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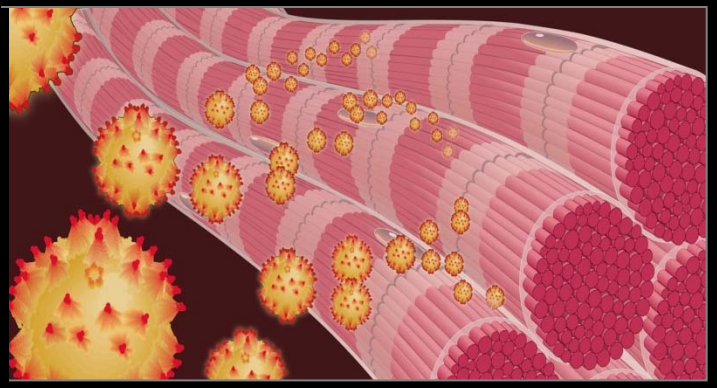


# Phase I Gene Therapy Trial using rAAV1.Follistatin in Becker Muscular Dystrophy and Sporadic Inclusion Body Myositis

RAC Protocol 10101074

Jerry R. Mendell, MD
Brian M. Kaspar, Ph.D
K. Reed Clark, Ph.D

# Presentation Objectives

- Describe Study Goals & Rationale for Trial
- Demonstrate merit of AAV1 serotype in achieving sustained gene expression in clinical trial
- Delineate the advantages of the alternatively spliced Follistatin isoform to be used in the proposed trial
- Describe pre-clinical and toxicology safety studies done in preparation for clinical trial
- Summarize the Clinical Protocol
- Address the Questions raised by reviewers during course of presentation**

# The Rationale

- Target Quadriceps muscle weakness
  - Becker muscular dystrophy
  - Sporadic inclusion body myositis
  - Highly predictable natural hx
    - 50-60 yo sIBM; 25-40 yo BMD*

- Frequent falls
  - Limb fractures
  - Loss of ambulation

- Improving quadriceps strength will preserve ambulation*

# No Alternative Treatment

- Weight training
- Electrical Stimulation
- Anabolic Steroids
- Immunosuppressive Therapy

Figure 1 - Experimental procedure

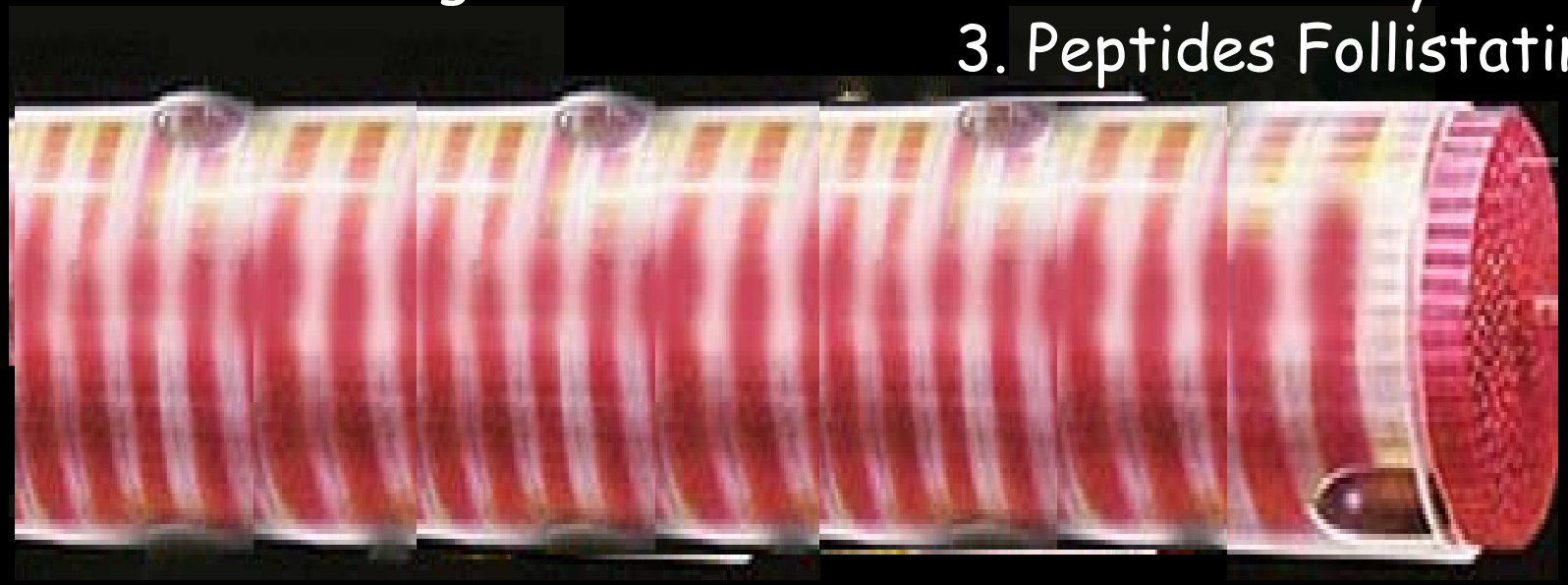
Circulating myostatin
Propeptide Complex

