NIH RAC Presentation

Study AGTC-AAT-002

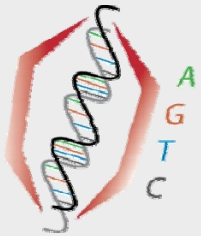
RAC Protocol 0910-1002

2 Dec 2009

Presentation Outline

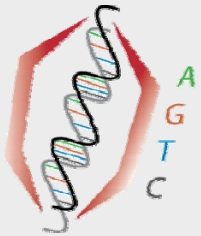
- Review of Phase 1 trial results
- Review of bridging toxicology study
- Phase 2 study overview
- Responses to RAC review

Review of Prior Phase 1 Study

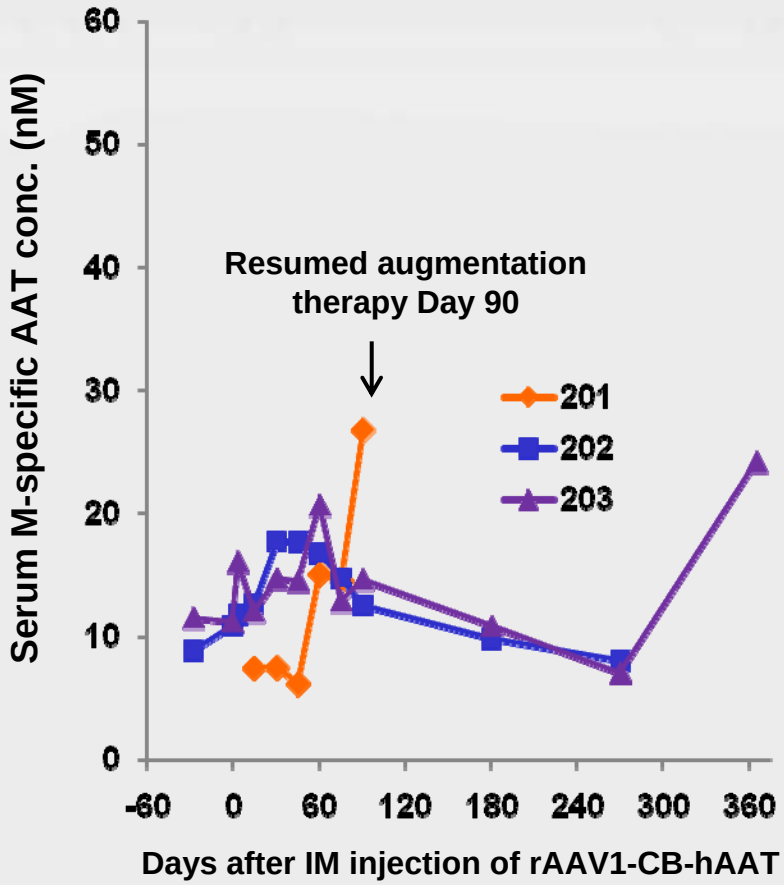


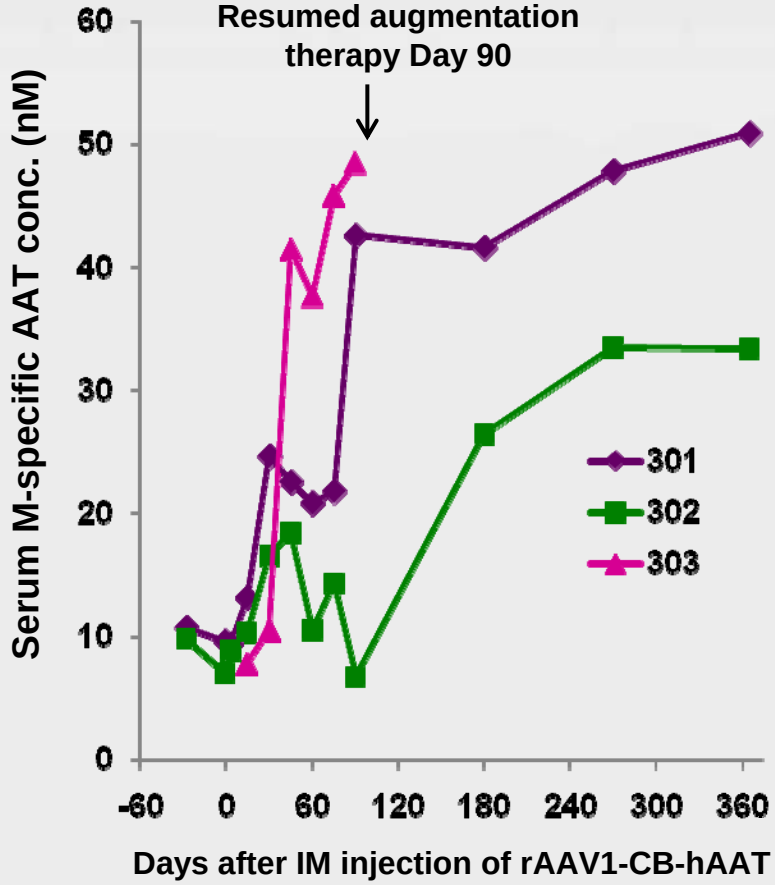
Phase 1 Study Results

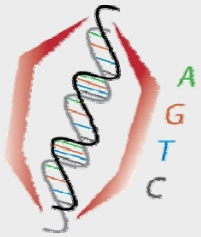
- Nine AAT-deficient subjects in three cohorts
  - Dosage 1×10^{11}, 3×10^{11} or 8×10^{11} vg/kg
  - Total volume 9.9 mL (9 x 1.1 mL) injected IM
- No safety issues
  - Mild, transient injection site reactions in 7 of 9 subjects
- Sustained M-specific AAT expression in two higher dose cohorts, despite anti-vector immune responses from Day 14 onward



