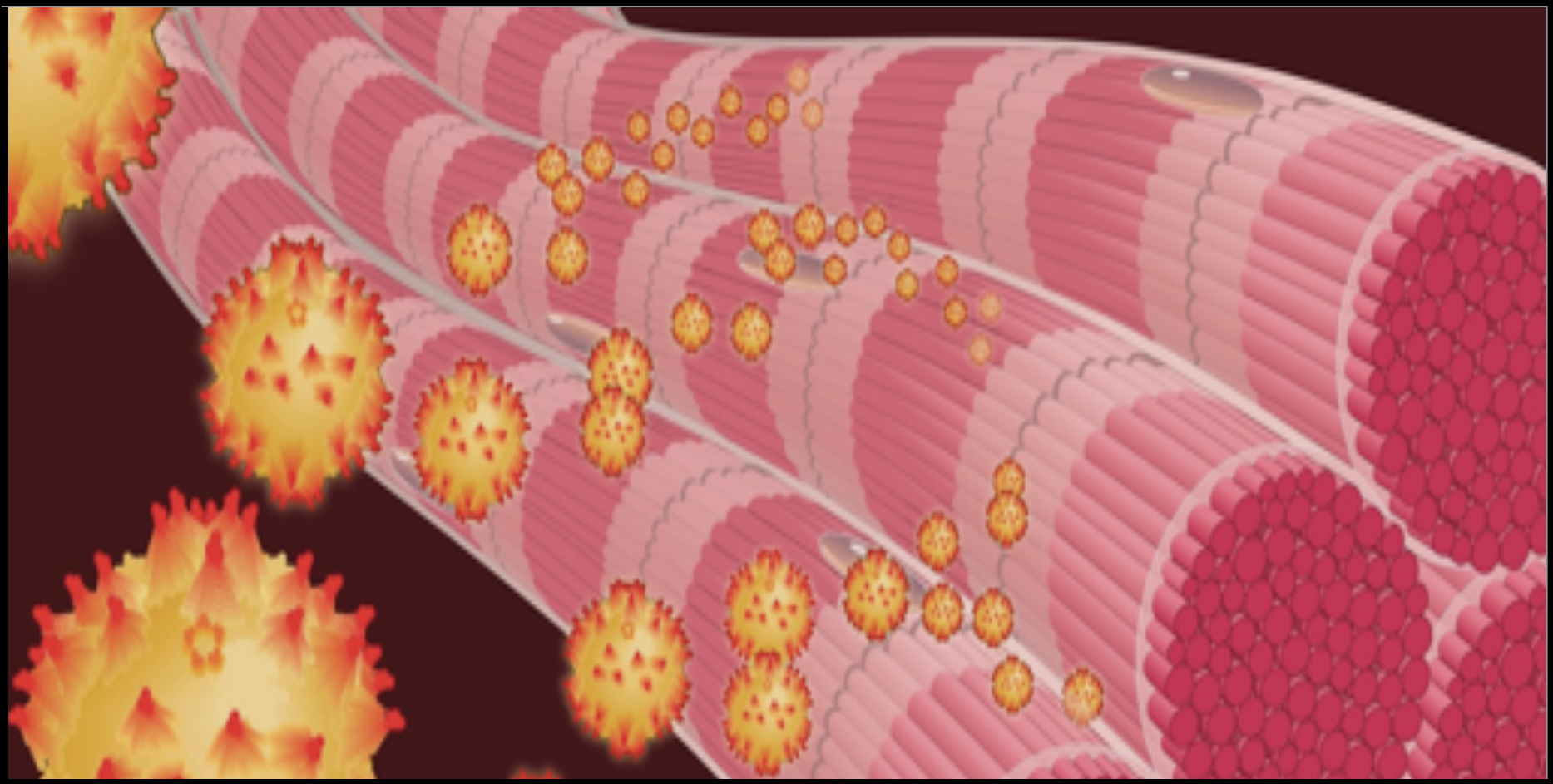
Road to Clinical Gene Therapy Through Translational Research



Jerry R Mendell, MD
Research Institute at Nationwide Children's Hospital

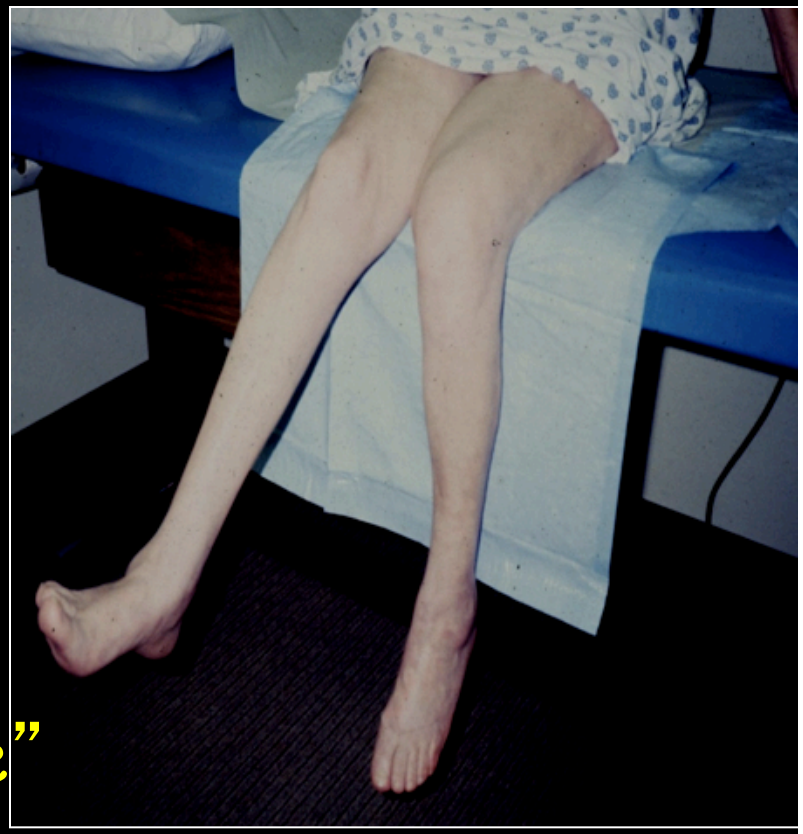
Steps to Clinical Trial

- Pre-clinical studies (proof-of-concept)
  - Take full advantage of experiments
- Discuss with FDA the issues in developing "product" for clinical trial
  - Pre-IND meeting (1 hour teleconference)
- Perform toxicology-biodistribution studies
  - Never proceed unless agreed upon by FDA
- Submit IND, obtain IRB and DSMB approval
- Perform Clinical Trial
  - Never deviate protocol from IND, IRB

Follistatin Gene Therapy

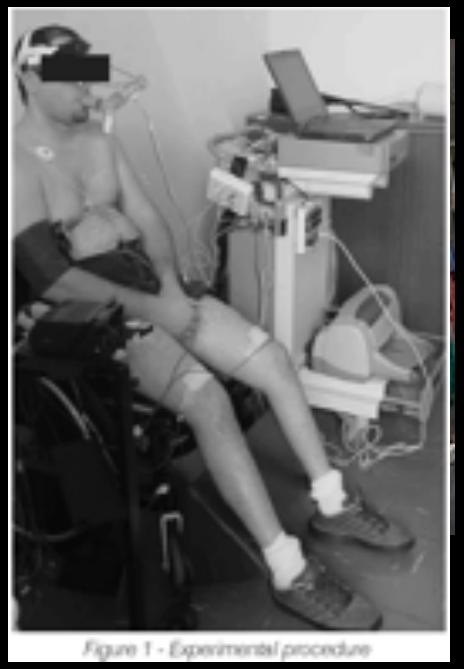
Conceptualize the Clinical Target

- Quadriceps muscle weakness
  - Becker muscular dystrophy
  - Inclusion body myositis
- Frequent falls
  - Limb fractures
  - Loss of ambulation
- Improving quadriceps muscle strength would result in a "clinically meaningful outcome"
  - The Central Issue at FDA



Are There Alternative Therapies to Gene Therapy?

