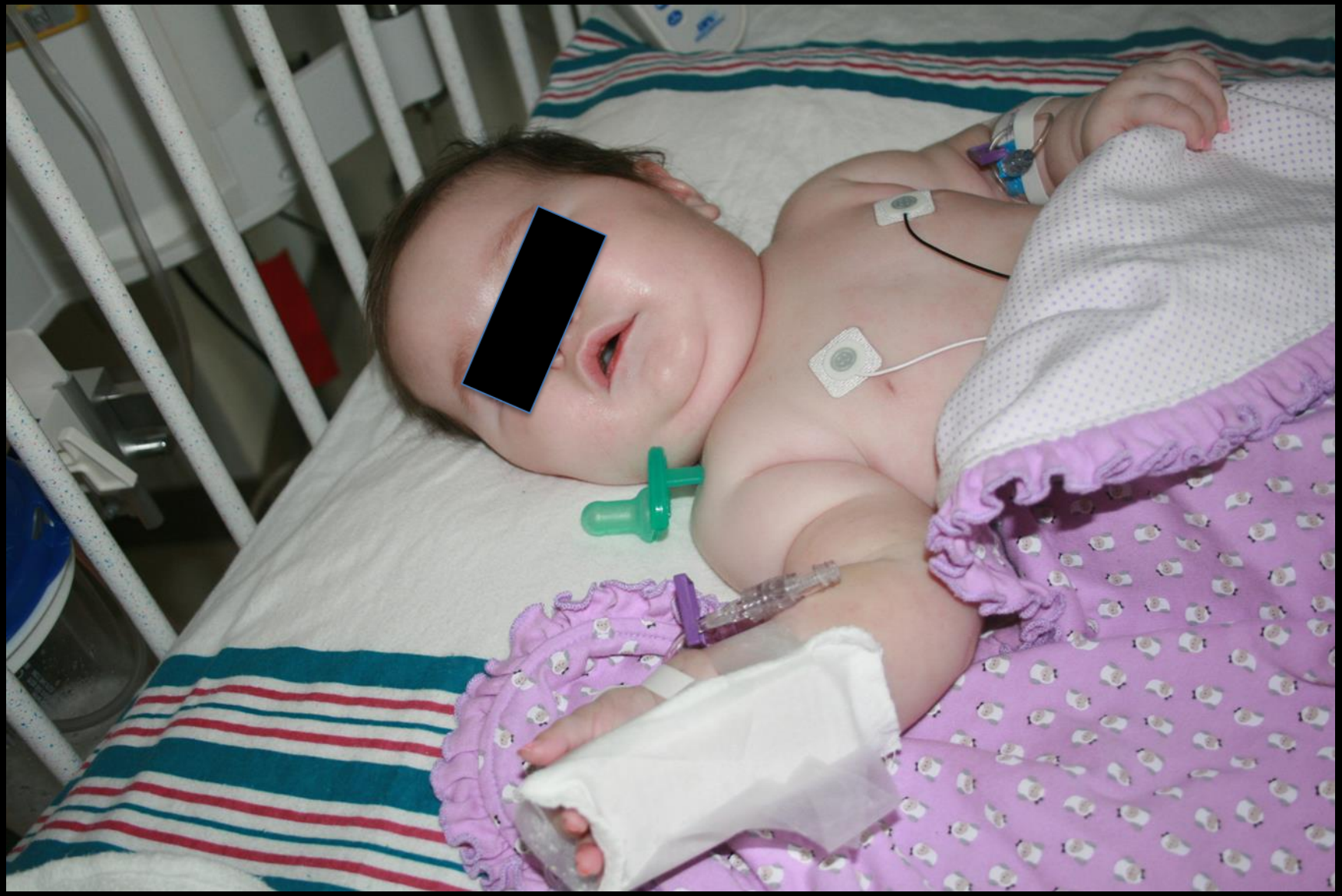




ILP Safe and Effective



Intravenous Delivery



Immune Responses following Gene Delivery

- Patient's mutation and Gene must be a perfect match to prevent rejection
- If the patient has previously been exposed to AAV, the gene could be rejected

The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

Dystrophin Immunity in Duchenne's Muscular Dystrophy

Jerry R. Mendell, M.D., Katherine Campbell, B.S., Louise Rodino-Klapac, Ph.D., Zarife Sahenk, M.D., Ph.D., Chris Shilling, M.S., Sarah Lewis, Dawn Bowles, Ph.D., Steven Gray, Ph.D., Chengwen Li, Ph.D., Gloria Galloway, M.D., Vinod Malik, Ph.D., Brian Coley, M.D., K. Reed Clark, Ph.D., Juan Li, M.D., Xiao Xiao, Ph.D., Jade Samulski, M.P.M., Scott W. McPhee, Ph.D., R. Jude Samulski, Ph.D., and Christopher M. Walker, Ph.D.

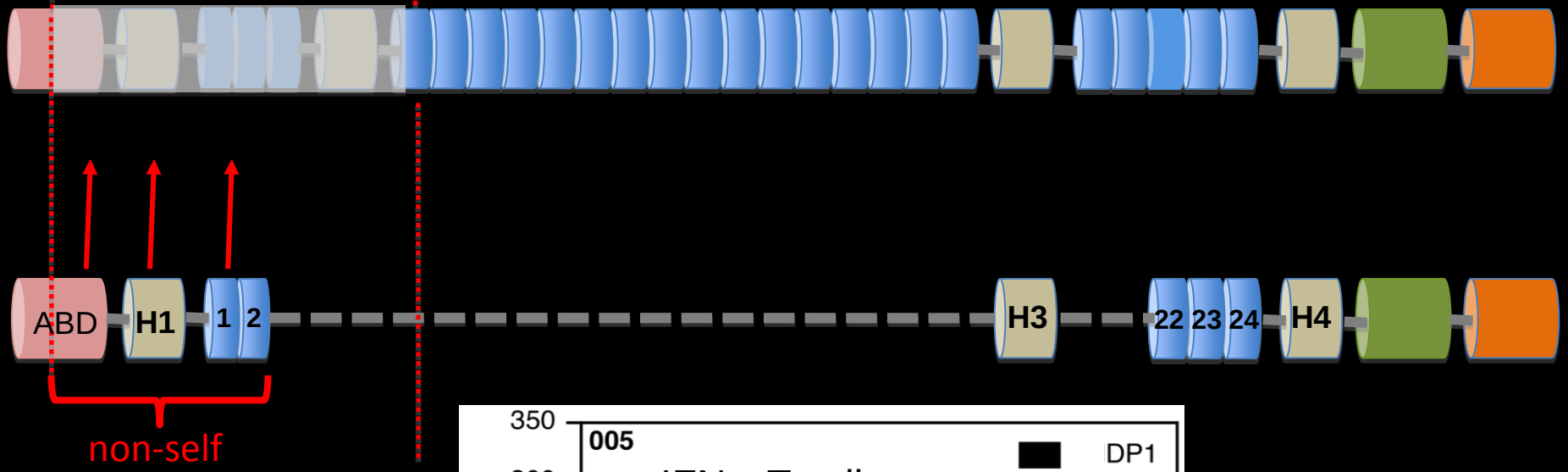
SUMMARY

We report on delivery of a functional dystrophin transgene to skeletal muscle in six patients with Duchenne's muscular dystrophy. Dystrophin-specific T cells were detected after treatment, providing evidence of transgene expression even when the functional protein was not visualized in skeletal muscle. Circulating dystrophin-specific T cells were unexpectedly detected in two patients before vector treatment. Revertant dystrophin fibers, which expressed functional, truncated dystrophin from the deleted endogenous gene after spontaneous in-frame splicing, contained epitopes targeted by the autoreactive T cells. The potential for T-cell immunity to self and nonself dystrophin epitopes should be considered in designing and monitoring experimental therapies for this disease. (Funded by the Muscular Dystrophy Association and others; ClinicalTrials.gov number, NCT00428935.)