**Myostatin Activation**
**Protease Cleavage**

BLOCKING
SIGNALLING PATHWAY

1. Gene mutation
2. MYOAntibody
3. Peptides Follistatin

Neg control muscle growth



# MYOSTATIN REGULATION OF MUSCLE SIZE

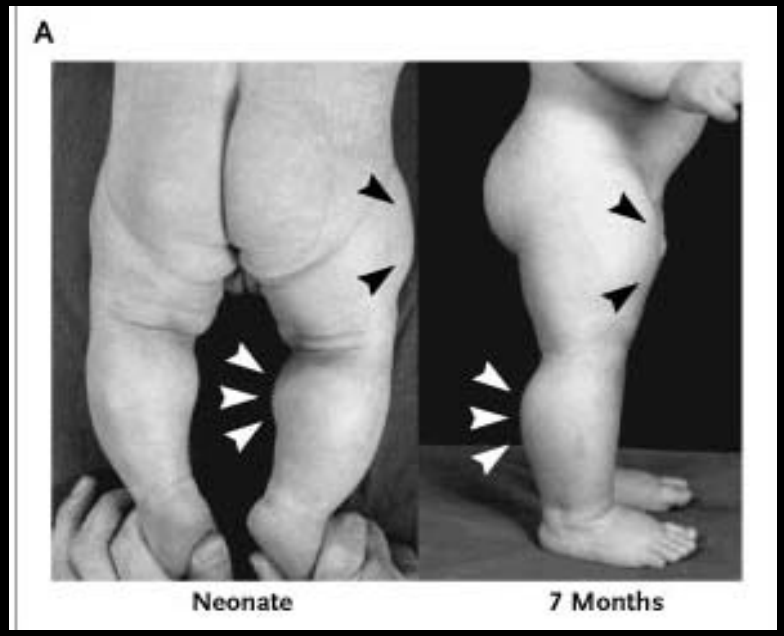
# Myostatin Gene Mutation

- Targeted disruption of the myostatin gene was a seminal observation SJ Lee:

- Renowned "Mighty" Mouse

- Spontaneous mutant Double-muscled Cow

- Even man regulates muscle growth through this pathway: Spontaneous gene mutation newborn N Engl J Med. 2004;350:2682-8



# Functional improvement of dystrophic muscle by myostatin blockade MYO ANTIBODY

Sasha Bogdanovich*†, Thomas O. B. Krag*†, Elisabeth R. Barton*, Linda D. Morris*, Lisa-Anne Whittemore‡, Rexford S. Ahima§ & Tejvir S. Khurana*

Blockade of endogenous myostatin by using intraperitoneal injections of blocking antibodies for three months resulted in an increase in body weight, muscle mass, muscle size and absolute muscle strength in *mdx* mouse muscle along with a significant decrease in muscle degeneration and concentrations of serum creatine kinase. The functional improvement of dystrophic muscle by myostatin blockade provides a novel, pharmacological strategy for treatment of diseases associated with muscle wasting such as DMD, and circumvents the major problems associated with conventional gene therapy in these disorders.

Nature 2002

ORIGINAL ARTICLE

Ann Neurology March 11, 2008

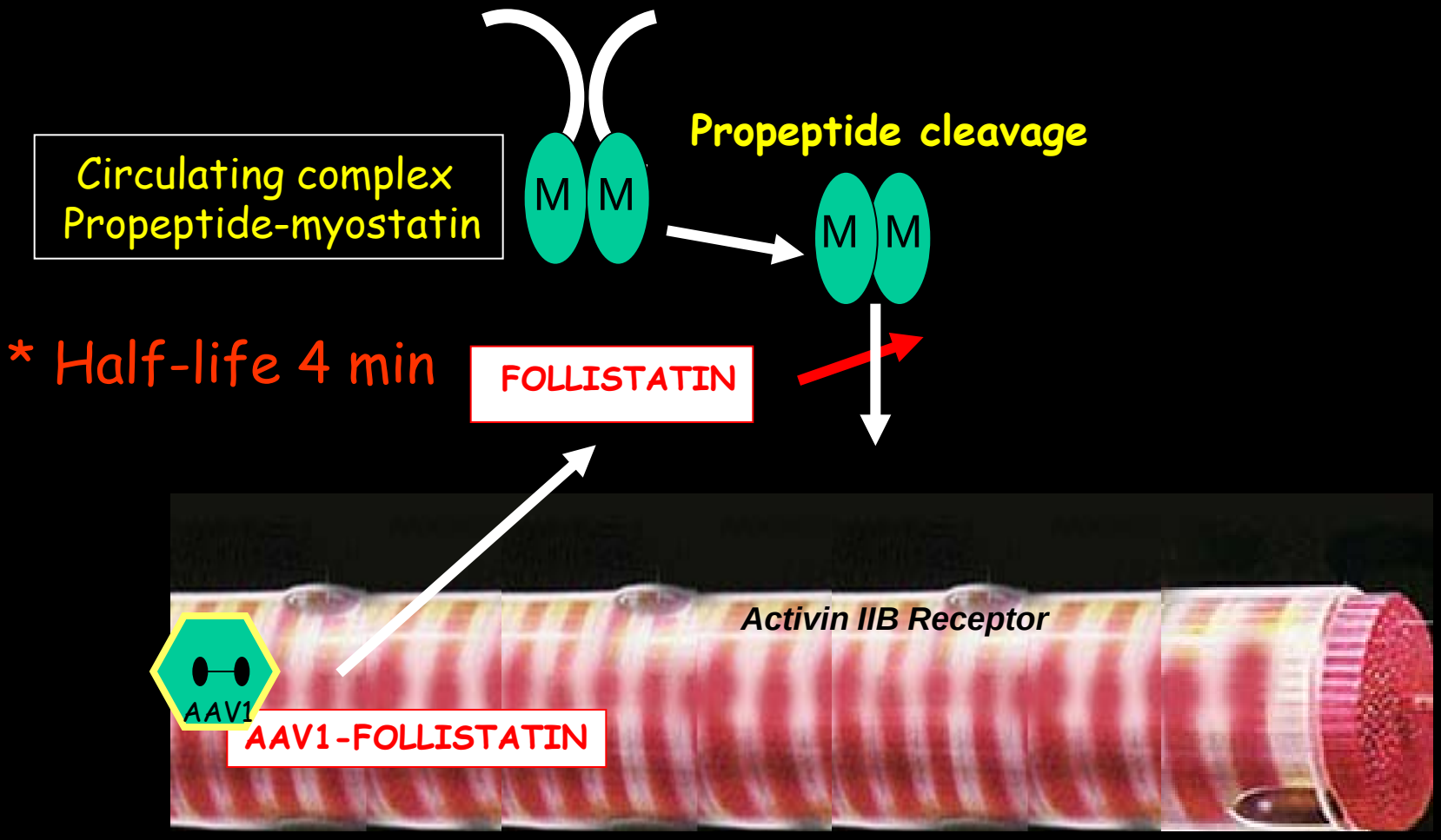
# A Phase I/II trial of MYO-029 in Adult Subjects with Muscular Dystrophy