- Weight training
- Electrical Stimulation
- Anabolic Steroids



Path to Proof-of-Concept

Circulating myostatin
Propeptide Complex

Myostatin Activation
Protease Cleavage of Complex

INHIBIT BINDING
Gene mutation
Antibody
Peptides

MYOSTATIN REGULATION OF MUSCLE SIZE

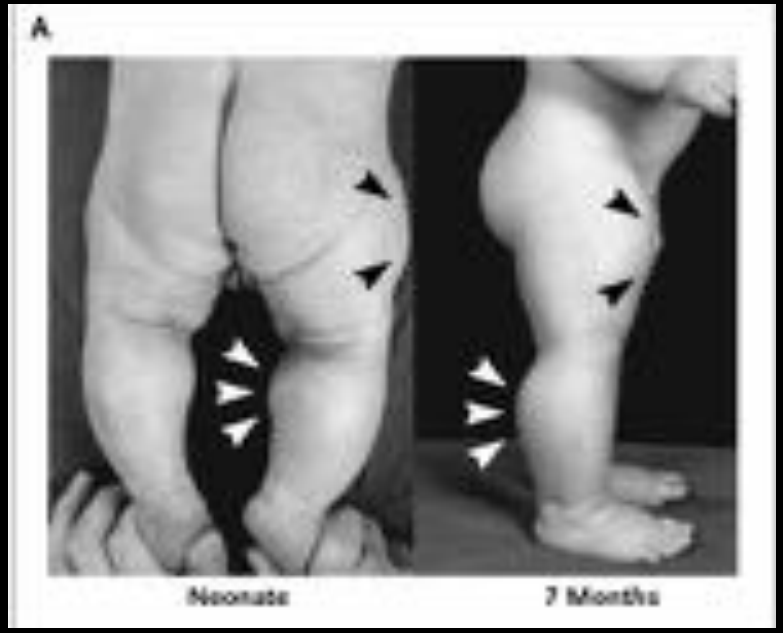
Myostatin Gene Mutation

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

  - "Mighty" Mouse

  - Double-muscled cow

  - Newborn with gene mutation mutation N Engl J Med. 2004;350:2682-8



ORIGINAL ARTICLE

Ann Neurology March 11, 2008

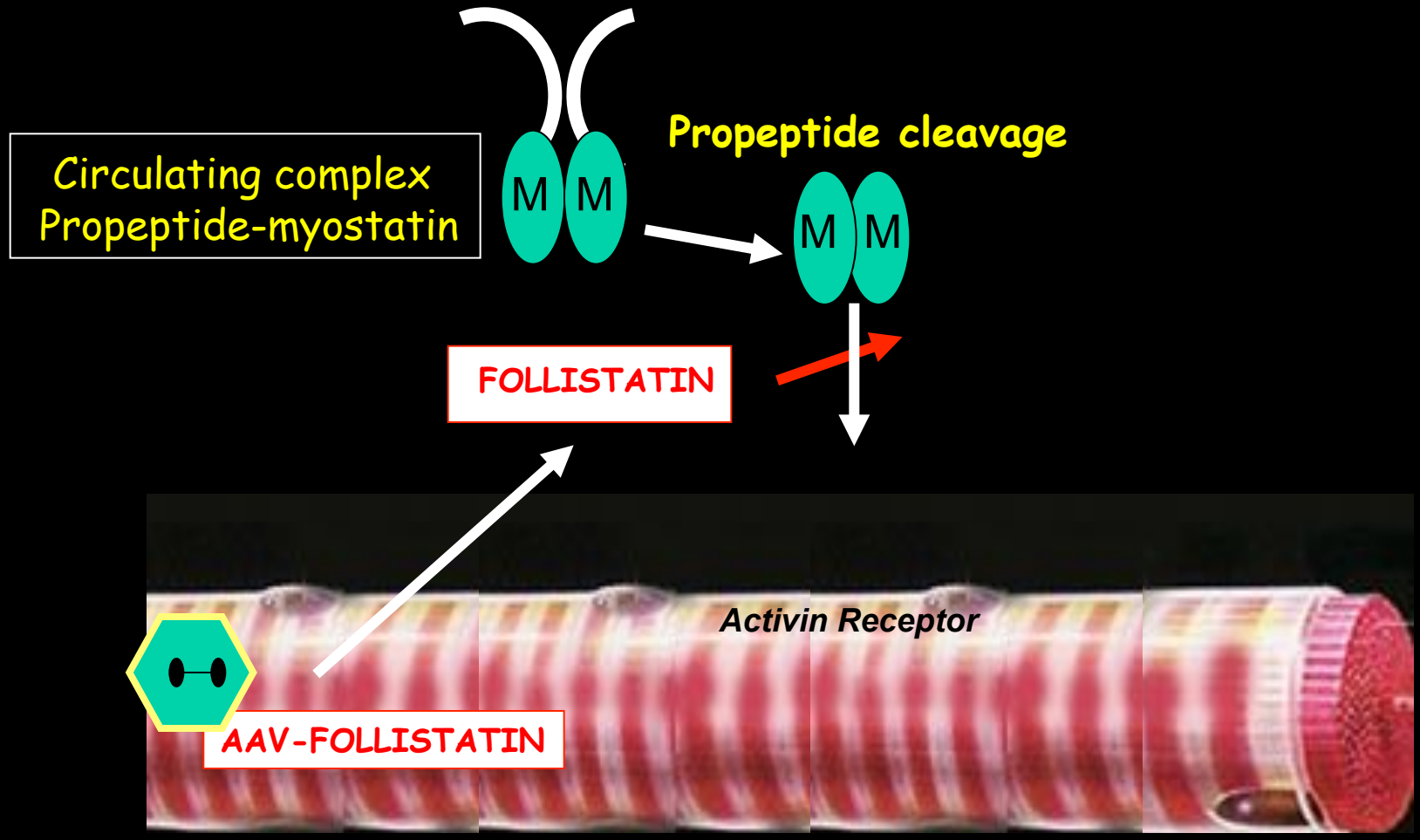
A Phase I/II trial of MYO-029 in Adult Subjects with Muscular Dystrophy

Kathryn R. Wagner, MD, PhD,[1] James L. Fleckenstein, MD,[2] Anthony A. Amato, MD,[3] Richard J. Barohn, MD,[4] Katharine Bushby, MD,[5] Diana M. Escolar, MD,[6] Kevin M. Flanigan, MD,[7] Alan Pestronk, MD,[8] Rabi Tawil, MD,[9] Gil I. Wolfe, MD,[10] Michelle Eagle, PhD, MSc, MCSP, SRP,[5] Julaine M. Florence, PT, DPT,[8] Wendy M. King, PT,[11] Shree Pandya, MS, PT,[9] Volker Straub, MD,[5] Paul Juneau, MS,[12] Kathleen Meyers, RN, BSN,[13] Cristina Csimma, PharmD, MHP,[14] Tracey Araujo, MSPharm,[14] Robert Allen, MD,[15] Stephanie A. Parsons, PhD,[15] John M. Worney, PhD,[14] Edward R. LaVallie, PhD,[14] and Jerry R. Mendell, MD[11]

Wyeth sponsored 11 Center Trial (10 USA;1GB)
Using antibody to myostatin

- No Clinical benefit
- High dose cohorts developed skin hypersensitivity reactions

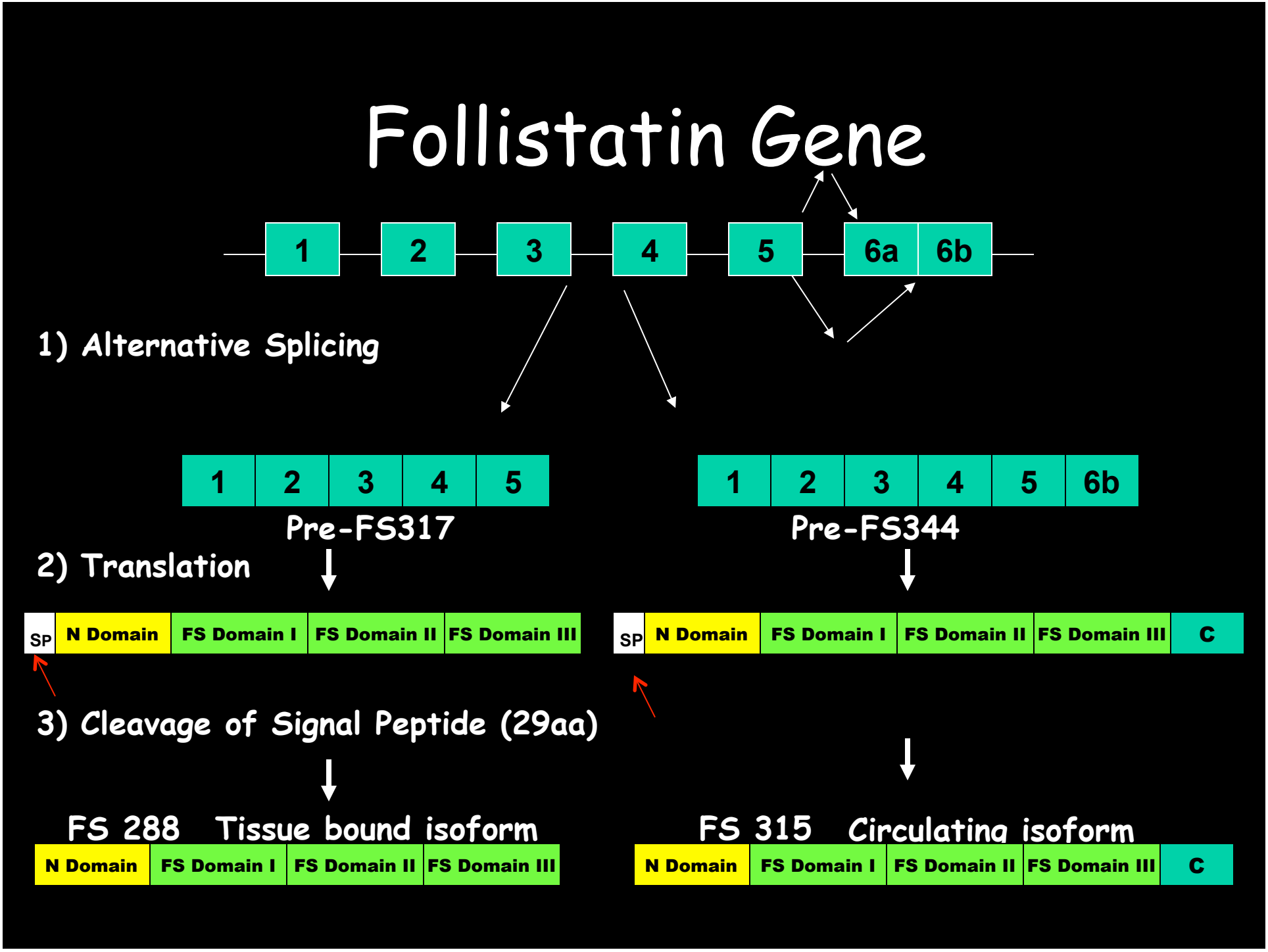
Follistatin Gene Therapy



INJECT AAV INTO MUSCLE

Choice for Transgene to Bring to Clinical Trial

- What would be acceptable to FDA and IRB

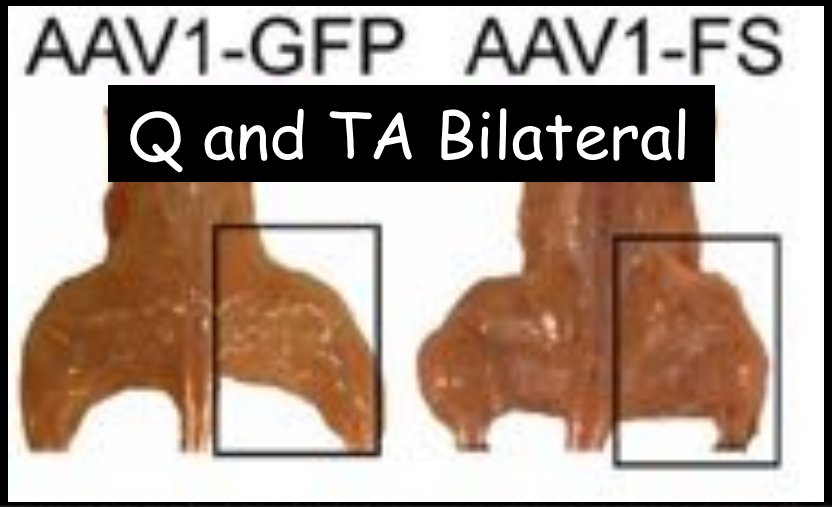

Follistatin Gene
1
2
3
4
5
6a
6b
1) Alternative Splicing
1
2
3
4
5
Pre-FS317
1
2
3
4
5
6b
Pre-FS344
2) Translation
SP
N Domain
FS Domain I
FS Domain II
FS Domain III
SP
N Domain
FS Domain I
FS Domain II
FS Domain III
C
3) Cleavage of Signal Peptide (29aa)
FS 288 Tissue bound isoform
N Domain
FS Domain I
FS Domain II
FS Domain III
FS 315 Circulating isoform
N Domain
FS Domain I
FS Domain II
FS Domain III
C