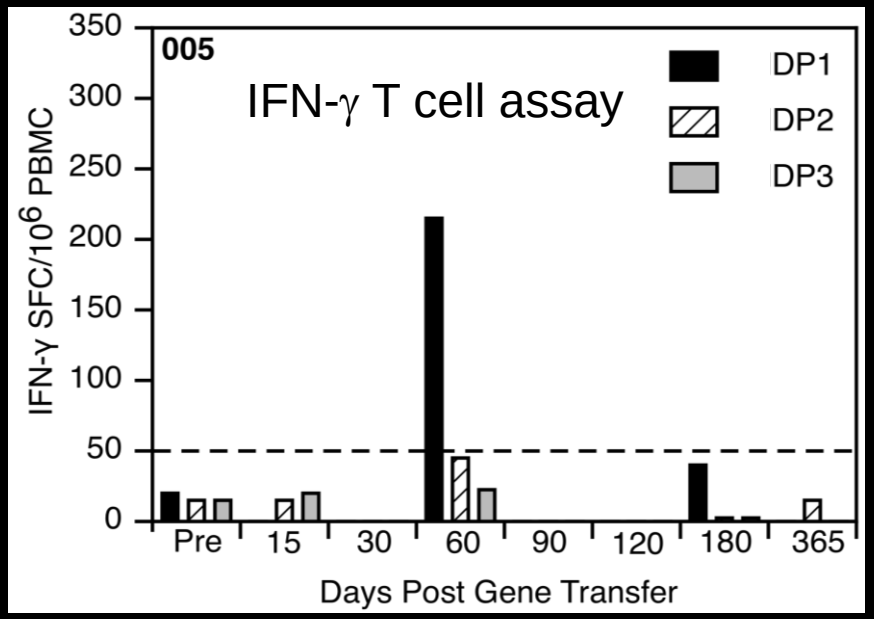
N Engl J Med 2010;363:33-41

DMD Gene Therapy Trial

Missing portion of Gene from mutation



non-self



Challenges Posed by Large Size of Gene for AAV Packaging

- Gene for transfer must be capable of fitting into AAV and if not Choices Include:
  - Reducing the size of the gene at the risk of not correcting the defect (dystrophin gene for DMD)
  - Packaging the gene in separate pieces more reliable for correcting defect

LGMD Classification

DISEASE	LINKAGE	GENE	GENE PRODUCT
LGMD1A	5q22.3-31.3	MYOT	Myotilin
LGMD1B	1q21.2	LMNA	Lamin A/C
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LGMD1D	6q23	X	Unknown
LGMD1E	7q	X	Unknown
LGMD1F	7q32.1	X	Unknown
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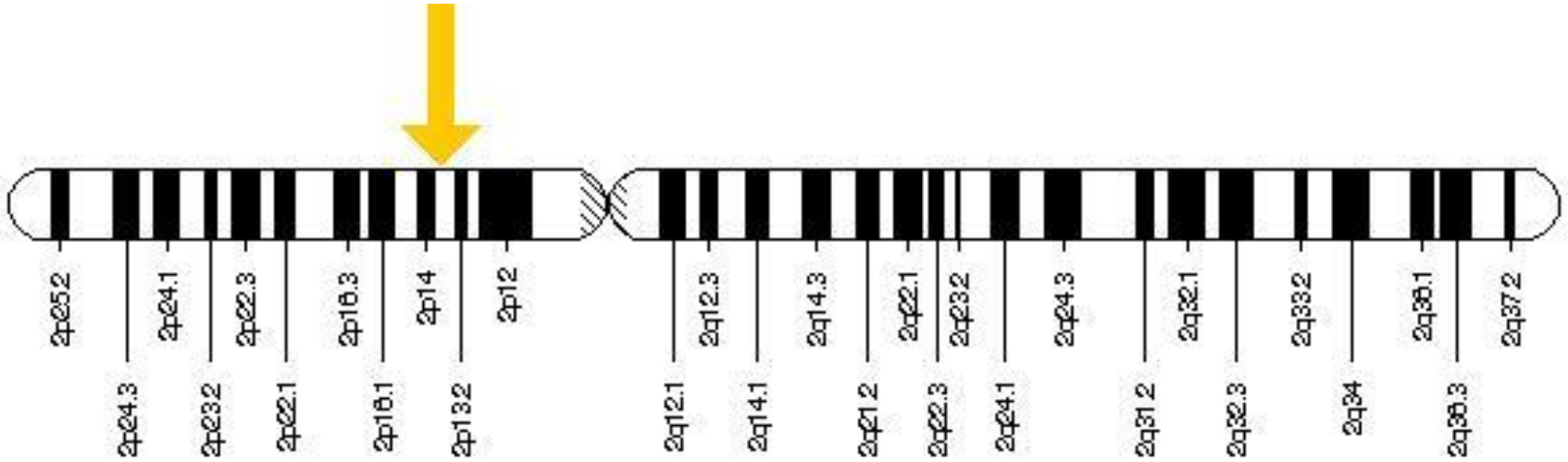
LGMD2E	4q12	SGCB	β-sarcoglycan
LGMD2F	5q33-34	SGCD	δ-sarcoglycan
LGMD2G	17q11-12	TCAP	Telethonin
LGMD2H	9q31-33	TRIM32	E3-ubiquitin-ligase
LGMD2I	19q13	FKRP	Fukutin related protein
LGMD2J	2q31	TTN	Titin
LGMD2K	9q34	POMT1	Protein O-Mannosyltransferase 1
LGMD2L	11p13	ANO5	Anoctamin
LGMD2M	9q31	FCMD	Fukutin
LGMD2N	19q13	POMT2	Protein O-Mannosyltransferase 2

Dysferlin Gene

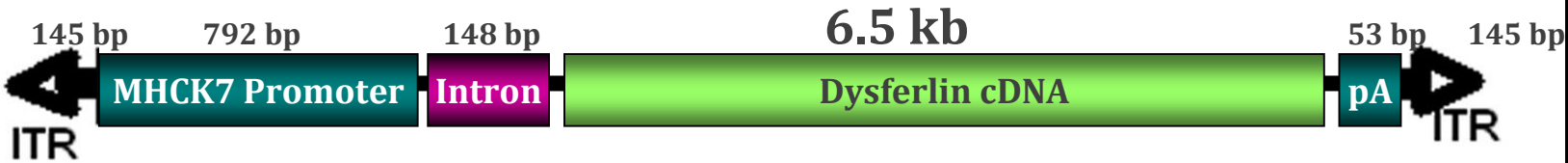


The DYSF gene is located on the short (p) arm of chromosome 2 at position 13.3. (2p13.3)