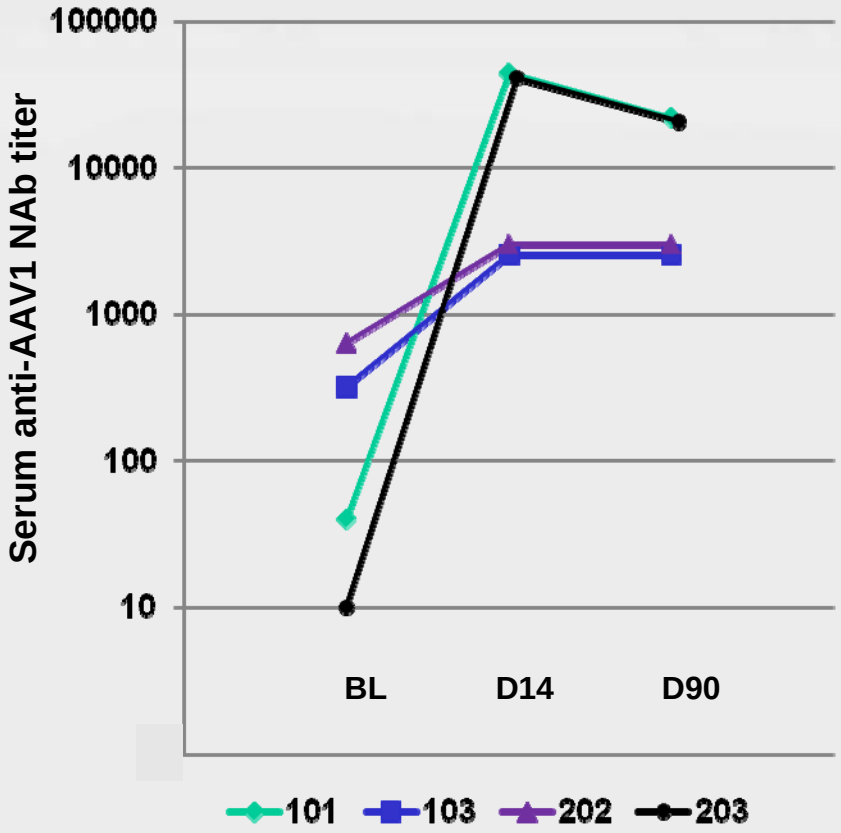
Sustained hAAT Expression



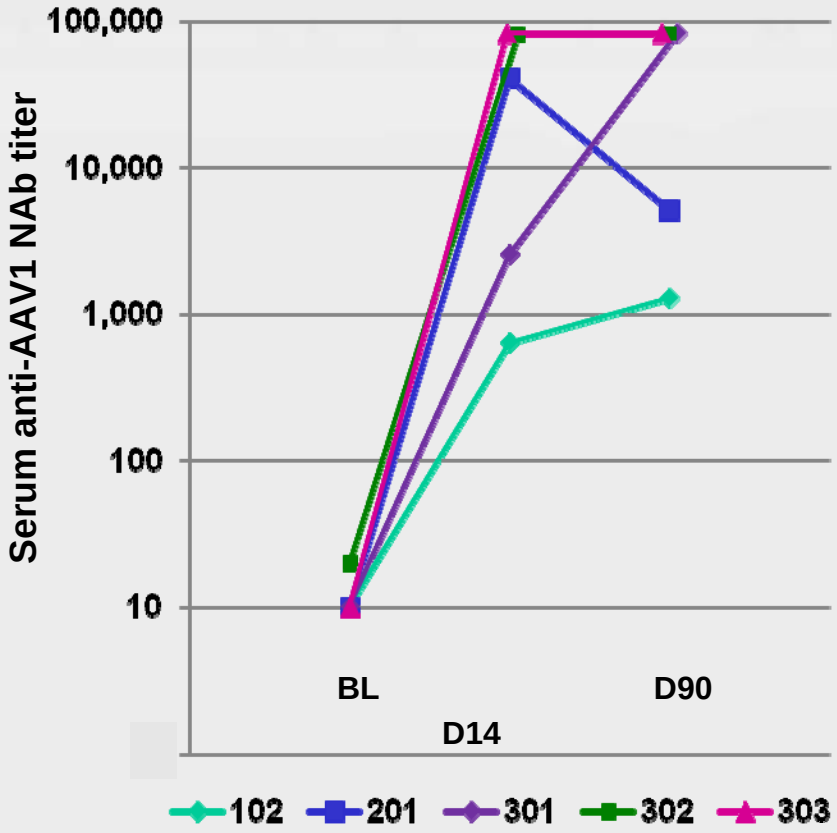




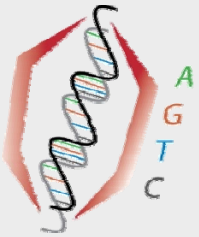