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,[13] Stephanie A. Parsons, PhD,[13] John M. Wozney, PhD,[14] Edward R. LaVallie, PhD,[14] and Jerry R. Mendell, MD[11]

Wyeth sponsored 11 Center Trial (10 USA;1UK)

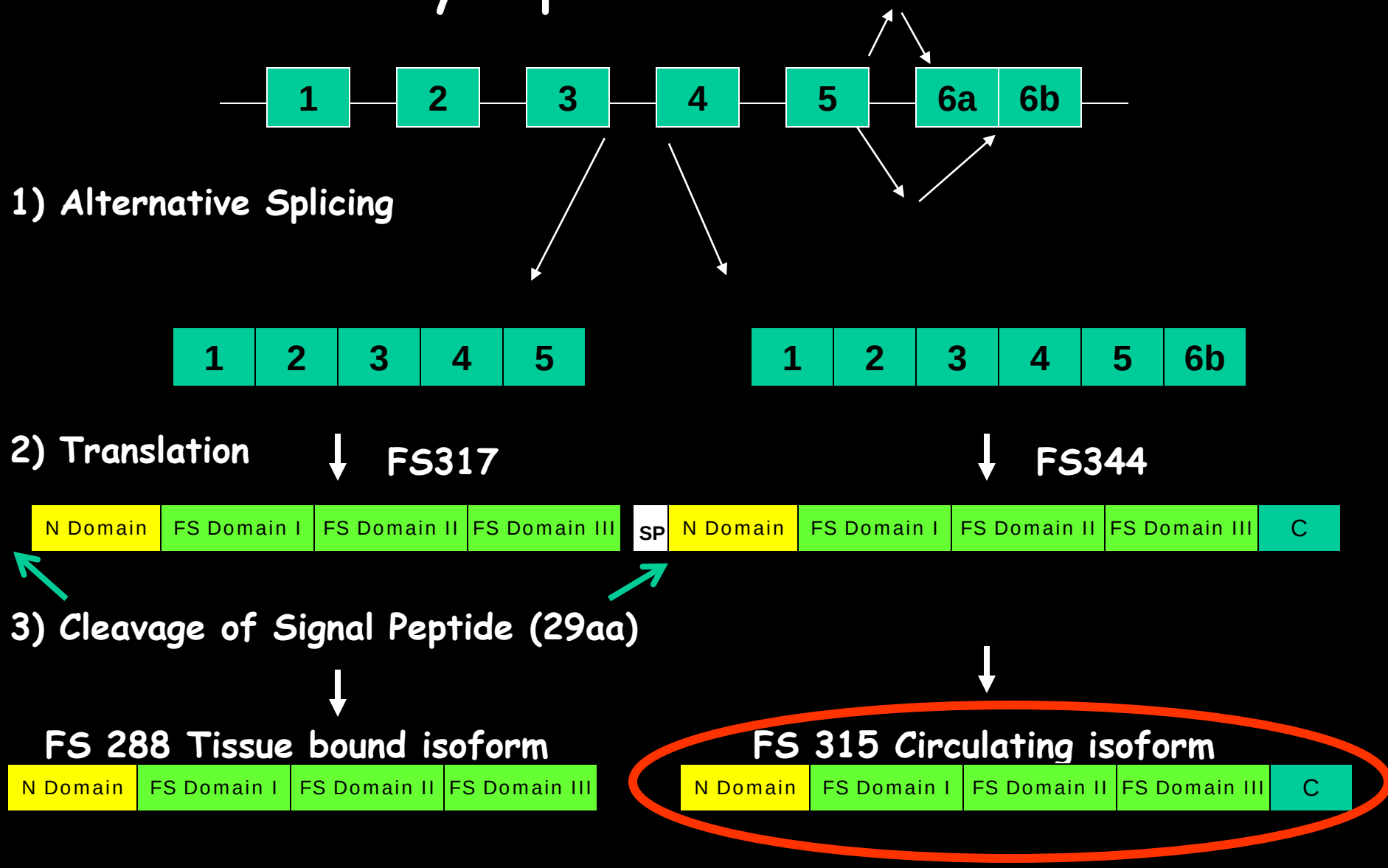
- No Clinical Benefit
- Trend toward efficacy in high dose cohort
- DLT skin hypersensivity
- Study supports safety of myostatin inhibition