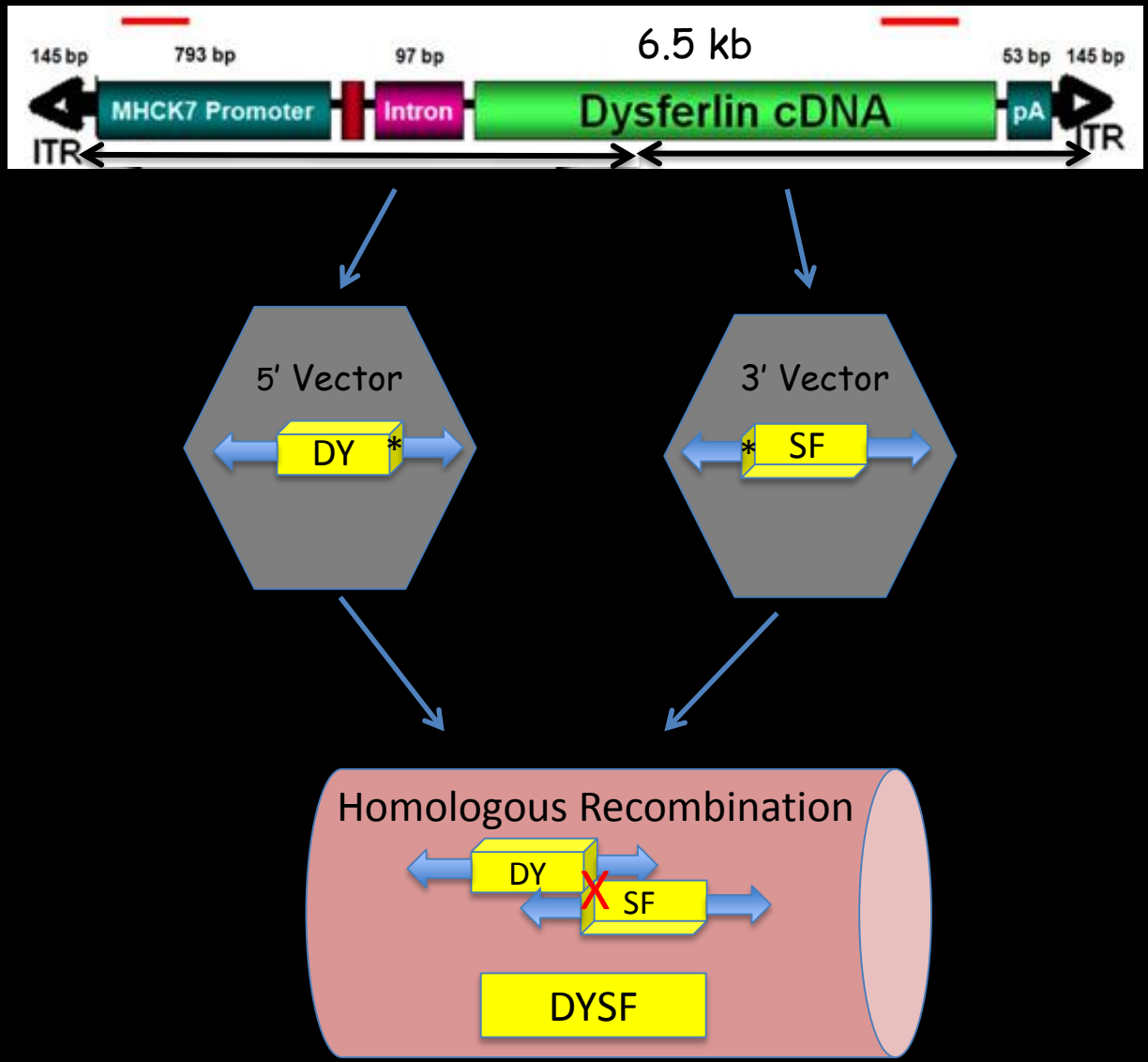
AAV Choice of Virus for Gene Replacement

- Efficient for gene transfer
- "Episomal"- does not disturb other genes
- Long-term persistence with single injection

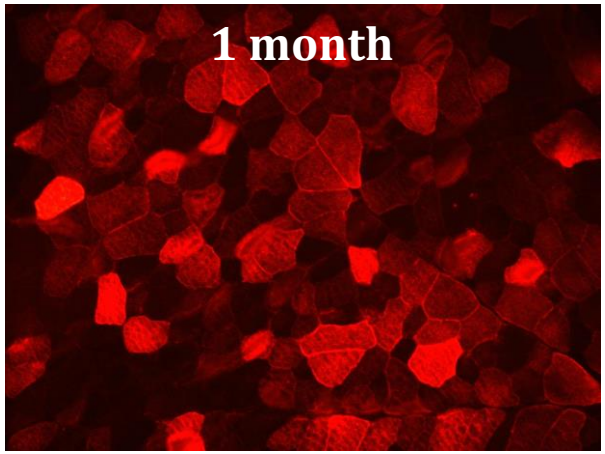
- AAV Size limitation – 4.7 kb

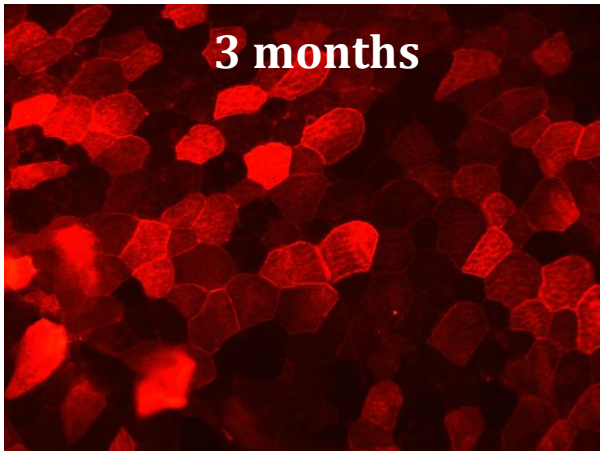


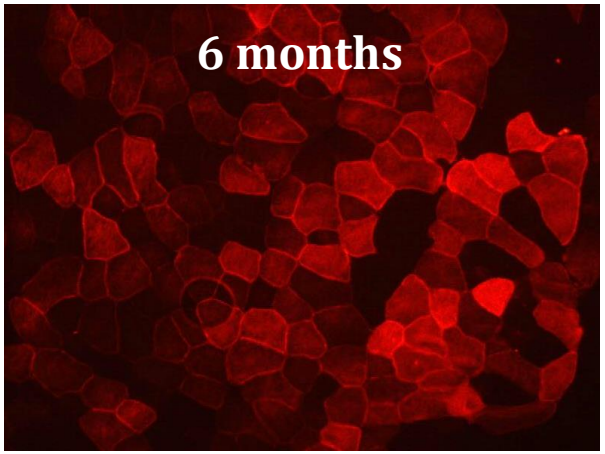
Package the Gene in Separate Pieces

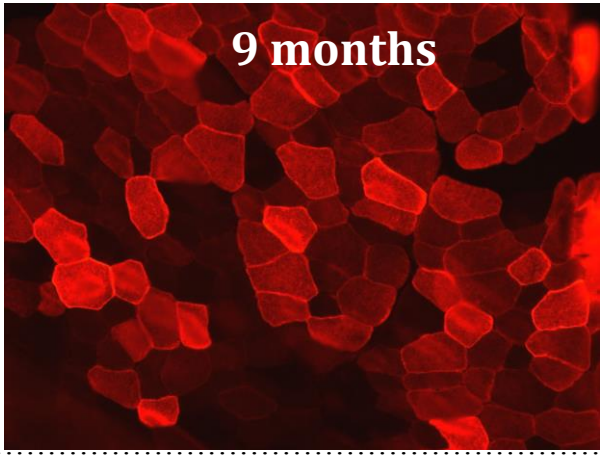


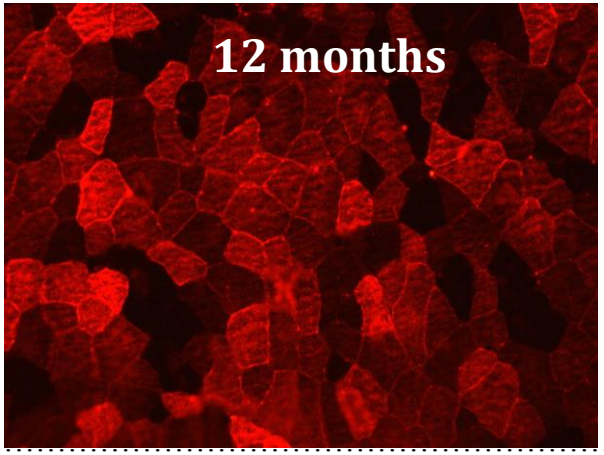
Dysferlin expression levels are maintained for at least 12 months