Anti-AAV Antibody Response



rAAV-experienced subjects

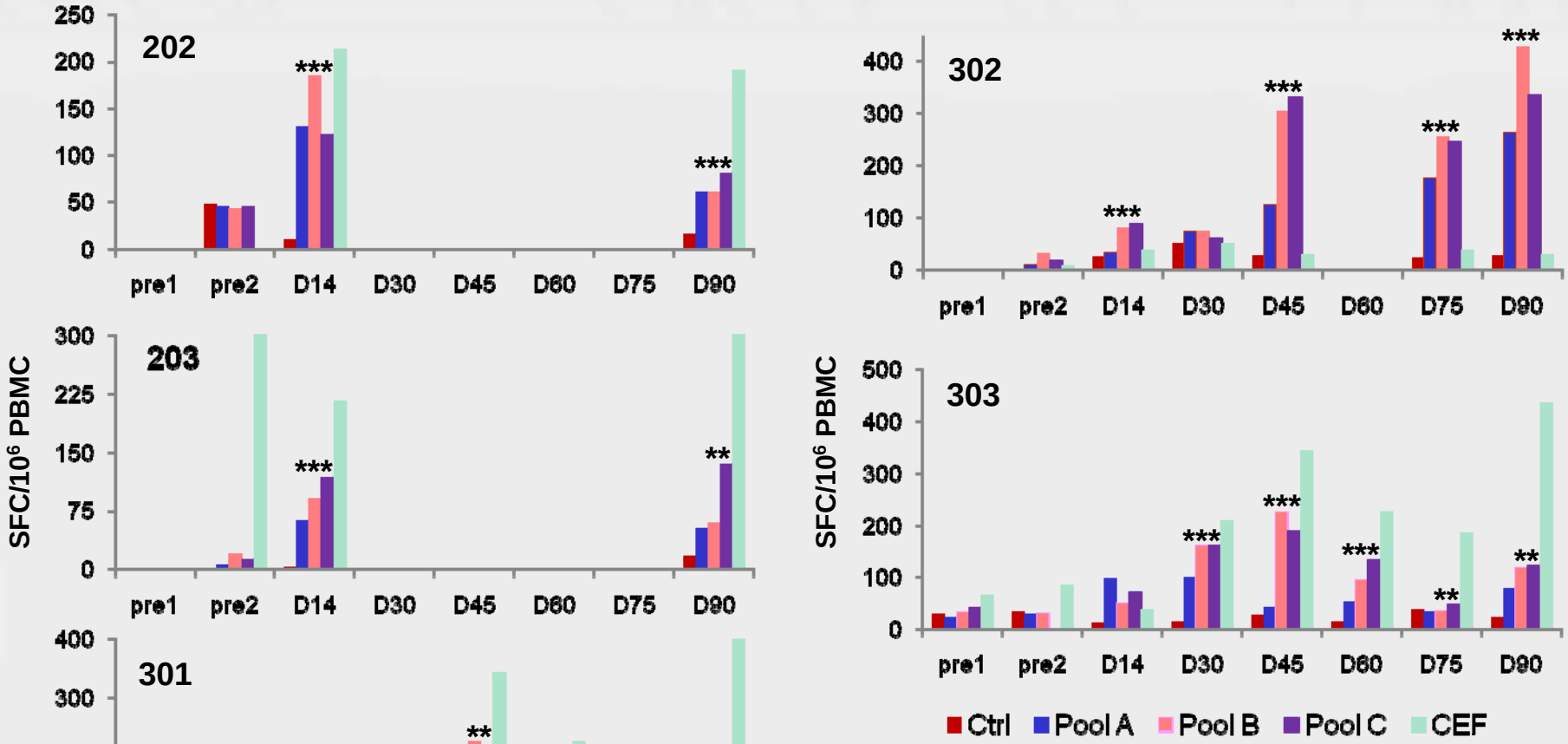


rAAV-naïve subjects

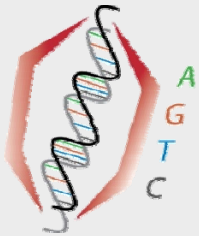