# Follistatin Peptide Blockade




INJECT AAV INTO MUSCLE

# Alternatively Spliced Follistatin Gene

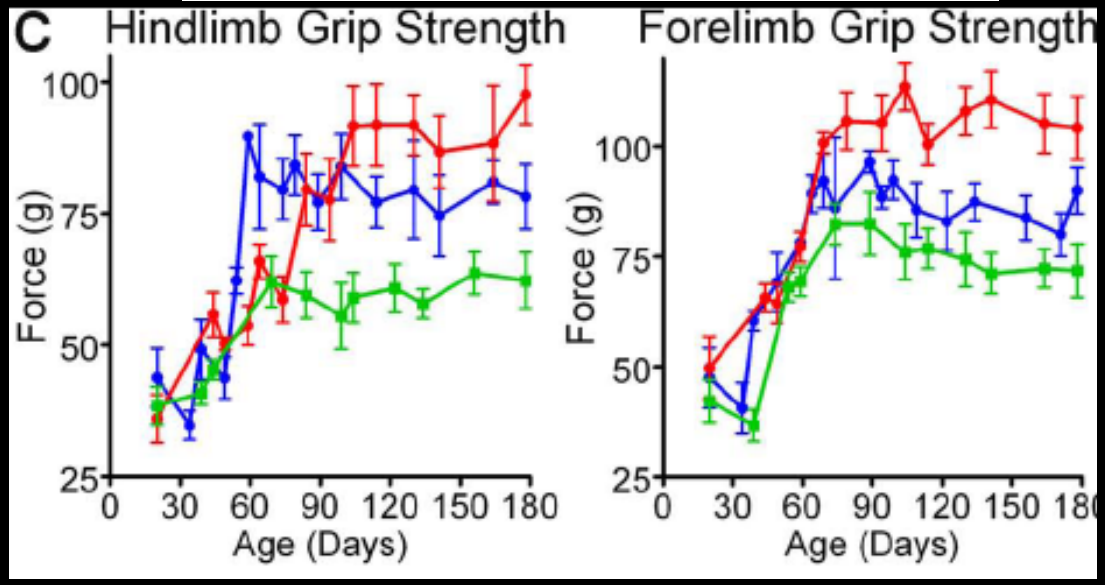


# Pre-Clinical Studies
# Treatment of *mdx* with AAV1.FS344

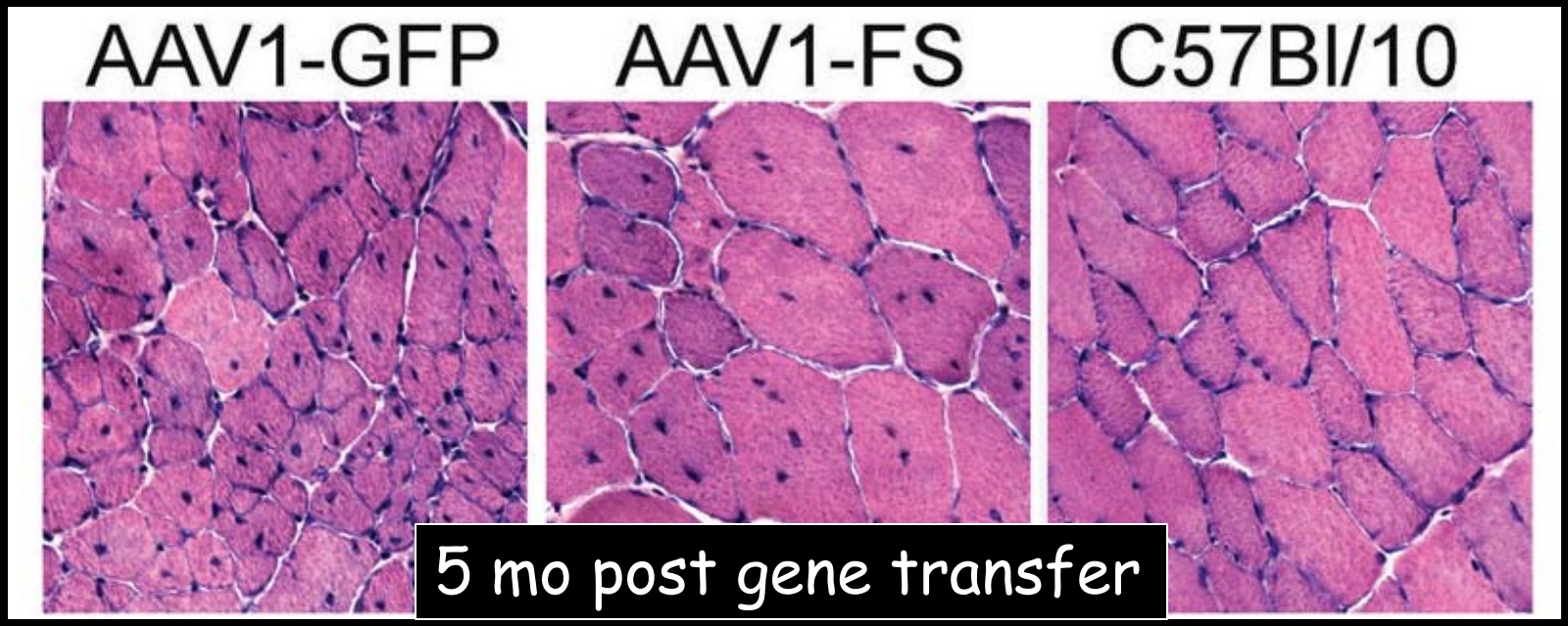
*Gene Expression 6mo

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

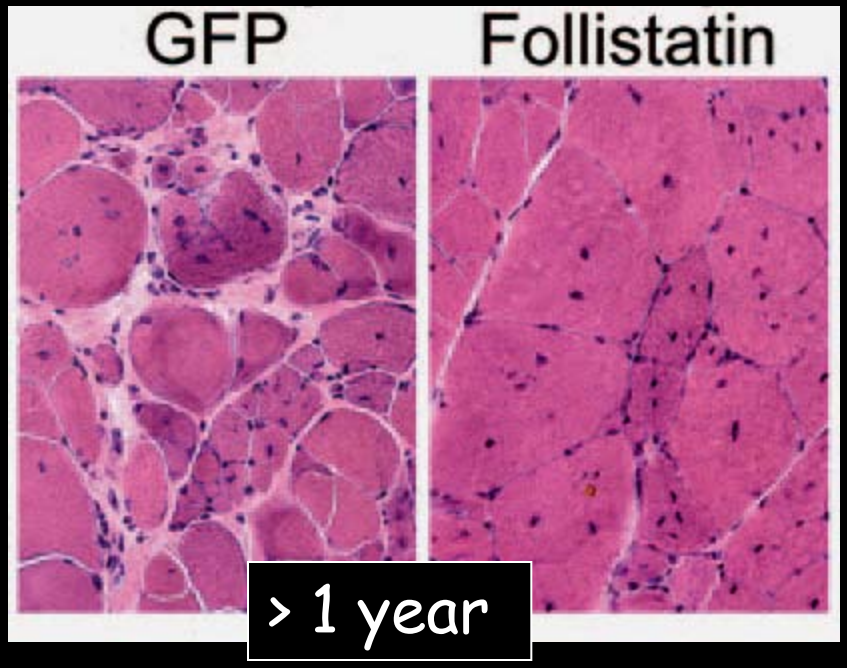