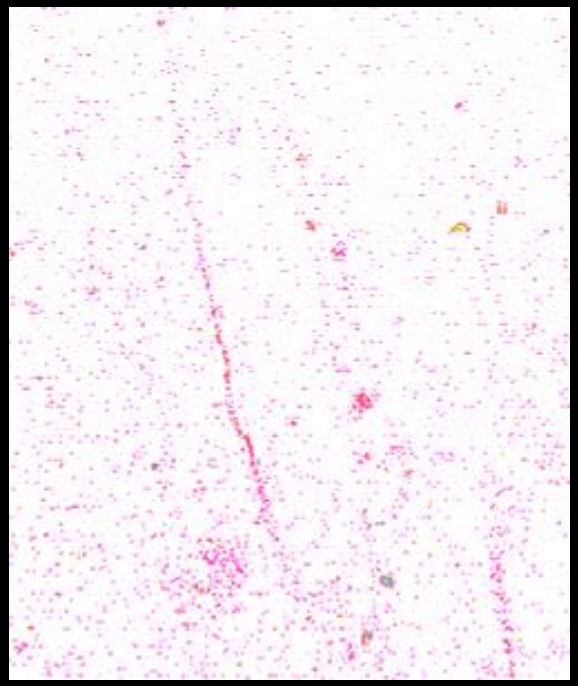




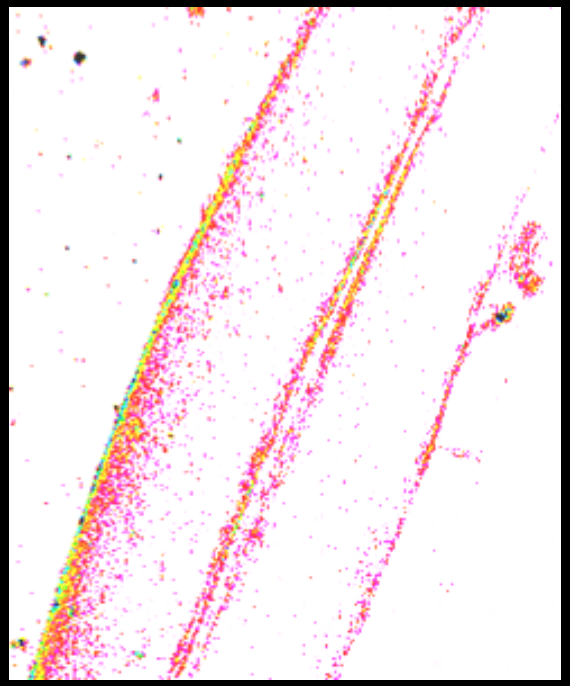


MEMBRANE REPAIR

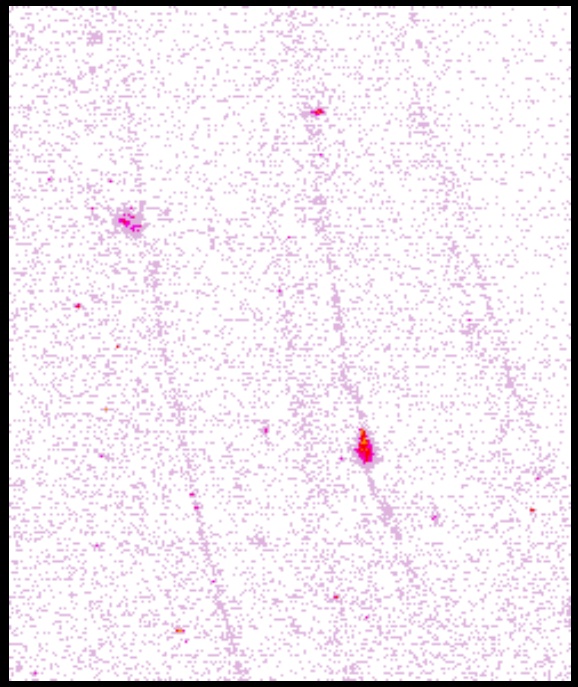
WT muscle fiber



129-Dysf KO



129-Dysf KO rAAV.Dysf



LGMD2B Gene Therapy

Gene Replacement for Dysferlin in LGMD2B

Looks Very Favorable

Will Move to Clinical Trial 2016

Choice of Potential Genes to Delivery

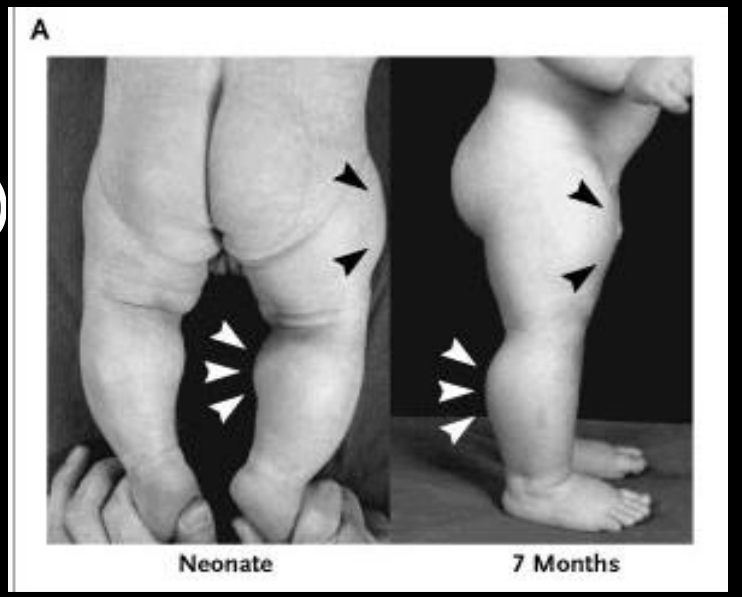
Follistatin Gene Therapy

Increasing Muscle Strength by Inhibition of Myostatin Pathway

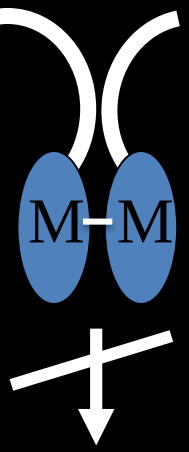
Myostatin Gene Mutation

- Targeted disruption of the myostatin gene: increases muscle size and body weight

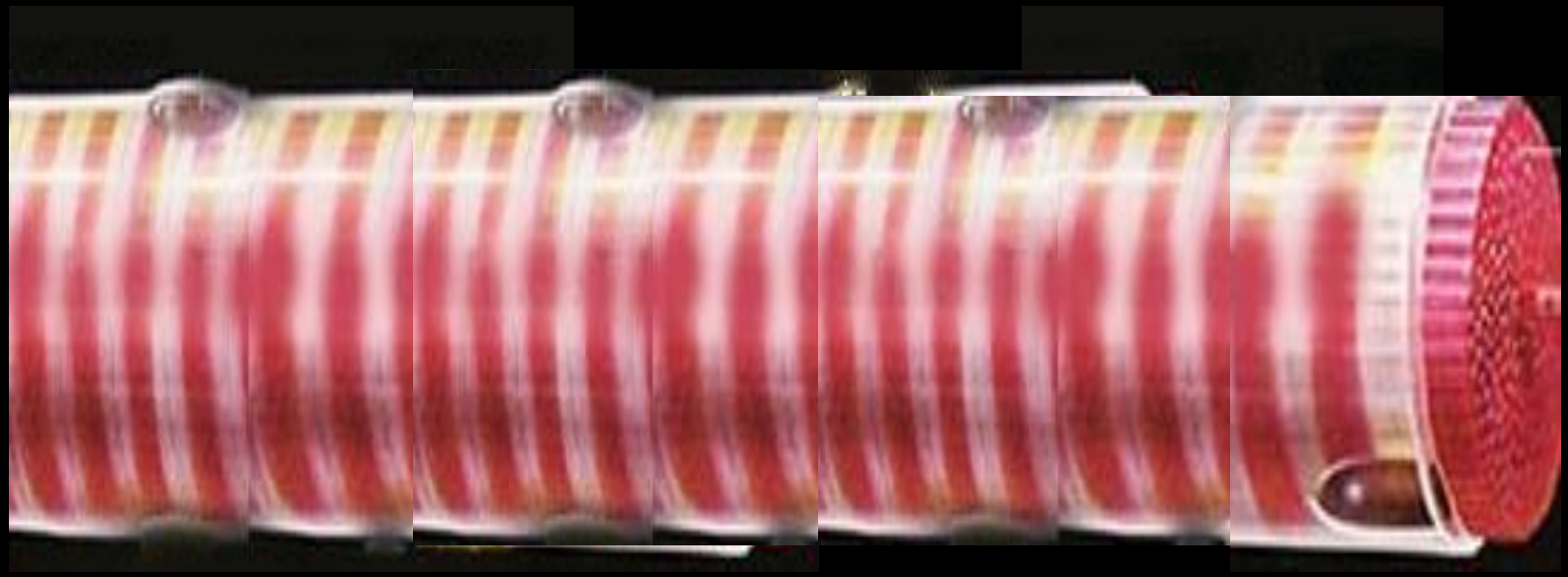
  - "Mighty" Mouse (Mstn KO)
  - Double-muscled cow (Mstn Het)
  - Newborn with gene mutation mutation N Engl J Med. 2004;350:2682-8