Go beyond Proof-of-Concept Studies

- Keep Clinical Trial in your Vision
- FDA must see functional changes to accompany gene expression and evidence of safety/tolerability

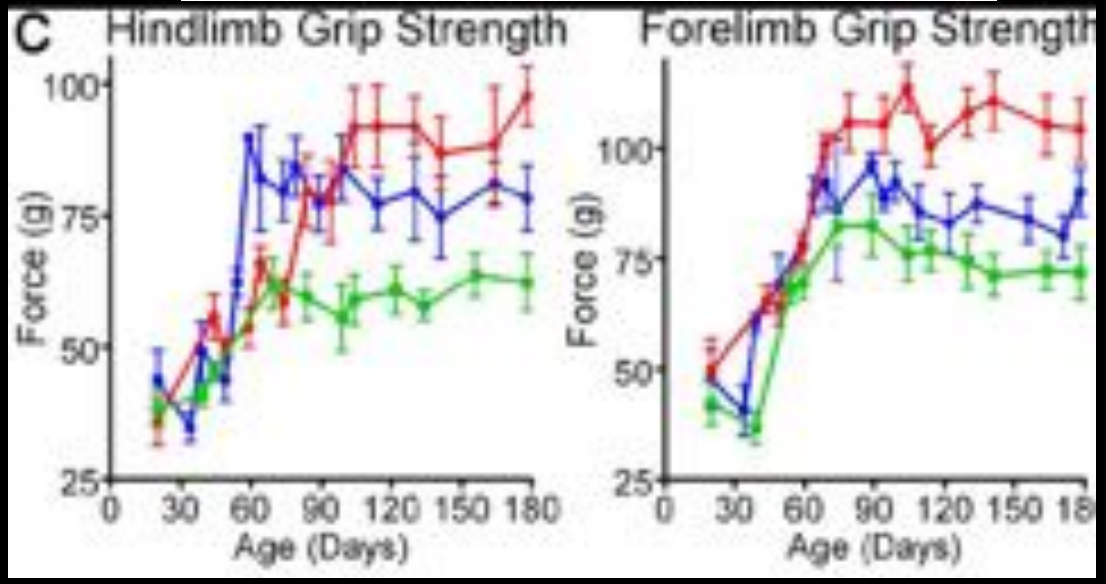
Pre-Clinical Studies
Treatment of *mdx* with AAV1.FS344

*Gene Expression 6mo

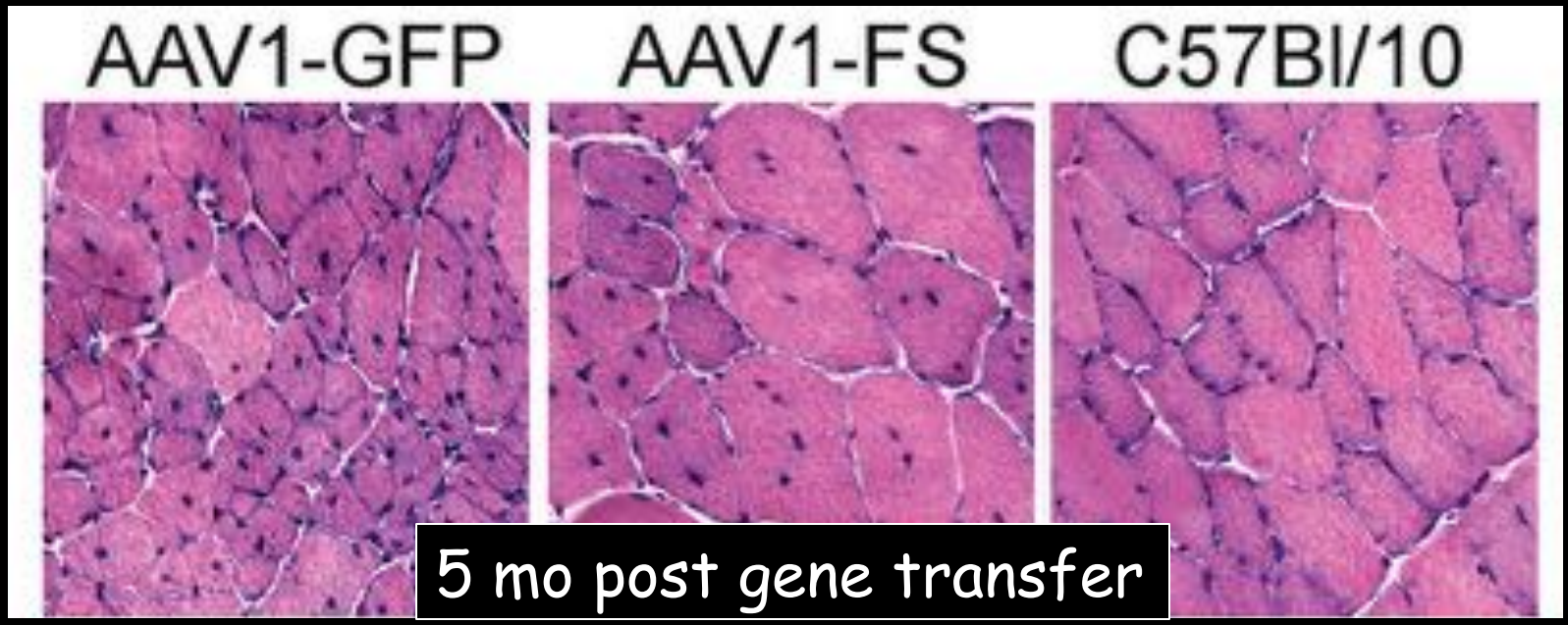


$1X10^{12}$ vg/kg**

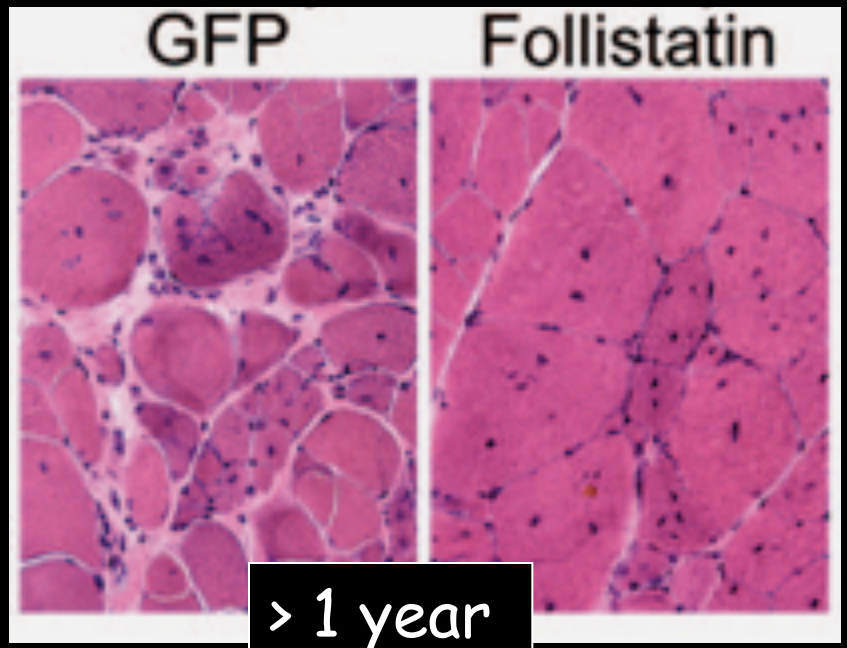
$1X10^{11}$ vg/kg

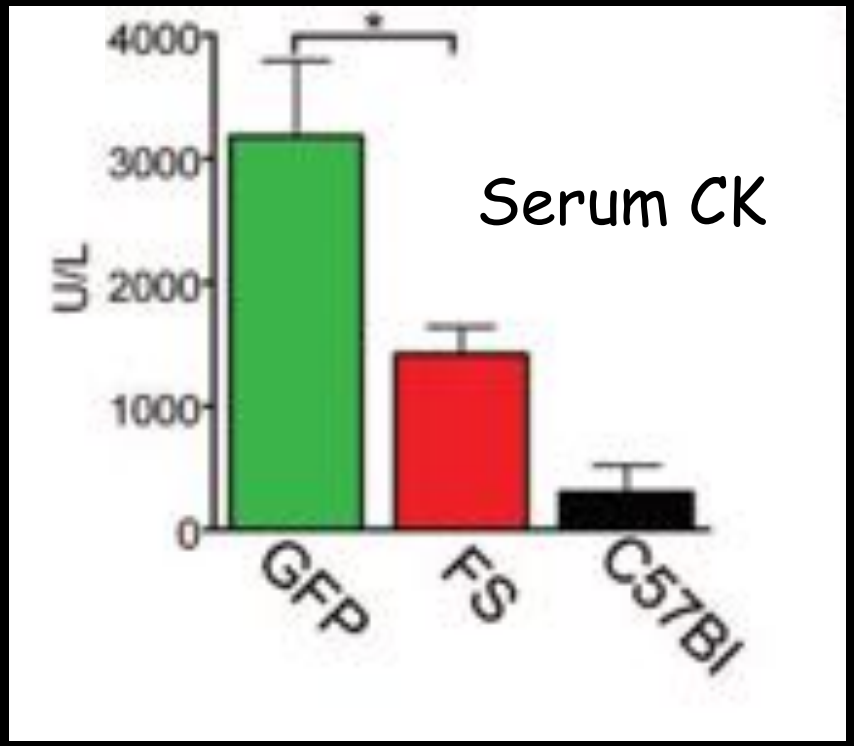


Haidet et al PNAS 2008;105:4318-4322



*Gene Expression for 1 year





3 months post gene transfer

Normal Reproduction in Mice

Reproductive Study (C57Bl/10)

Group	Mean Litter Size (SD)	
AAV1-FS Male Treated x Untreated Female (n=4)	9.0 (2.582)	T
AAV1-FS Female Treated x Untreated Male (n=4)	9.25 (1.708)	T
Untreated Male x Untreated Female (n=4)	9.0 (2.160)	U

Reproductive Study (*mdx*)

Group	Mean Litter Size (SD)	
AAV1-FS Male Treated x Untreated Female (n=3)	4.5 (0.707)	T
AAV1-FS Female Treated x Untreated Male (n=2)	2.0 (0)	T
Untreated Male x Untreated Female (n=6)	3.83 (1.169)	U