$1X10^{11}$ vg/kg

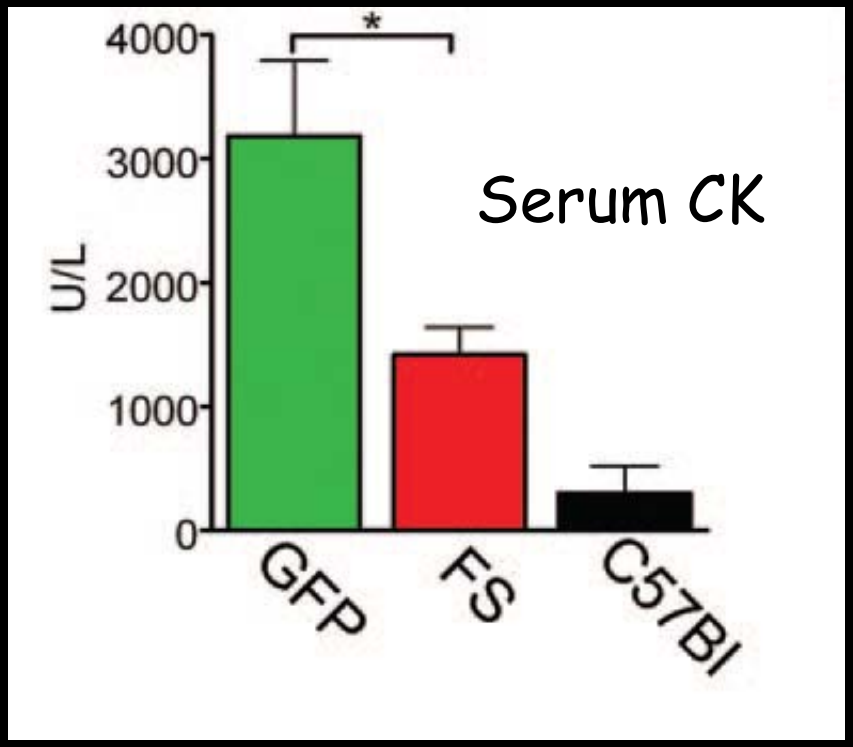



Haidet et al PNAS 2008;105:4318-4322




*Gene Expression for 1 year



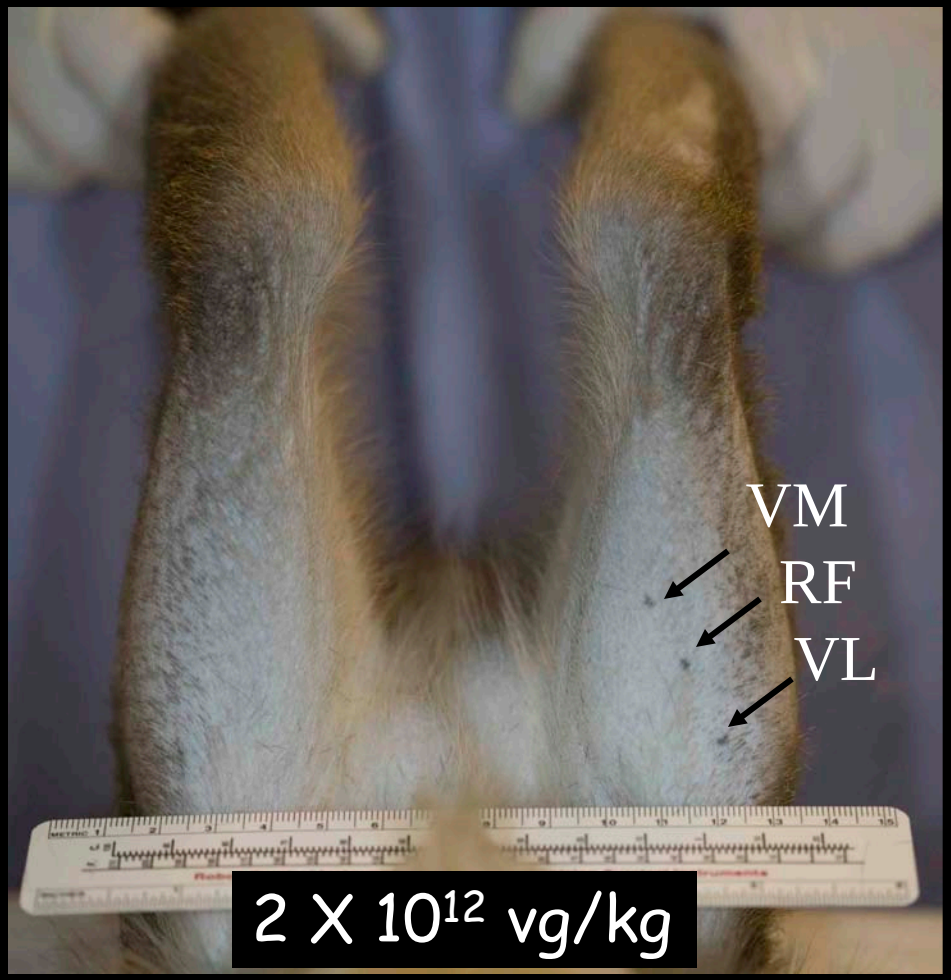


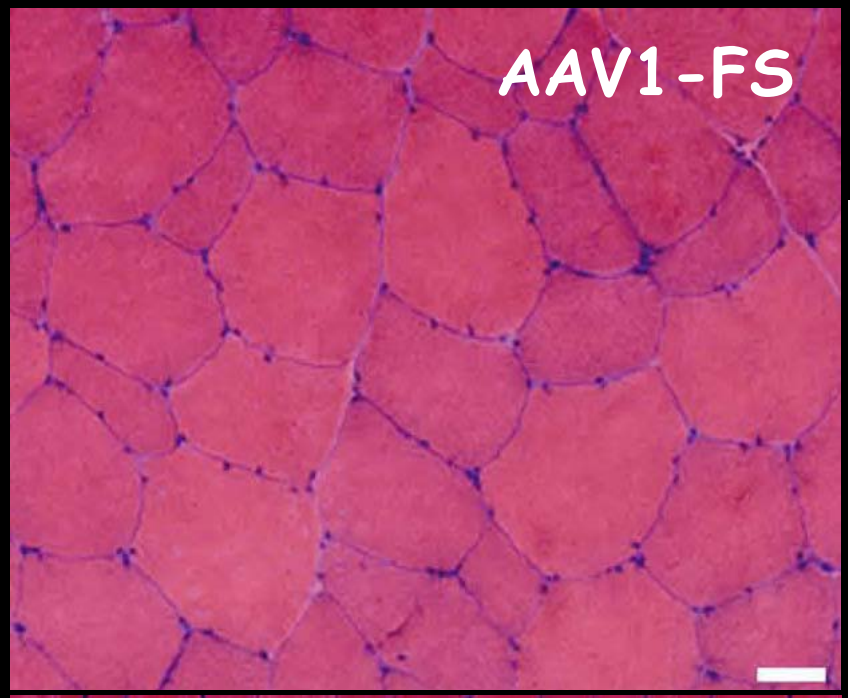

3 months post gene transfer

# Can the Mouse Studies Predict Efficacy in a Clinical Trial ?

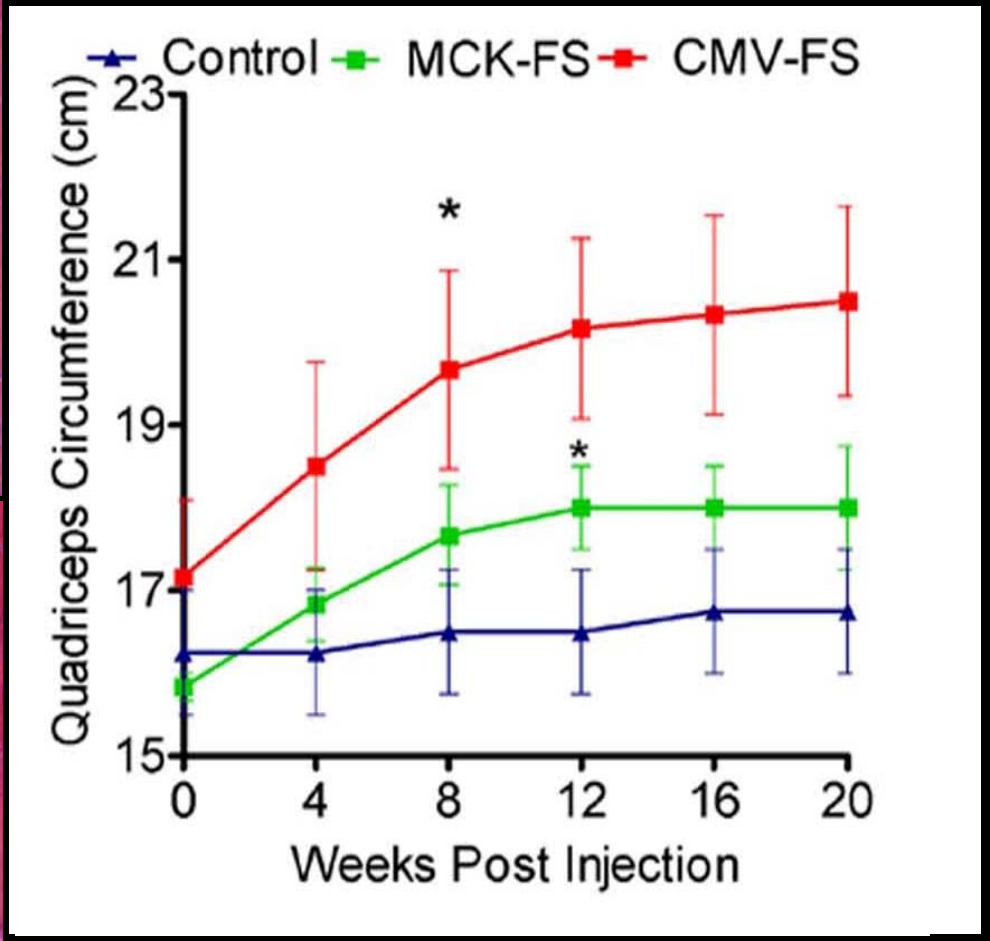
# MOVING TO NON-HUMAN PRIMATE

# FS344 Gene Transfer to Monkey





5 MO POST GENE TRANSFER *




2 X $10^{12}$ vg/kg

# 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%) |

# Safety Issues

## Follistatin member of TGFβ Superfamily

- Reproduction
- Pituitary Gonadal Axis Hormones
- Bone growth (inhibits BMPs)
- Cellular Growth and Differentiation