Circulating myostatin
Propeptide Complex

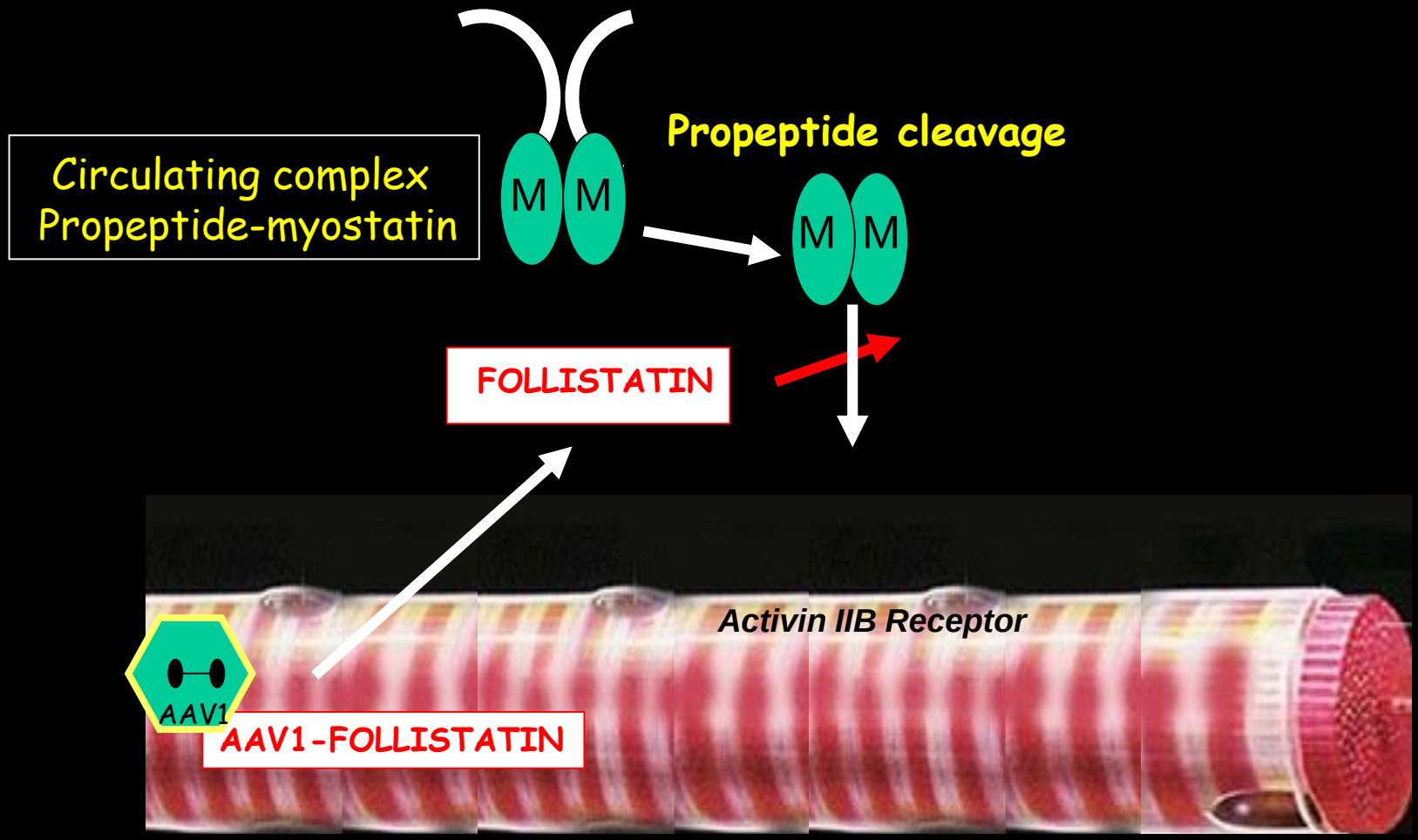


INHIBIT BINDING
Follistatin



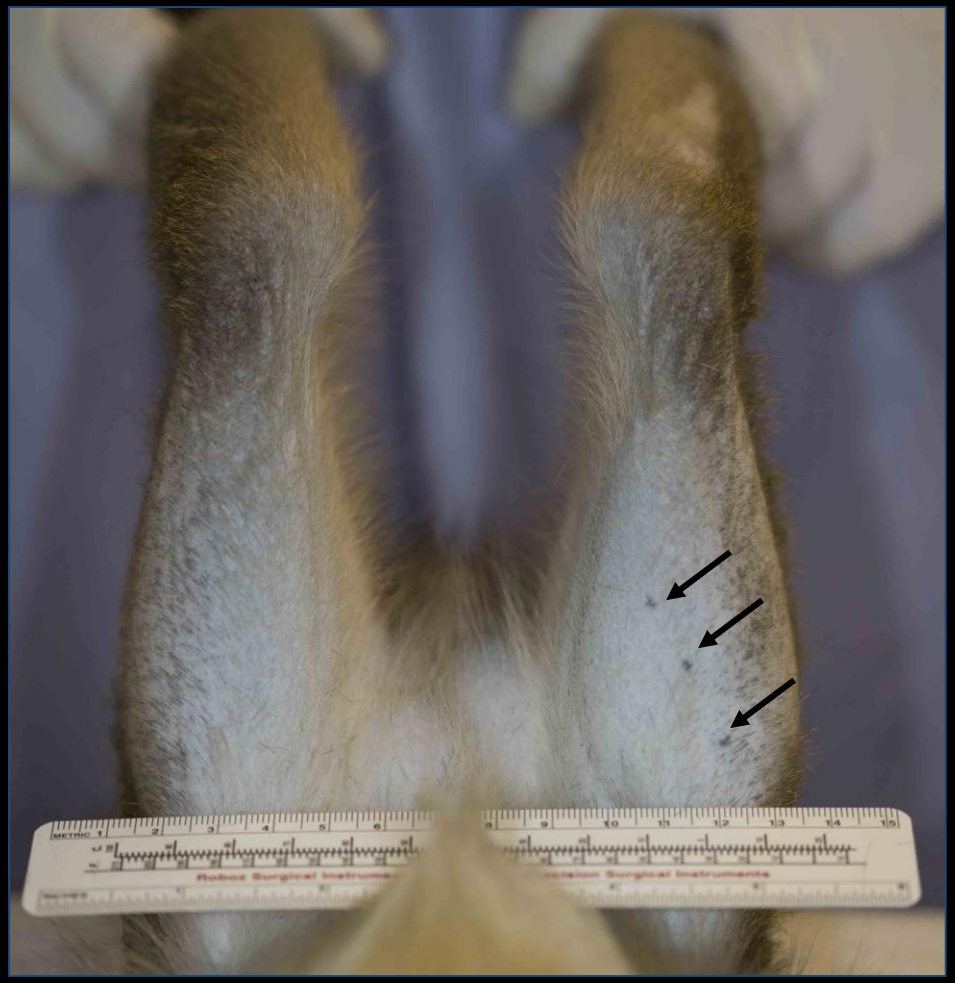
MYOSTATIN REGULATION OF MUSCLE SIZE

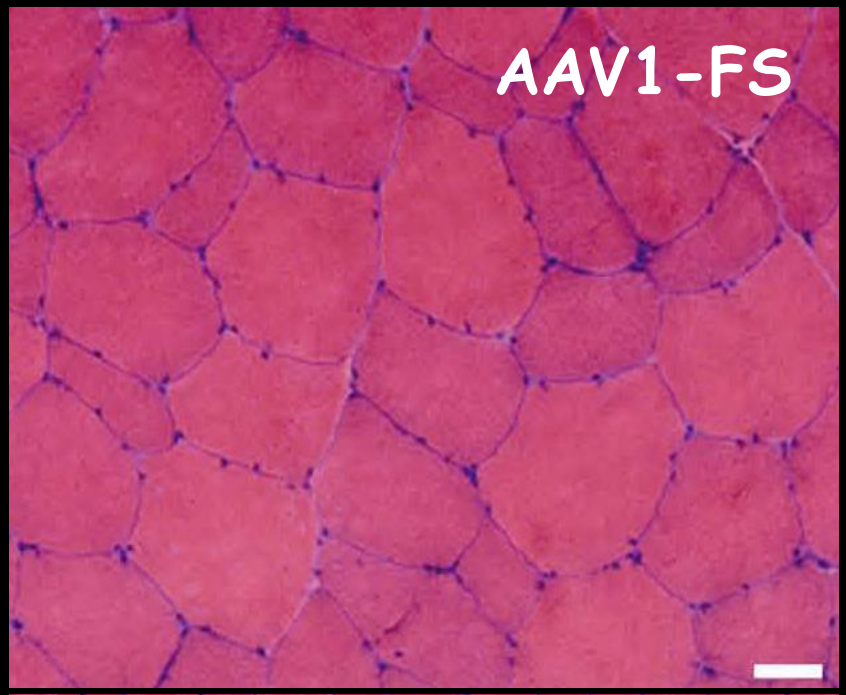
Follistatin Peptide Blockade

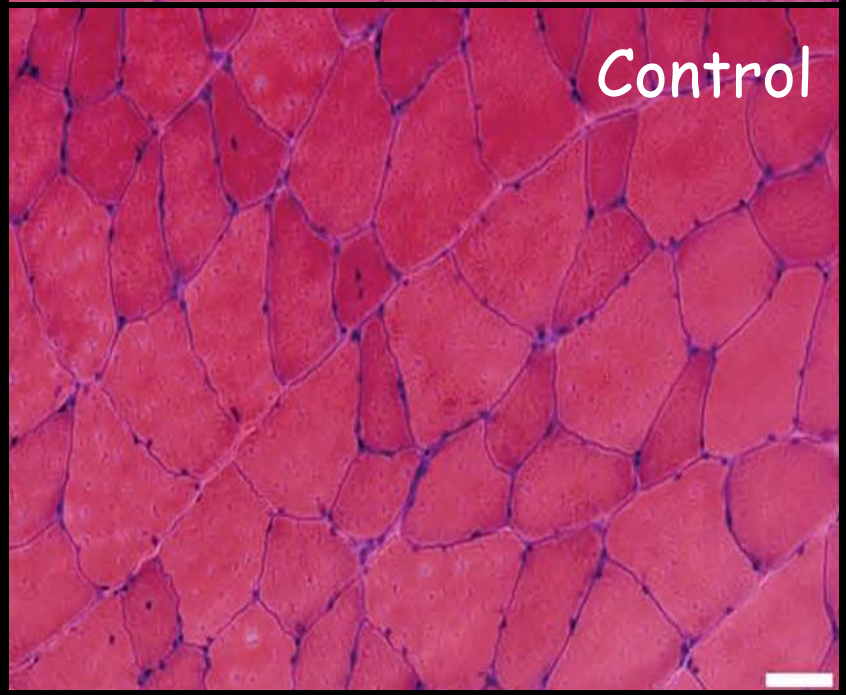


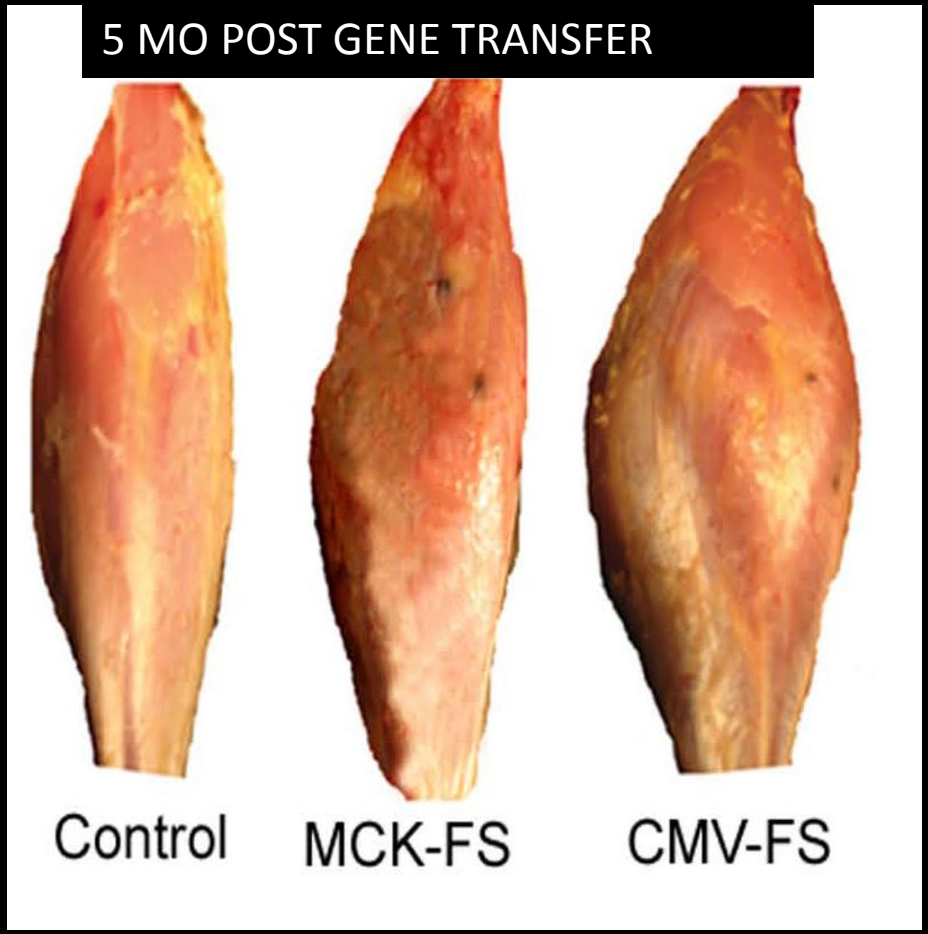
INJECT AAV INTO MUSCLE

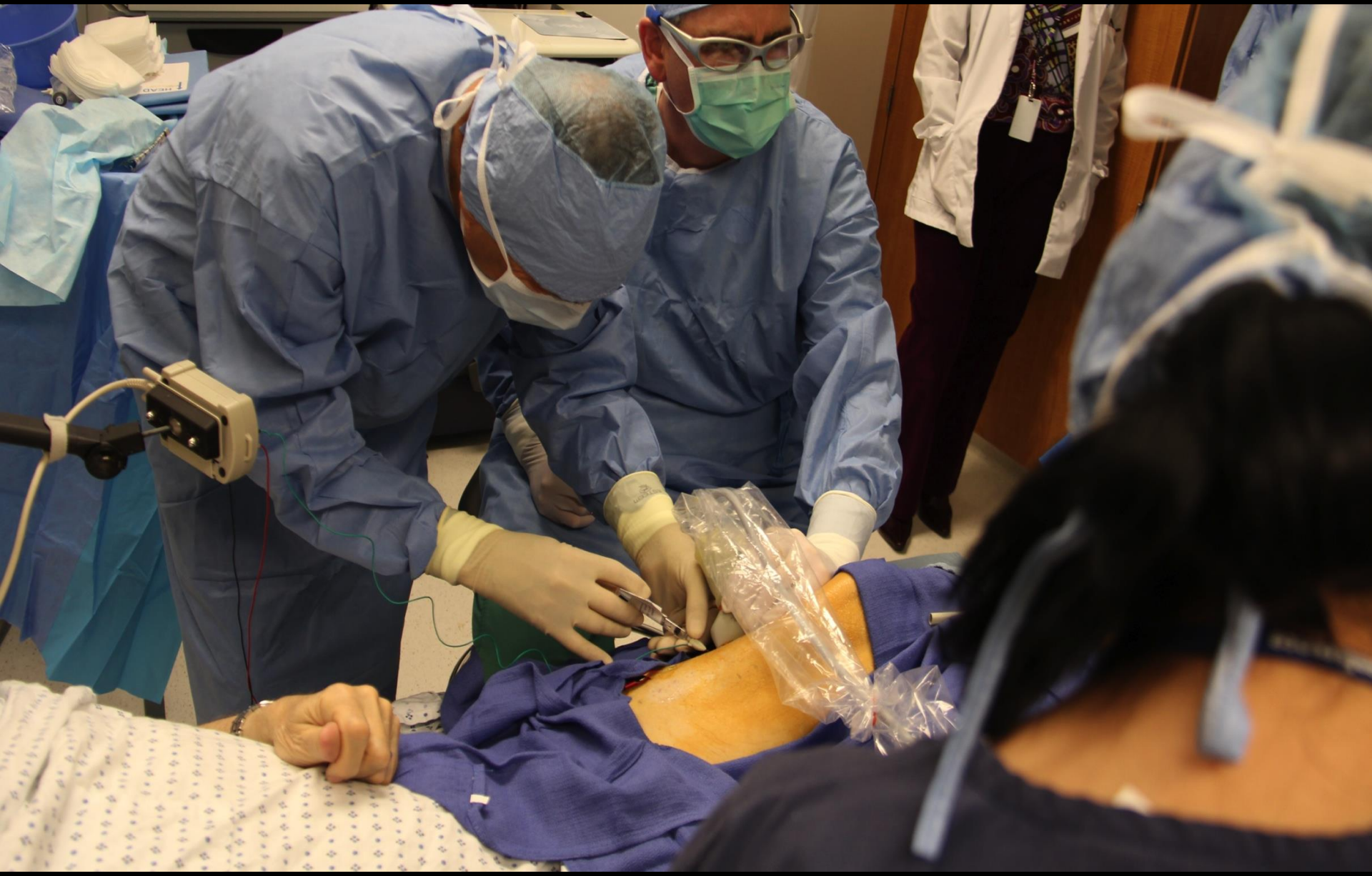
FS344 Gene Transfer to Monkey




AAV1-FS


Control

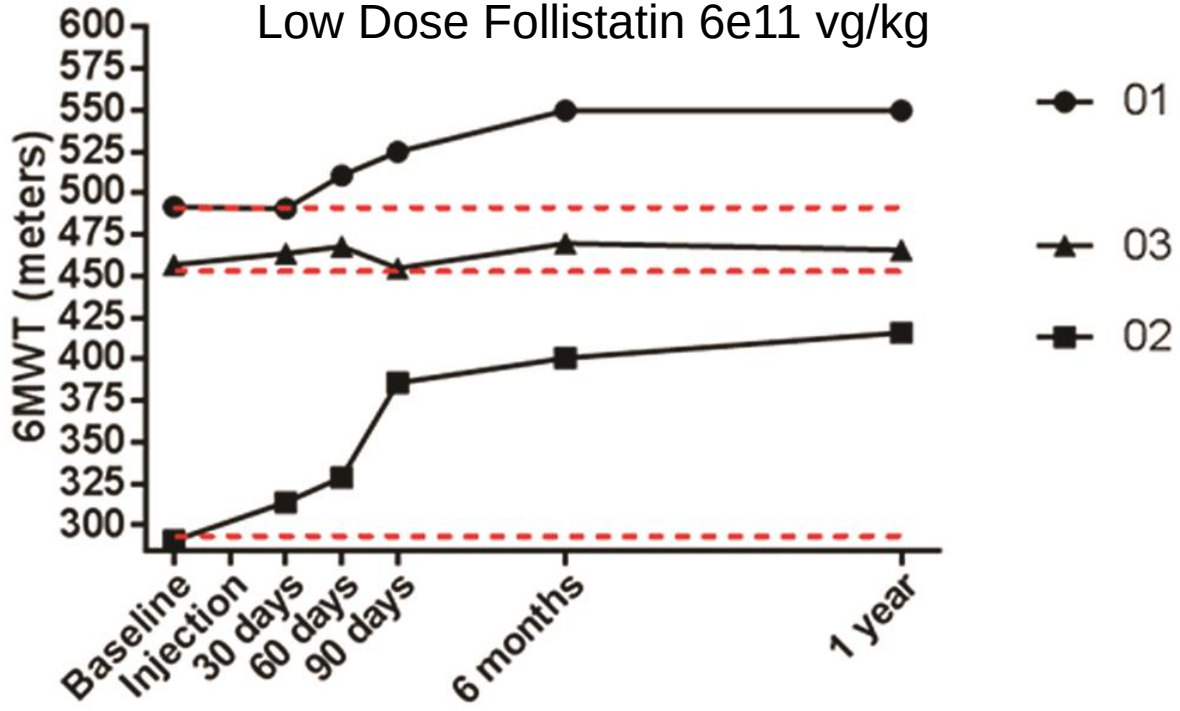