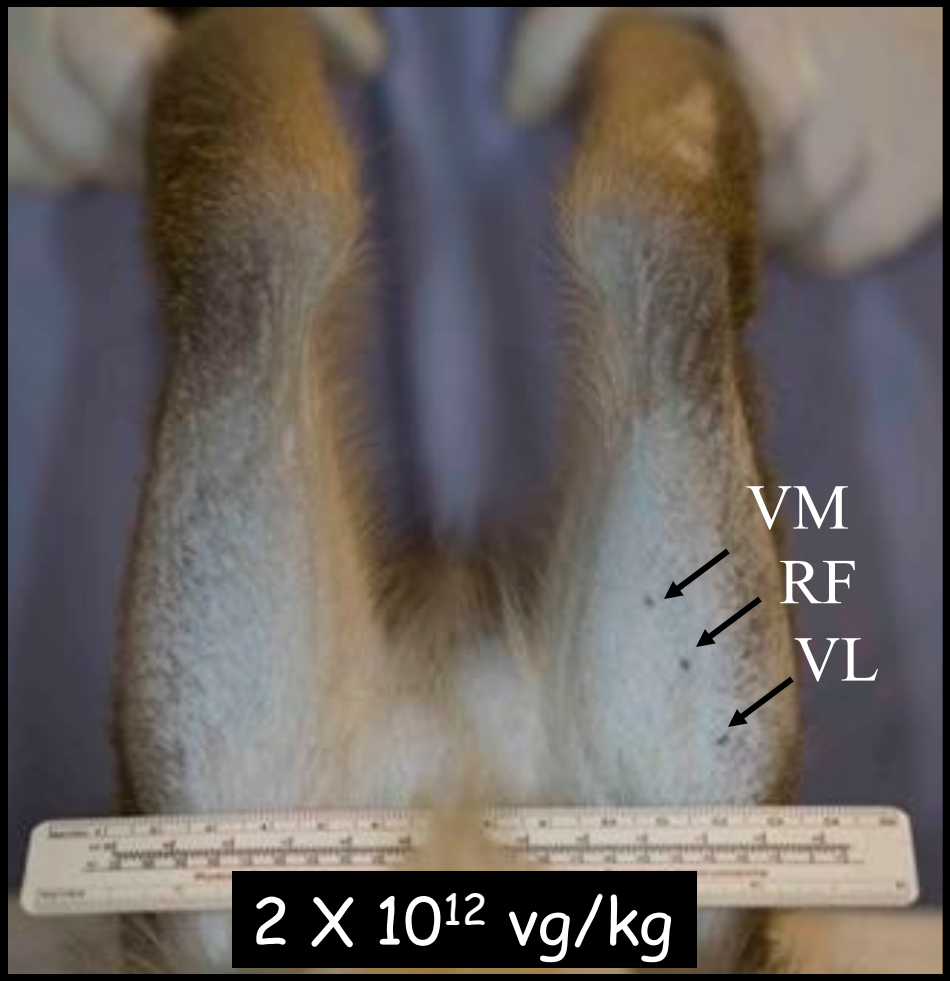
Follicle Stimulating Hormone in C57/Bl10Mice

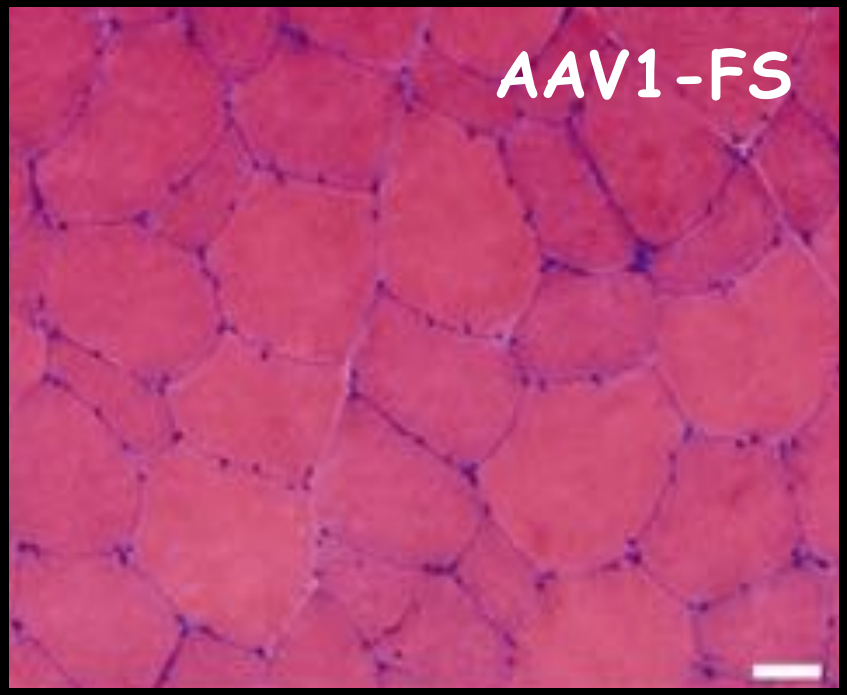
	4 wks	12wks	20wks	
1763	2.56	14.2	13.64	Female Controls
1764	13.44	2	2.65	
1765	2.78	1.68	4.56	4.650 ± 5.2
1766	2.11	2.43	1.05	
1767	2.56	4.85	1.35	
1741	2.43	2.25	1.54	Female FS
1743	4.58	1.82	10.81	
1744	3.16	2.28	2.06	4.305 ± 4.3
1745	9.33	2.15	2.81	
1758	20.02	22.63	19.84	Male Controls
1759	23.14	21.71	18.55	
1760	22.38	21.92	22.7	20.258 ± 1.5
1761	17.68	21.18	19.69	
1762	17.64	23.23	20.51	
1746	18.42	18.84	17.63	Male FS
1771	18.84	23.09	21.95	
1748	18.35	16.04	19.32	21.13 ± 2.6
1749	17.8	23.13	23.88	
1750	18.33	18.54	22.87	

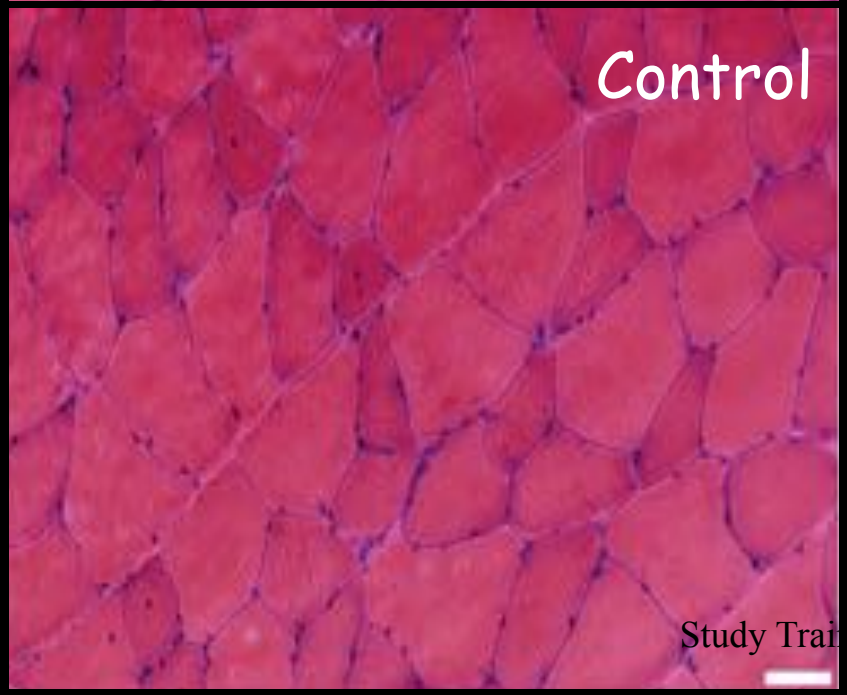
Can the Mouse Studies Predict Safety and Efficacy in a Clinical Trial ?