# 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 |
| 1765 | 2.78 | 1.68 | 4.56 |
| 1766 | 2.11 | 2.43 | 1.05 |
| 1767 | 2.56 | 4.85 | 1.35 |
| 1741 | 2.43 | 2.25 | 1.54 |
| 1743 | 4.58 | 1.82 | 10.81 |
| 1744 | 3.16 | 2.28 | 2.06 |
| 1745 | 9.33 | 2.15 | 2.81 |
| 1758 | 20.02 | 22.63 | 19.84 |
| 1759 | 23.14 | 21.71 | 18.55 |
| 1760 | 22.38 | 21.92 | 22.7 |
| 1761 | 17.68 | 21.18 | 19.69 |
| 1762 | 17.64 | 23.23 | 20.51 |
| 1746 | 18.42 | 18.84 | 17.63 |
| 1771 | 18.84 | 23.09 | 21.95 |
| 1748 | 18.35 | 16.04 | 19.32 |
| 1749 | 17.8 | 23.13 | 23.88 |
| 1750 | 18.33 | 18.54 | 22.87 |

Female Controls

4.650 ± 5.2

Female FS

4.305 ± 4.3

Male Controls

20.258 ± 1.5

Male FS

21.13 ± 2.6

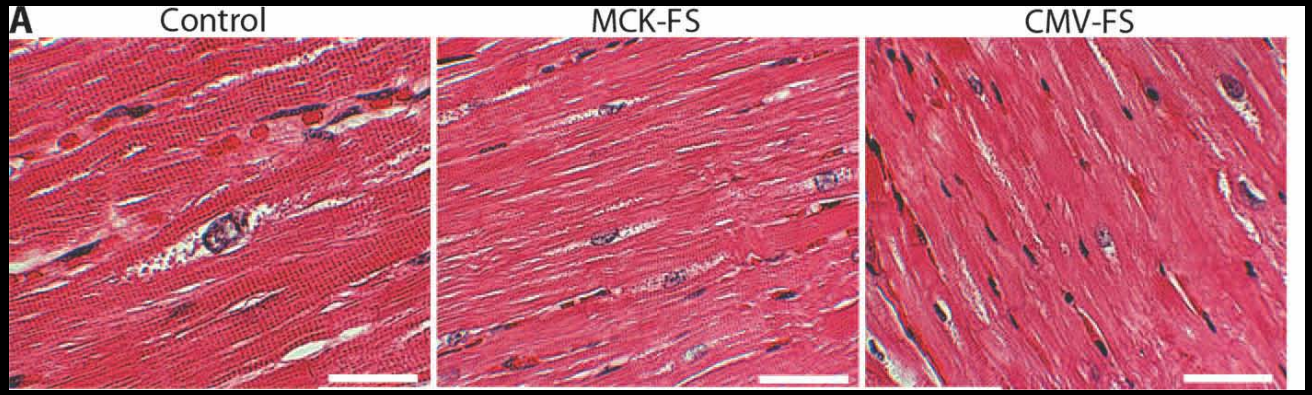
# Hormone Levels Post AAV1-FS Treatment NHP in Pre-clinical Studies

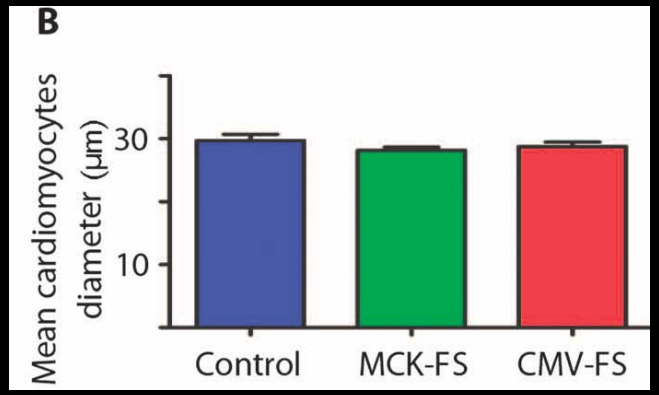
Table 3. Hormonal data of rAAV1- FS344 treated non-human primates