Anti-AAV T Cell Responses

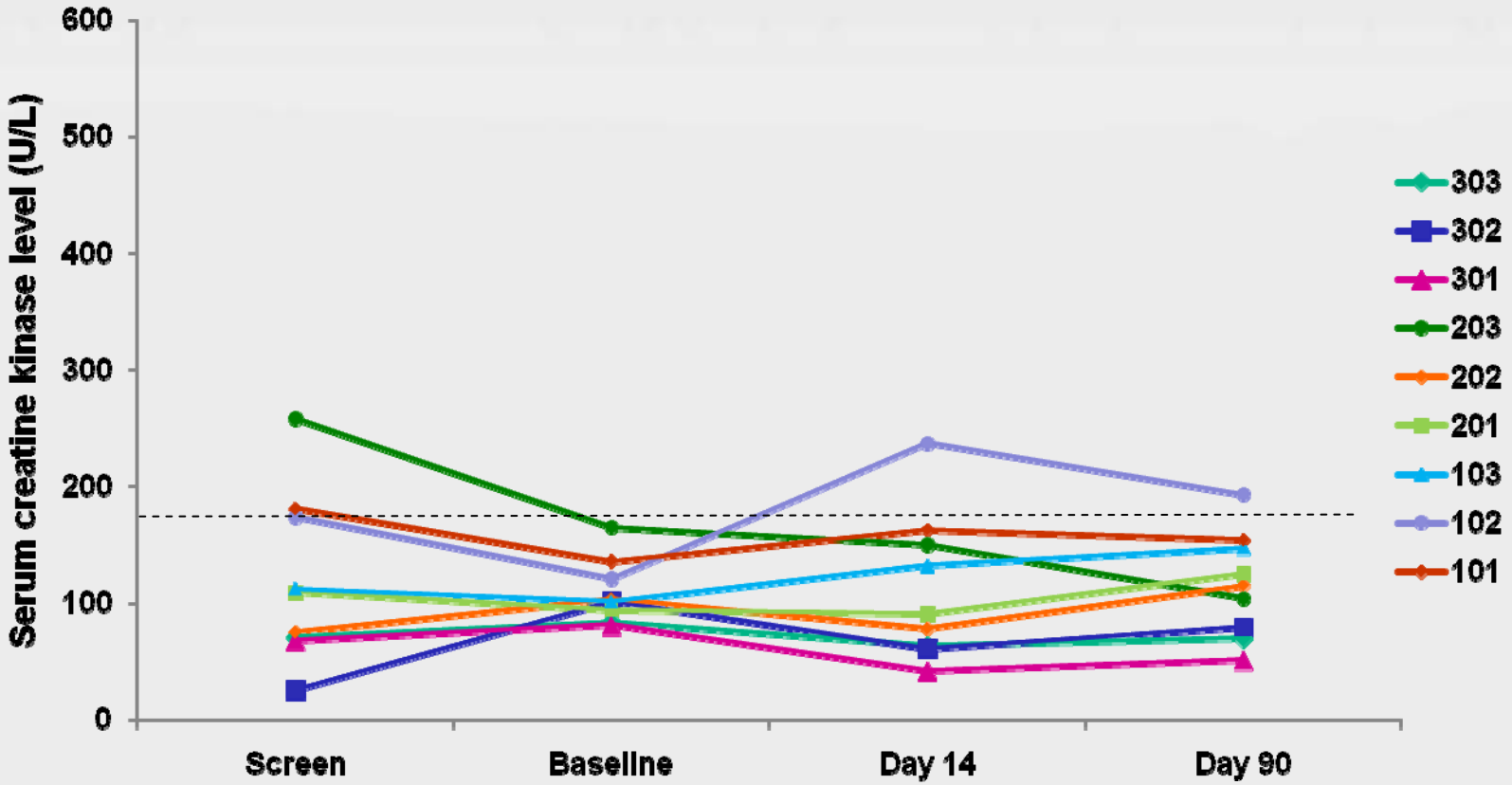
IFN-γ ELISPOT Assay with AAV Capsid or CEF Peptide Pools