Moving to Non-Human Primate to Simulate Clinical Trial

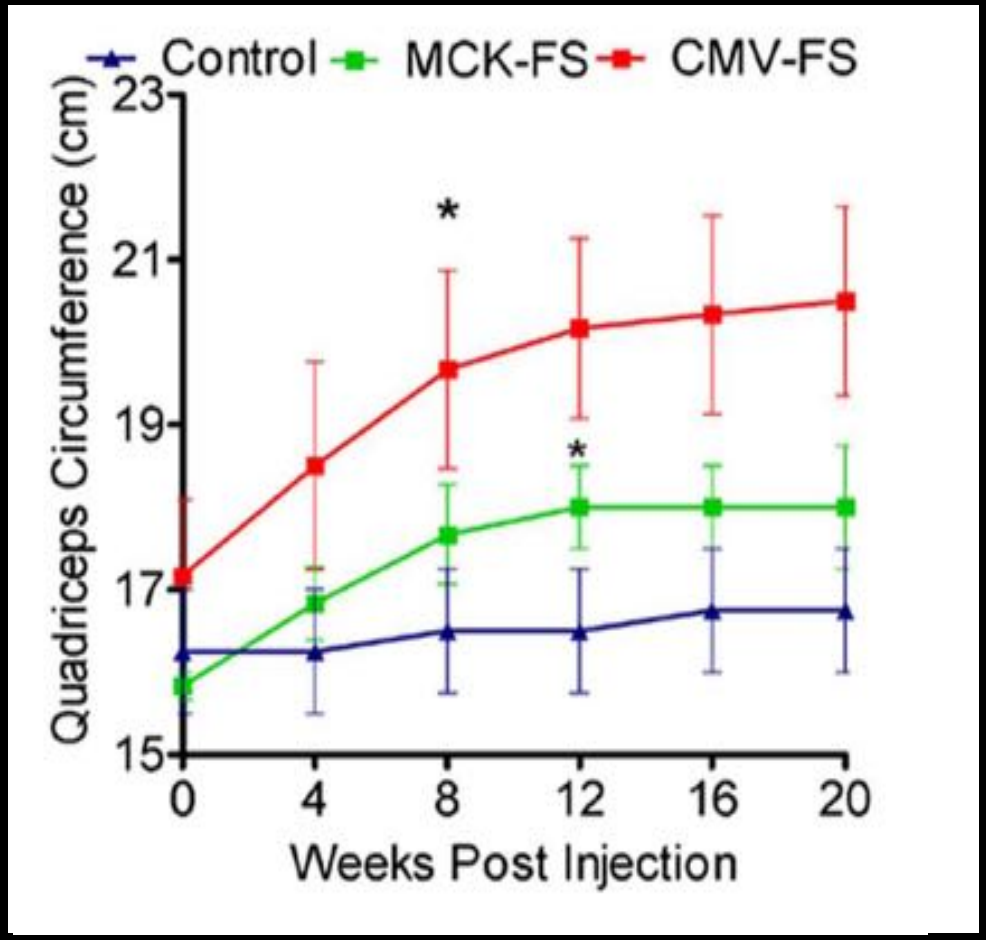
FS344 Gene Transfer to Monkey







5 MO POST GENE TRANSFER *



2 X 10^{12} vg/kg

Study Trai

Functional Improvement

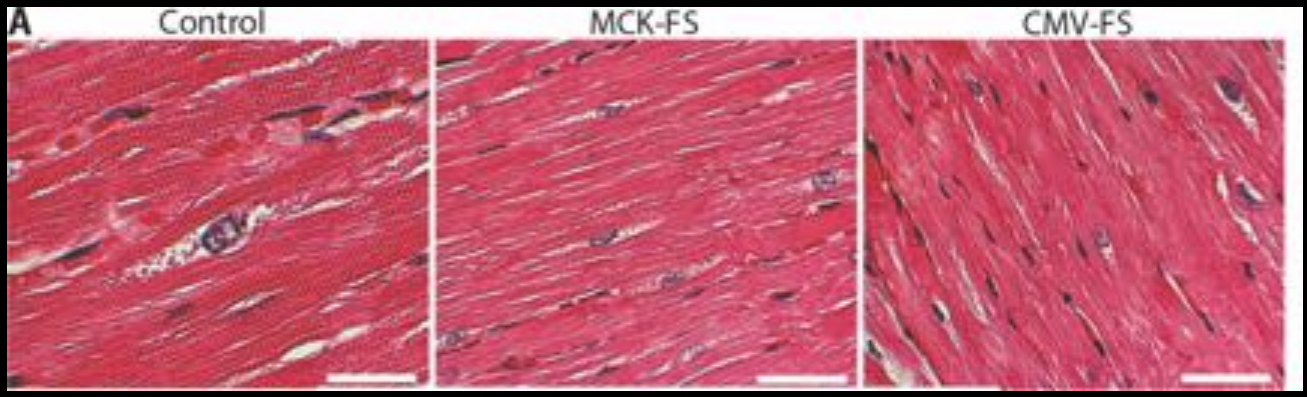
Promoter	Twitch Force		Tetanic Force	
	Untreated Leg	FS-Treated	Untreated leg	FS-Treated
MCK	17.0	19.0 (11.8%)	65.0	73.0 (12.3%)
CMV	19.0	24.0 (26.3%)	64.0	72.0 (12.5%)

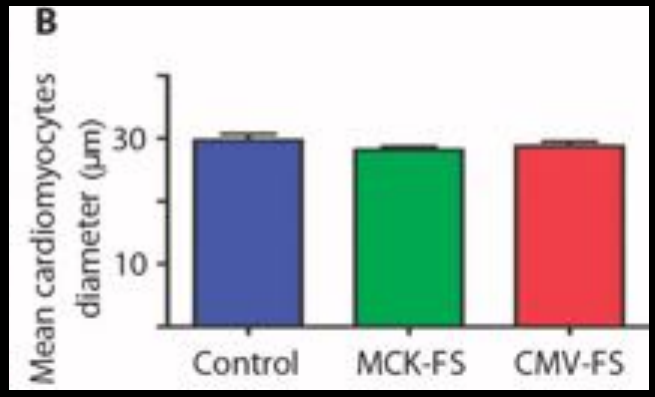
Hormone Levels Post AAV1-FS Treatment NHP in Pre-clinical Studies

Table 3. Hormonal data of rAAV1- FS344 treated non-human primates

Time Point	Animal	FSH (ng/ml)		LH (ng/ml)		Estradiol (ng/ml)	Testosterone (ng/ml)
Baseline	No	Males	Females	Males	Females	Females	Males
	1	0.53 √	1.7	0.34	0.57 √	49.08 √	0.13
	2	0.74	1.39	2.21	1.03	17.65	8.28 √
	3	0.39		0.35			1
	4	0.34		0.18			0.12
5 months	1	0.52 √	1.65	0.68	0.51 √	74.22 √	0.27
	2	0.42	1.39	0.78	2.35	61.3	9.02 √
	3	0.5		0.55			4.99
	4	0.36		0.25			1.67
15 months	1		1.21		0.64	72.01	
	2	0.29	2.42	0.23	2.34	56.54	5.48
	3						
	4	0.45		0.42			7.42

No Cardiotoxicity**
5 and 15 months



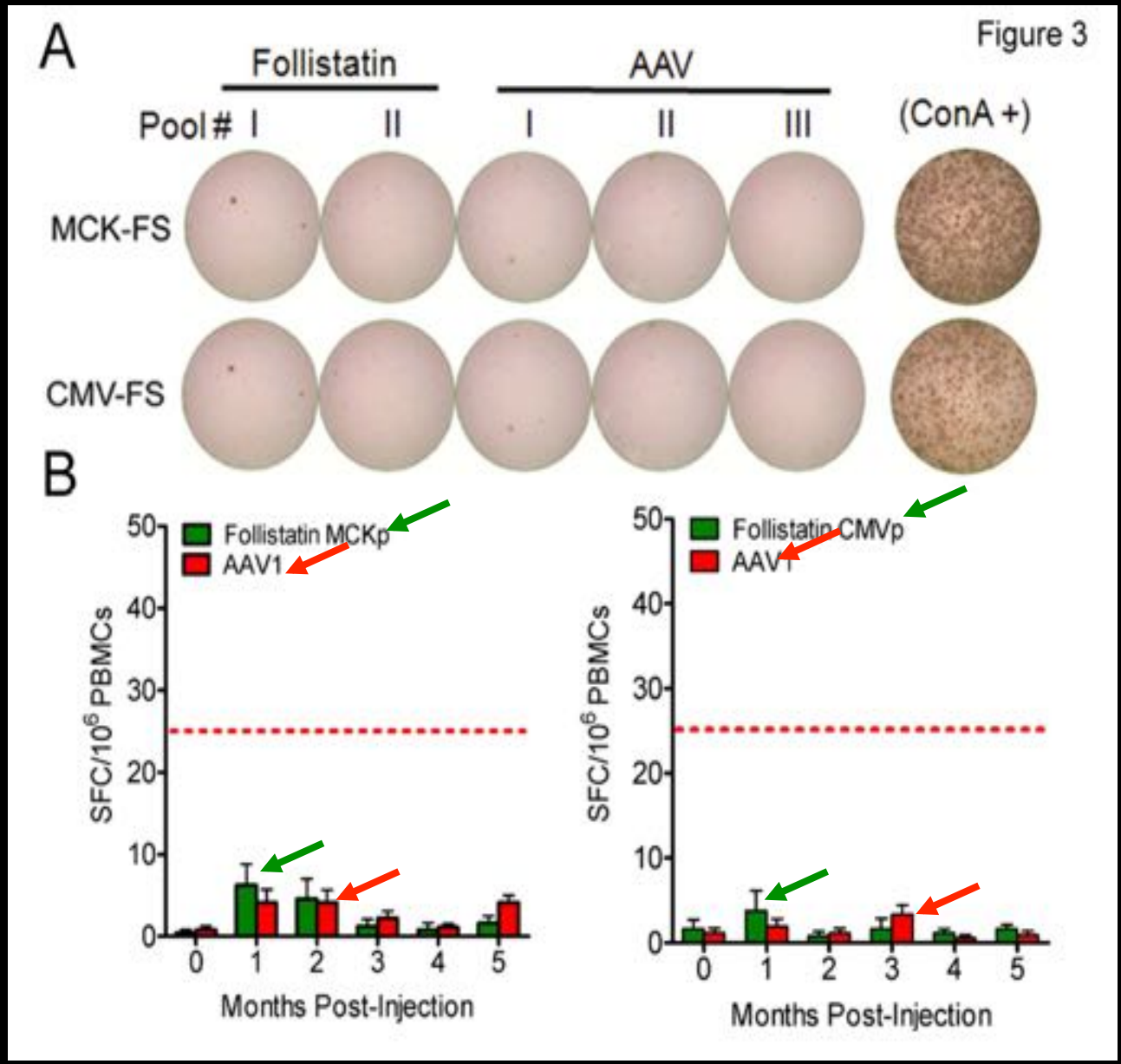


Clinical Chemistries
Monkeys used in Pre-clinical Studies

Table 2. CBC Chemistry of rAAV1-FS344 treated non- human primates

Parameter	MCK-FS			CMV-FS		
	Baseline	5 months Post Injection	15 months Post Injection	Baseline	5 months Post Injection	15 months Post Injection
Hgb (mg/dL)	11.7 ± 1.2	12.3 ± 0.7	13.5 ± 0.6	12.9 ± 0.9	12.9 ± 0.3	12.6 ± 0.8
WBC (K/cu mm)	9.4 ± 3.6	11.0 ± 1.8	7.5 ± 1.7	13.2 ± 1.7	10.8 ± 2.8	15.5 ± 8.9
Platelets (K/cu mm)	444.7 ± 78.6	473.7 ± 101.5	448.5 ± 34.6	475.3 ± 21.2	470.0 ± 10.8	432.0 ± 39.6
CK (U/L)	282.3 ± 123.3	103.3 ± 34.0	261.0 ± 97.6	315.1 ± 436.8	–	141.0 ± 5.7
ALT (U/L)	29.7 ± 12.9	19.7 ± 2.1	31.5 ± 2.1	28.7 ± 10.3	21.7 ± 4.6	29.5 ± 6.4
AST (U/L)	35.3 ± 3.51	34.7 ± 9.9	37.5 ± 6.4	44.3 ± 11.4	31.7 ± 6.0	35.5 ± 4.9
BUN (mg/dL)	19.0 ± 1.0	12.3 ± 1.5	16.0 ± 1.4	16.3 ± 4.9	16.0 ± 4.4	18.5 ± 7.8
Creatinine (mg/dL)	0.5 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.9 ± 0.2	0.9 ± 0.1	0.9 ± 0.1
GGT (U/L)	72.0 ± 28.8	92.0 ± 38.7	77.5 ± 51.6	77.0 ± 20.7	71.3 ± 18.2	75.0 ± 9.8

IFN-γ ELISpot to FS344 and AAV1



Necropsies of NHP used in Pre-Clinical Studies

- Full necropsy on all monkeys
  - slides on each organ evaluated by a board certified veterinary pathologist blinded to treatment group (control vs FS)
- No treatment-related abnormalities found in heart, liver, lung, spleen, kidney, testis, ovary and uterus (5 &15 months)