5 MO POST GENE TRANSFER
Control
MCK-FS
CMV-FS



Becker Muscular Dystrophy

Low Dose Follistatin 6e11 vg/kg

a



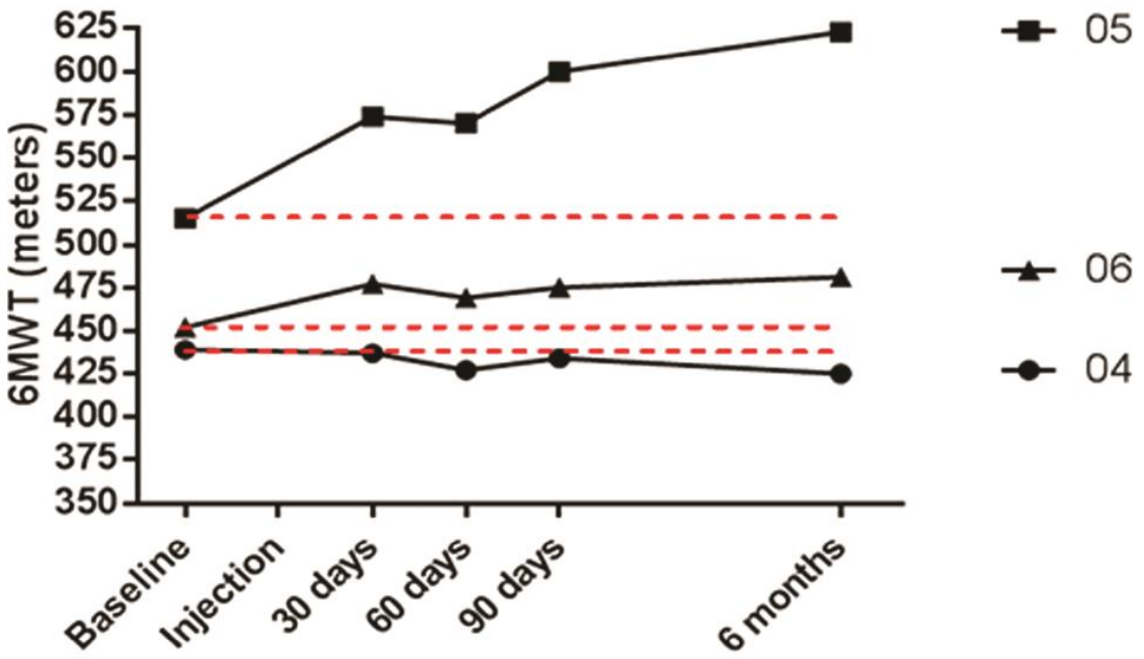
b

Visit	Low Dose Cohort		
	01	02	03
BL	492	291	457
D30	491	314	464
D60	511	329	468
D90	525	386	455
D180	550	401	470
1 YR	550	416	466
12-mo change	**+58 m**	**+125 m**	**+9 m**

Becker Muscular Dystrophy

High Dose Follistatin 1.2e12 vg/k

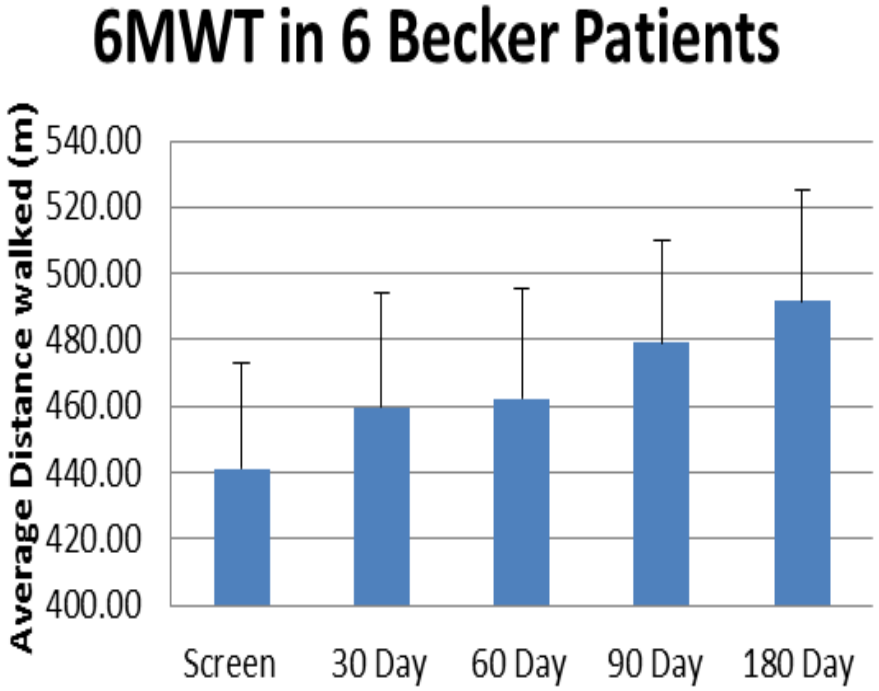
a



b

Visit	High Dose Cohort		
	04	05	06
BL	439	515	452
D30	437	574	477