* = significant response

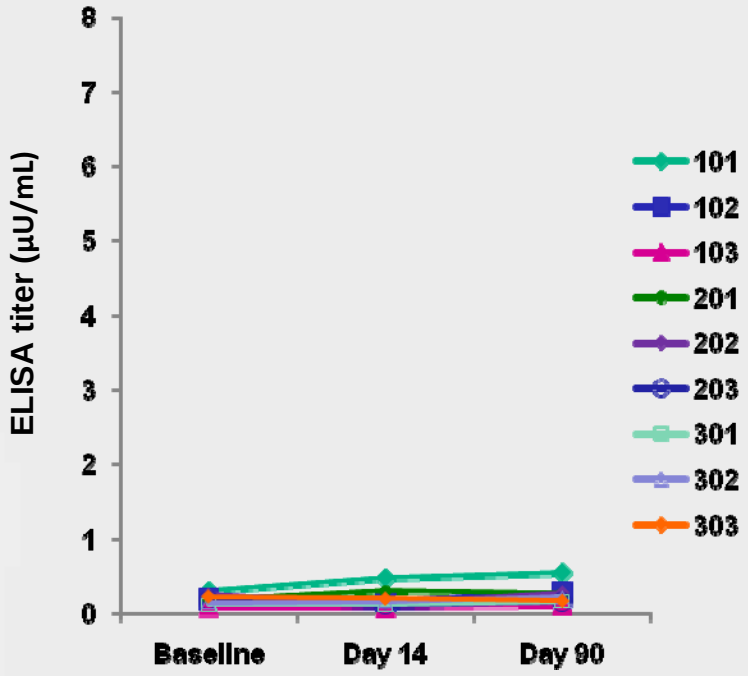


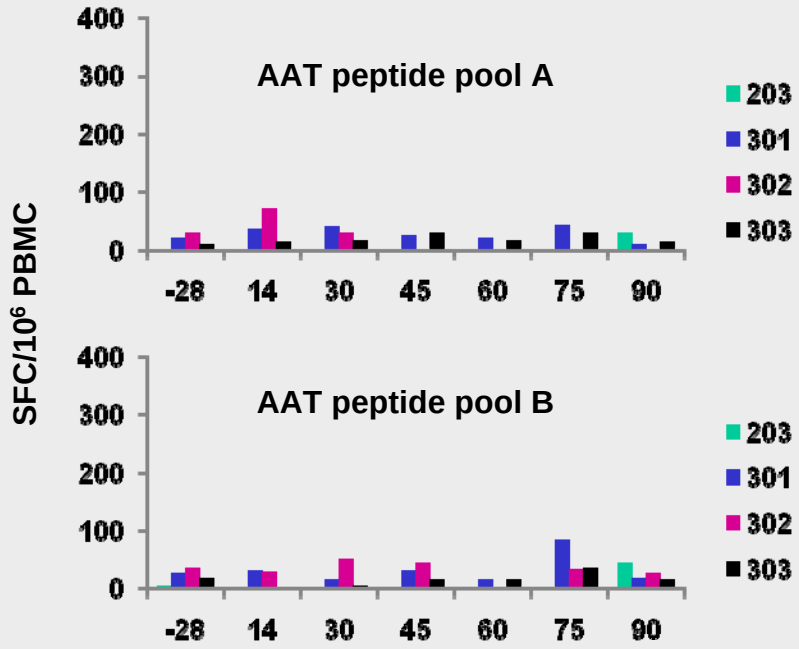
No Significant CK Elevations



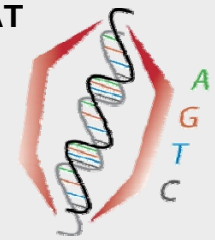
No Anti-AAT Immune Responses

- No antibody responses (ELISA)
- No T cell responses (IFN-γ ELISPOT)



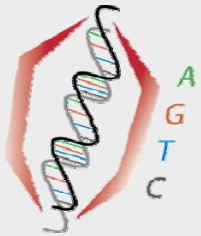


No significant responses



Conclusions from Phase 1 Study

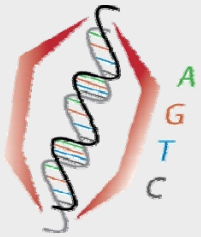
- IM injection of rAAV1-CB-hAAT caused only minimal to mild local reactions of short duration
- Serum expression of hAAT was sustained for at least 1 year despite antibody and T cell response to AAV
- No antibody or T cell responses to AAT