| 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

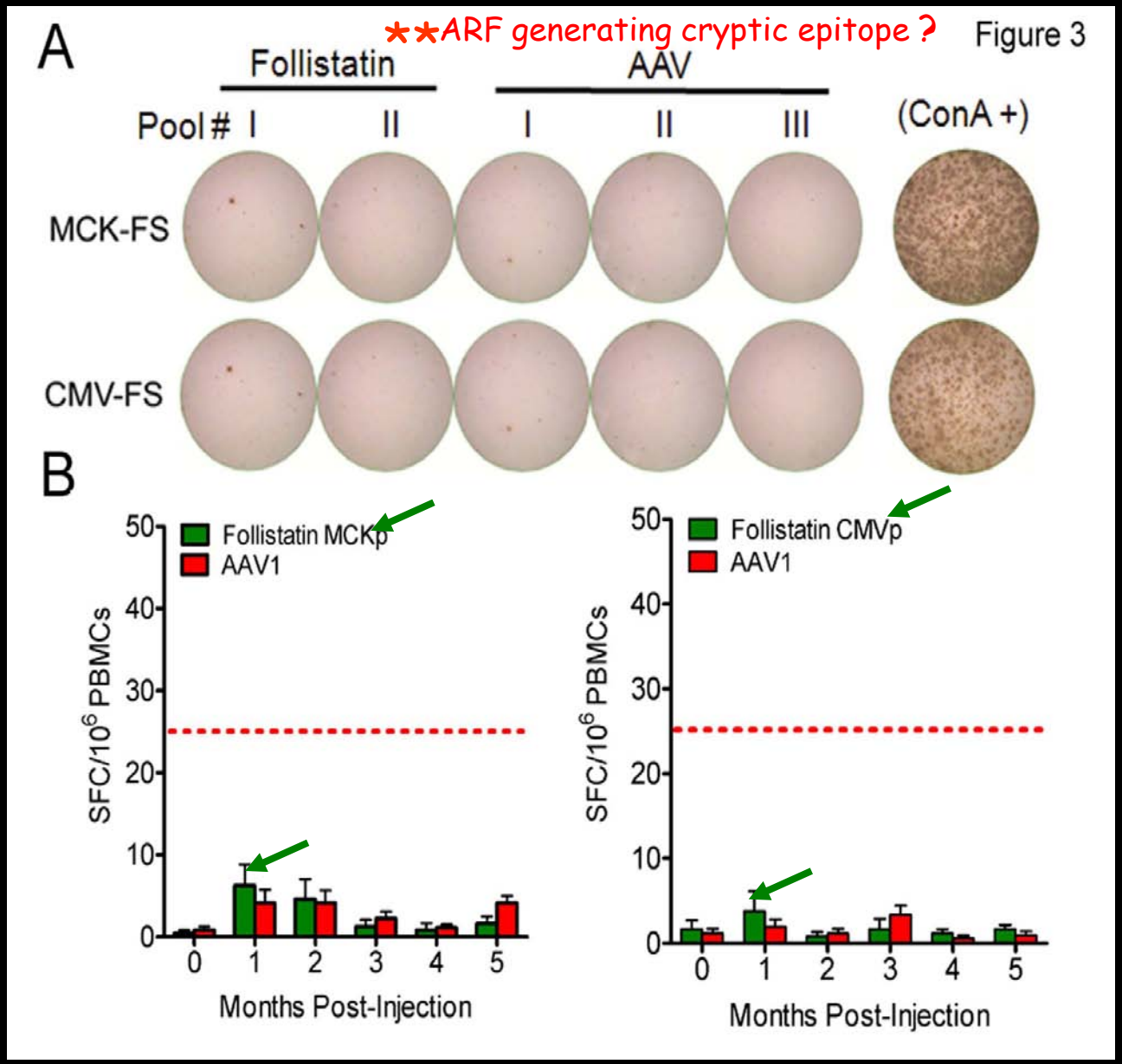
Table 2. CBC Chemistry of rAAV1-FS344 treated non- human primates

| 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 |

# Necropsies of NHP used in Pre-Clinical Studies

- Full necropsy on all monkeys
  - No treatment-related abnormalities found in heart, liver, lung, spleen, kidney, testis, ovary and uterus** (5 &15 months)

# IFN-γ ELISpot to FS344 and AAV1



# Toxicology Study

- Recommendation Pre-IND Meeting Held 2-25-08
- 36 week study Evals 6 *, 12 *, 24, 36 weeks

| 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 | $2.0 \times 10^{12}$ | 30 |
| 3 | 40 | 40 | 5 | 5 | AAV1.FS344 | $2.0 \times 10^{13}$ | 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

# 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 |

# AAV1.Follistatin Clinical Trial

- 18 subjects (9 sIBM /9 Becker muscular dystrophy patients)
- Patients with pre-existing immunity (Neutralizing ab 1:1600) to AAV1 will be excluded **

| Neutralizing antibody titer against AAV1 | | | | | |
|---|---|---|---|---|---|
| Time Post Injection | Patient #4 (E02-004) | | Patient #5 (E02-005) | | Patient #6 E02-006 |
| Pre-treatment | 1:800 | | <1:50 | | 1:1600 |
| Day 7 | 1:1600 | | 1:3200 | | >1:102400 |
| 2 weeks | 1:25600 | | 1:6400 | | >1:102400 |
| 6 weeks | 1:25600 | | 1:3200 | | >1:102400 |
| 12 weeks | 1:51200 | | 1:12800 | | >1:102400 |
| 26 weeks (6 months) | >1:102400 | | 1:12800 | | >1:102400 |

# AAV1.Follistatin Clinical Trial

- Immune Studies to AAV1 and Follistatin ELISA neutralizing ab / ELISpot throughout the trial**
- Muscle biopsies Pre-treatment and 6 months post GT **
- Grade II will be used as dose limiting toxicity unless Clinical FDA reviewer sets different standards for this protocol**
- Patients will be followed for 2 years **

## Participants

- Brian M. Kaspar, PhD
- K. Reed Clark, Ph.D
- Kevin Flanigan, MD
- Zarife Sahenk, MD, PhD
- Louise Rodino-Klapac, PhD
- Chris Walker, PhD
- Xiomara Rosales, MD
- Brian Coley, MD
- Janaiah Kota, PhD
- Chris Shilling MS

## DSMB

- Lee Sweeney, PhD UPenn
- Guangping Gao, PhD UMass
- Carrie Miceli, PhD UCLA
- Volker Straub, Newcastle