D60	427	570	469
D90	434	600	475
D180	425	623	481
6-mo change	**-14 m**	**+108 m**	**+29 m**

6 Minute Walk Distance



Statistically Significant 11.5% average improvement after 180 days

Path to Gene Therapy Trial

1. Establish unequivocal Proof of Principle

2. Discuss with FDA – Pre-IND Meeting
   - Plan Clinical Trial (age, inclusion criteria)
   - Plan Toxicology Study
     - Mice only or include non-human primates

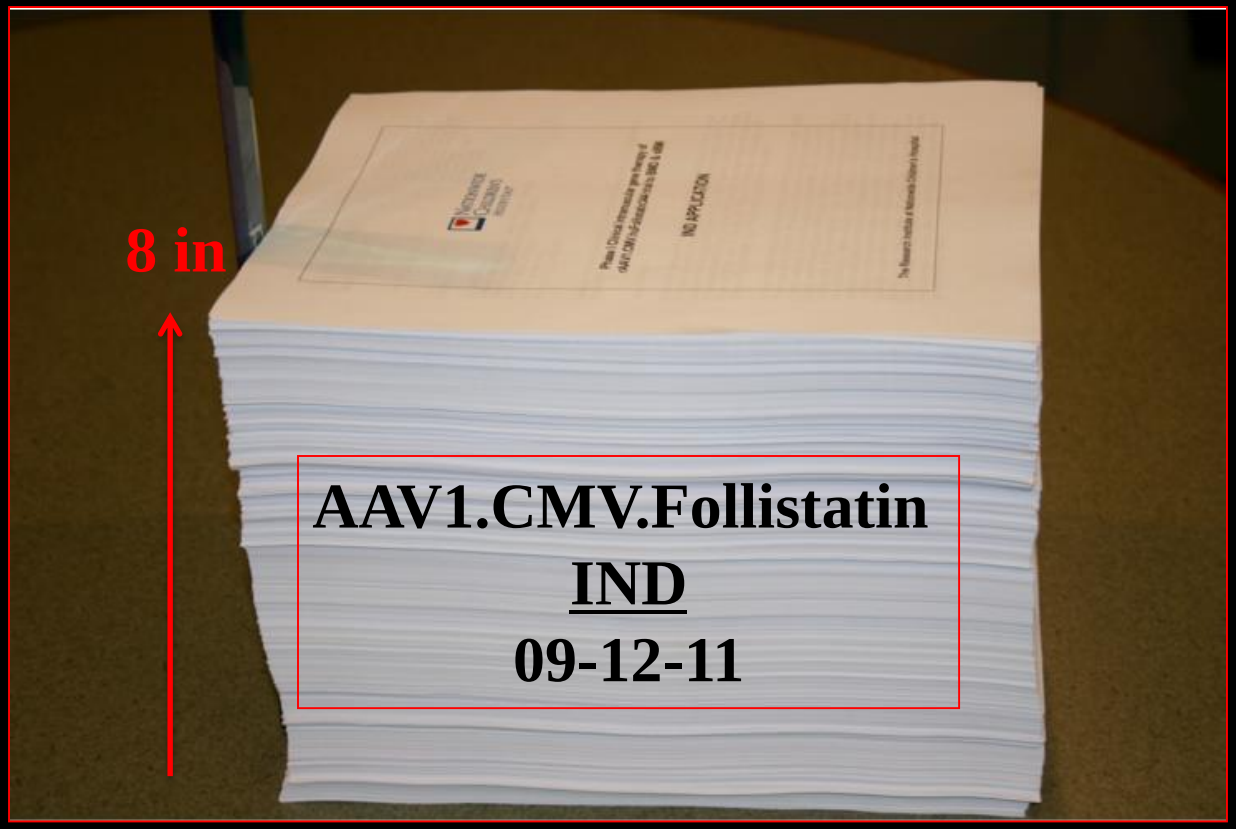
3. Perform Toxicology study using same virus as for clinical trials

4. Present study to Recombinant DNA Advisory Committee (RAC)

5. Apply for approval to Internal Review Board

6. Final Step submitting the IND

Final Step Before Clinical Trial
Submit the IND



Nationwide Children's Research Institute

JAIN FOUNDATION

Parent Project Muscular Dystrophy
LEADING THE FIGHT TO END DUCHENNE

Eunice Kennedy Shriver NICHD
National Institute of Child Health & Human Development

SENATOR PAUL D. WELLSTONE MUSCULAR DYSTROPHY COOPERATIVE RESEARCH CENTER