Pre-IND Meeting
Discussion of Toxicology-Biodistribution

IND-Enabling Toxicology

- InVivo study in mice
- Study Evals: 6, 12 , 24, 36 weeks post-injection

Group No.	No. of Toxicity Animals		No. of Alternate Animals[a]		Dose Material	Dose Level (vg/kg)	Dose Volume (µL[b])
	Males	Females	Males	Females			
1	40	40	5	5	Diluent	0	30
2	40	40	5	5	AAV1.FS344	2.0×10^{12}	30
3	40	40	5	5	AAV1.FS344	2.0×10^{13}	30

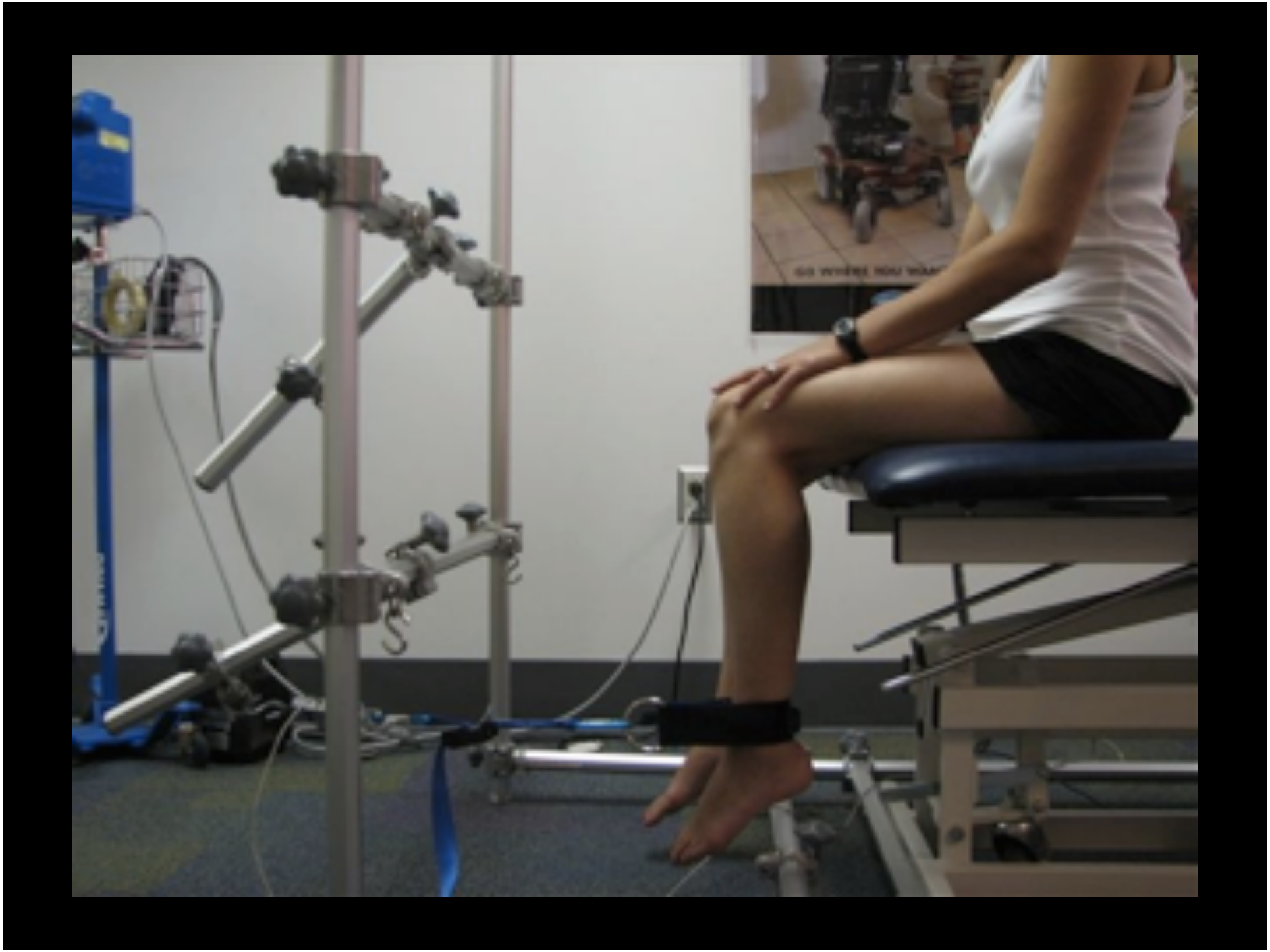
Table 7: [a]Five alternate animals/sex/group were dosed. If needed during the study period, an alternate animal was used to replace a toxicity phase animal. [b]30 µL per quadriceps muscle.

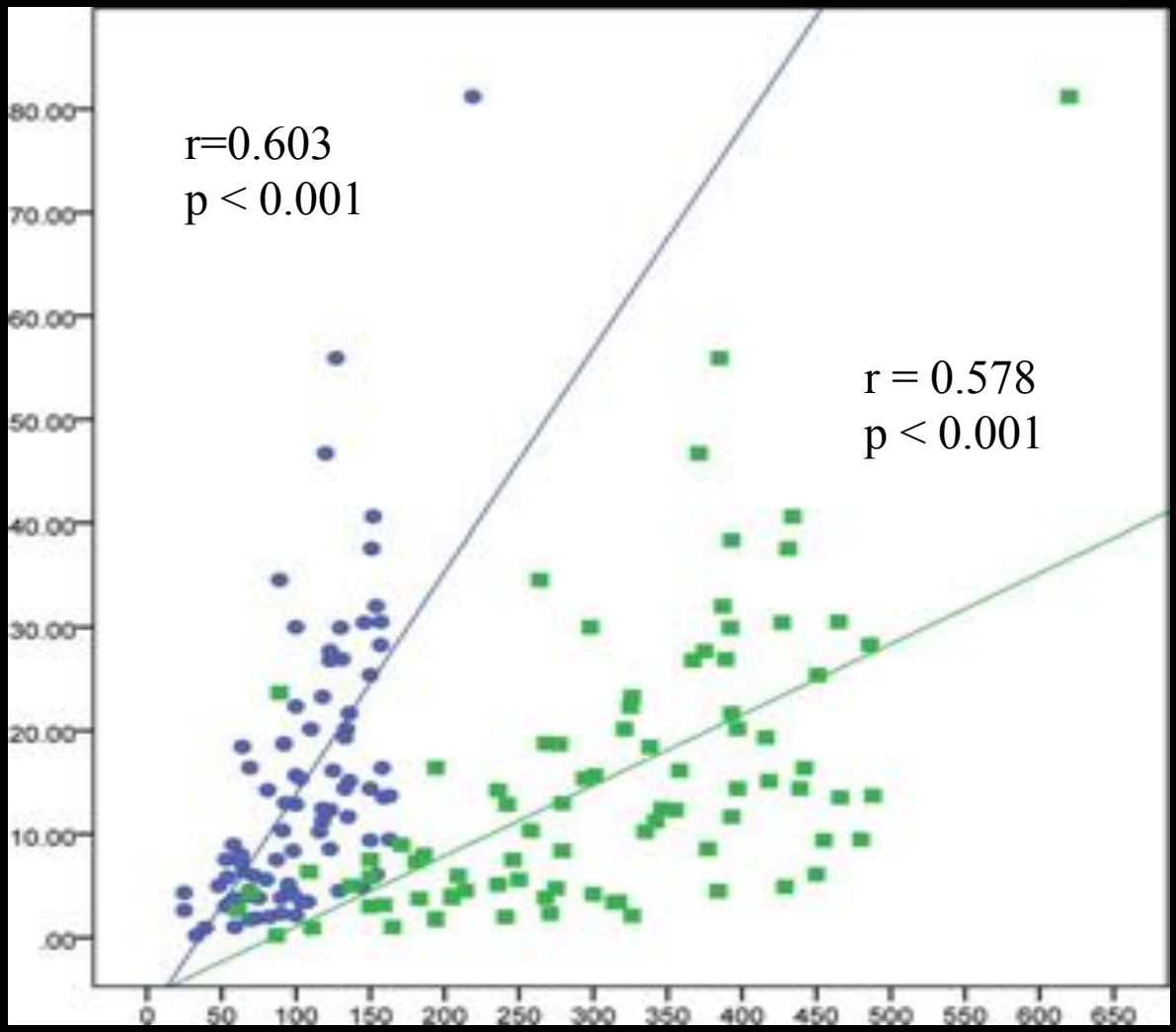
Taking this to clinical trial

Biopotency Assay
Dose Escalation AAV1.FS344 Study
Percent increase in mean fiber diameters

#	Treatment group	Mean Fiber Diameter (μm)	Increase in fiber diameter over control	% increase in fiber size over control
1	Vehicle Control	43.5 ± 3.9	-	-
2	2e11vg/kg	45.7 ± 4.2	2.2	5.0
3	6e11vg/kg	56.9 ± 4.0	13.4*	30.8
4	2e12vg/kg	62.1 ± 5.0	18.6*	42.7
5	2e13vg/kg	66.7 ± 4.8	23.1*	53.2

Prepare for Clinical Trial
Develop Outcome Measures



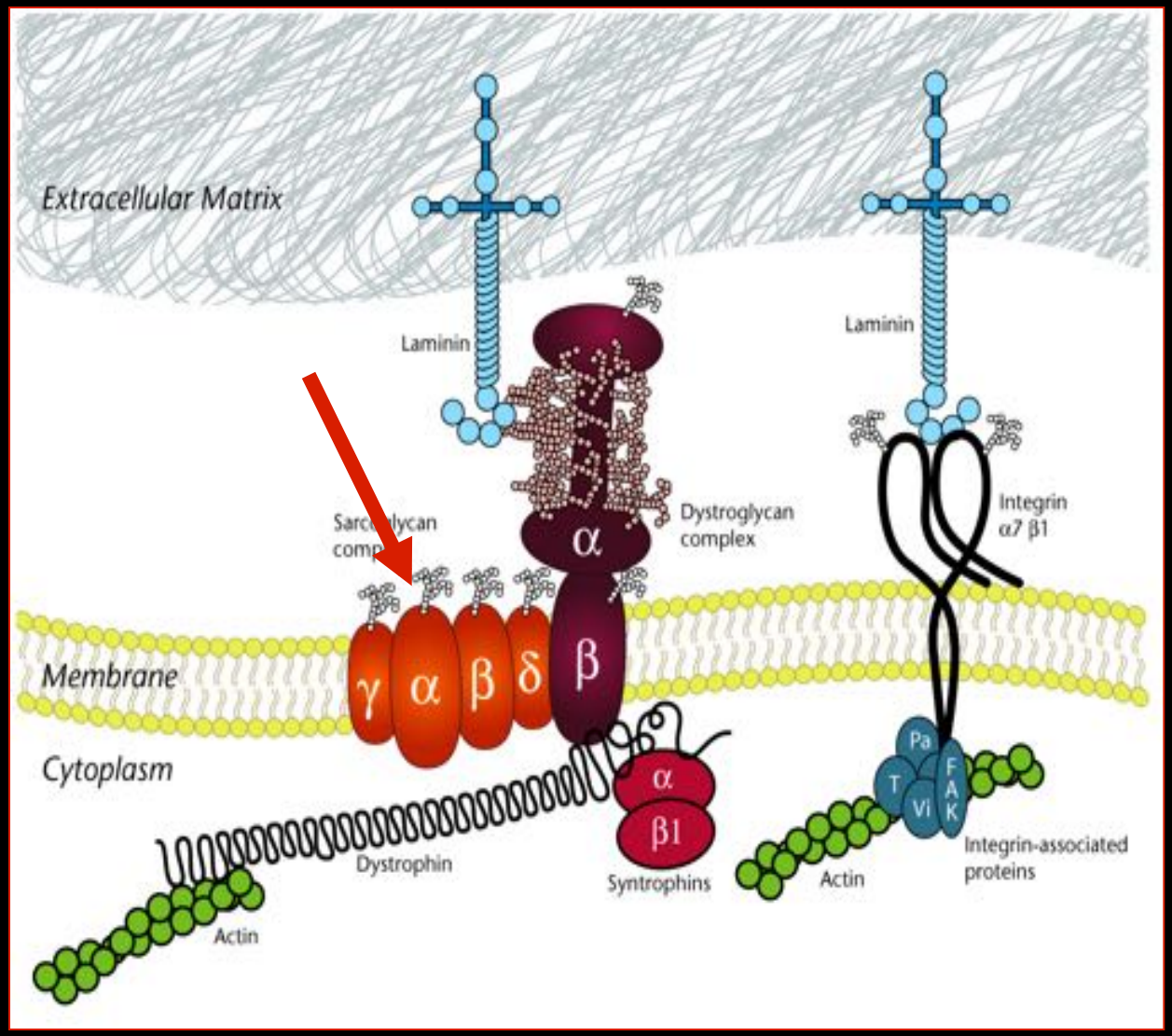

r=0.603
p < 0.001
r = 0.578
p < 0.001
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
.00
0
50
100
150
200
250
300
350
400
450
500
550
600
650

AAV1.Follistatin Clinical Trial

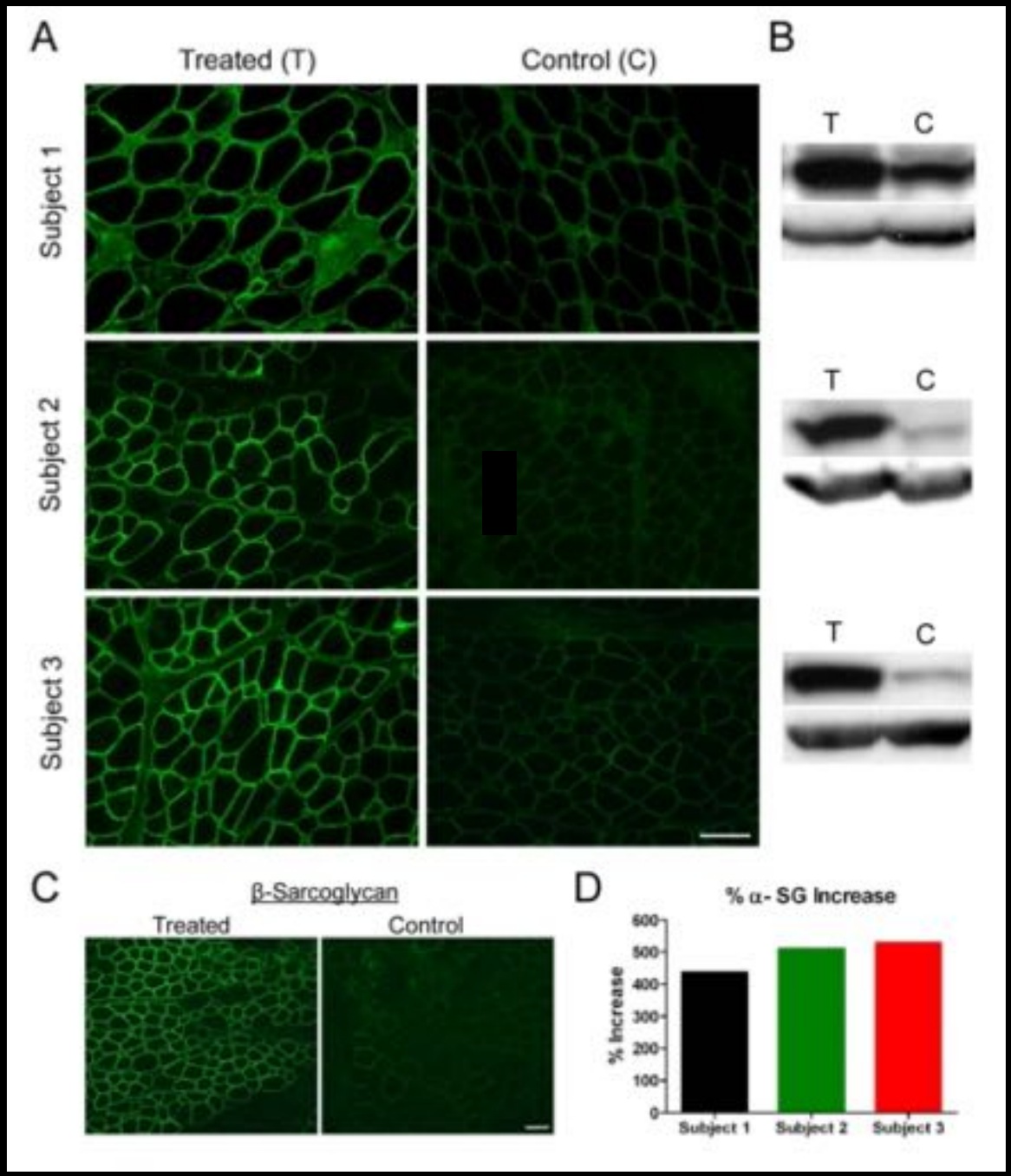
- 18 subjects (9 sIBM /9 Becker muscular dystrophy patients)
- Dose escalation study (2e11vg/kg) injection of AAV1.CMV.FS344 into quadriceps
- Outcome: 6MWT and Quantitative myometry of Knee extensors
- Muscle biopsies at 3 months and 6 months
- Patients will be followed for 2 years

Circumventing Barriers to Gene Expression

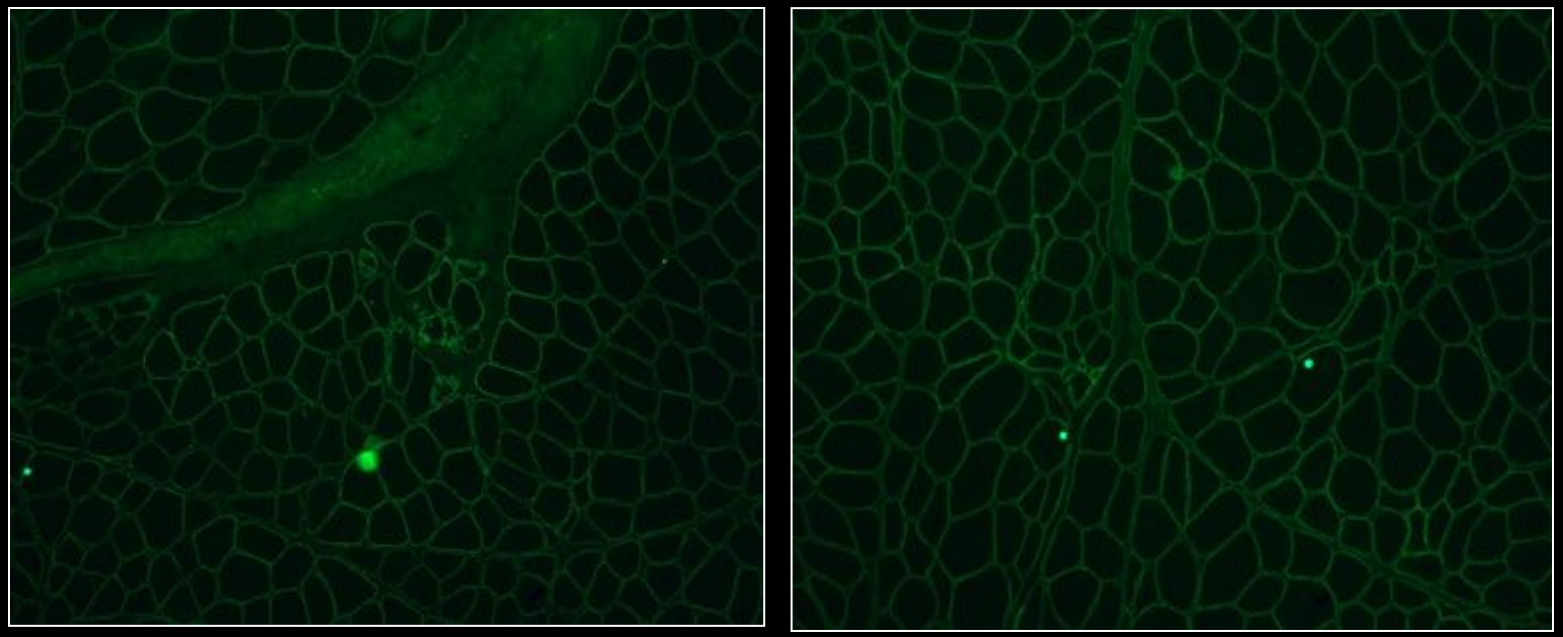
- Avoid pre-existing immunity to AAV
- Potential for immune response to transgene



Sarcoglycans

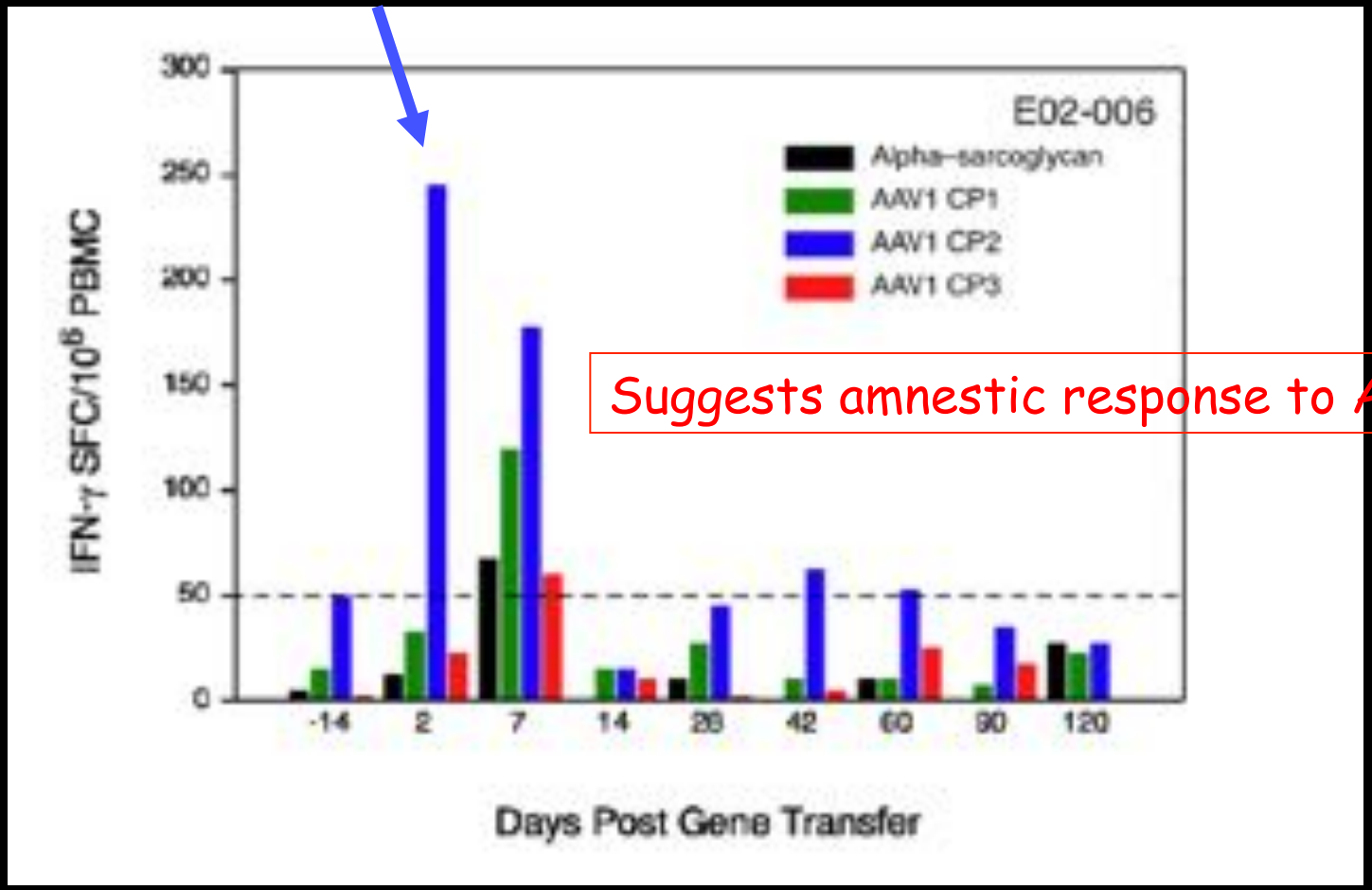

A
Treated (T)
Control (C)
Subject 1
Subject 2
Subject 3
B
T
C
T
C
T
C
C
β-Sarcoglycan
Treated
Control
D
% α- SG Increase
% Increase
600
500
400
300
200
100
0
Subject 1
Subject 2
Subject 3

Patient 02-006
EDB Injection June 24, 2009
Muscle Biopsies Dec 17, 2009



- No significant increase in Gene expression at 6 months by IF Bioquant analysis or WB

T cell Immunity to AAV Capsid
INF-γ ELISpot Assay



Suggests amnestic response to AAV1

Pre-existing immunity to transgene product

The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

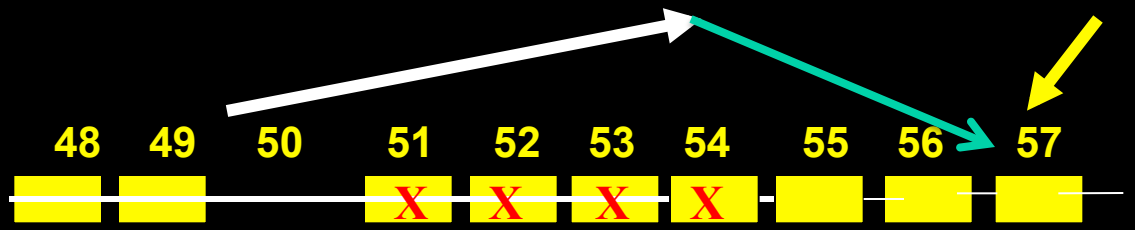
Dystrophin Immunity in Duchenne's Muscular Dystrophy

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.

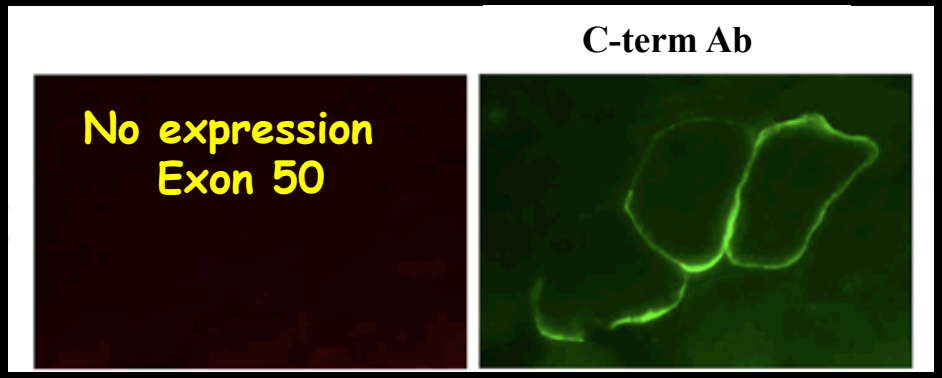
SUMMARY

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 363:1429-37



- Patient Deleted for Exon 50
  - Frameshift mutation
  - Revertant Fibers

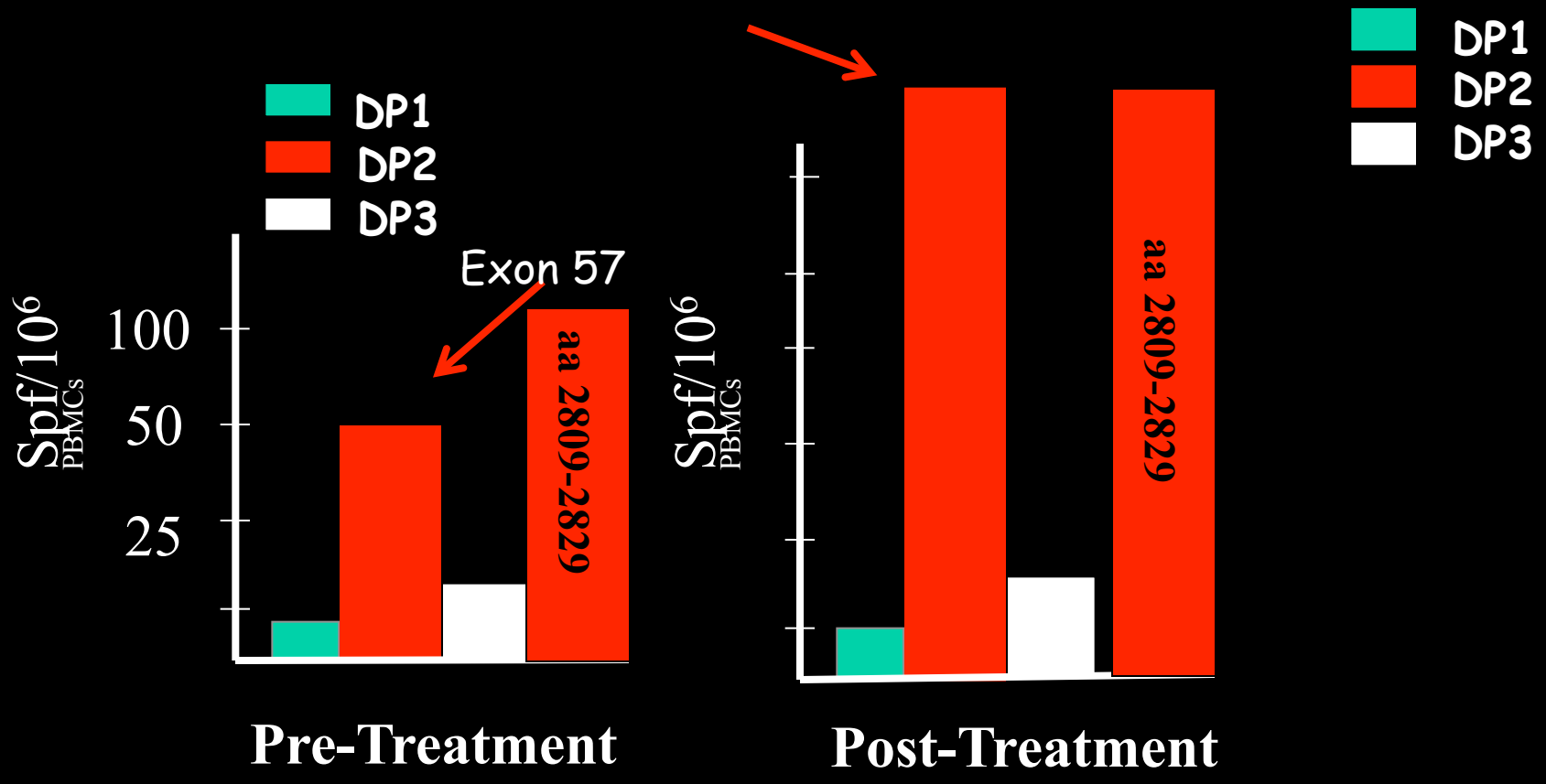


- Revertant Fibers did not express dystrophin in exons 50-54

- Spontaneous exon skipping with re-expression of dys exon 57

- Immunogenic epitope in exon 57

T Cell Immune Response in EXON 57



Immunogenic epitope specific to peptide fragment 74 spanning aa 2809- 2829 in exon 57

Formula for Success:
Plan the full translation study
From Pre-Clinical to Clinical

Cellular Immune
Christopher Walker
Katie Campbell

Gene Therapy Center
Brian Kaspar
K Reed Clark
Louise Rodino
Janaiah Kota
Brian Coley
Chris Shilling
Xiomara Rosales
Zarife Sahenk
Sarah Lewis