- Serum levels sub-therapeutic
  - Need increased doses of a more potent vector



Bridging Toxicology Study

Previous Toxicology Studies

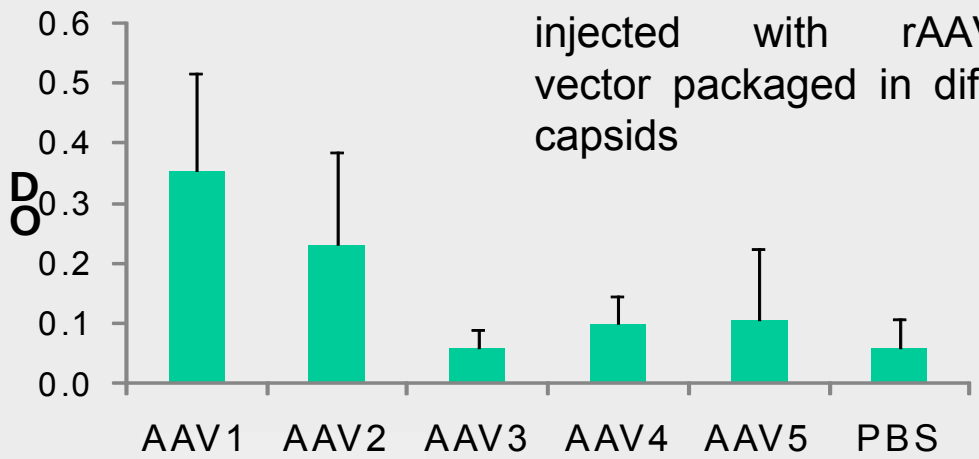
- Previous toxicology studies for AAV2 and AAV1 vectors
  - Only finding was chronic inflammation with myofiber degeneration at injection site
  - More with AAV1 than AAV2
  - Dose-dependent in mice but not rabbits
  - Consistent with response to human AAT expressed at injection site



Immunogenicity of Human AAT

- Cross-species immunogenicity of human AAT in all species studied
  - Mice, rabbits, rats, baboons, macaques
  - Less immunogenic in C57BL/6 than BALB/c mice (but not zero)

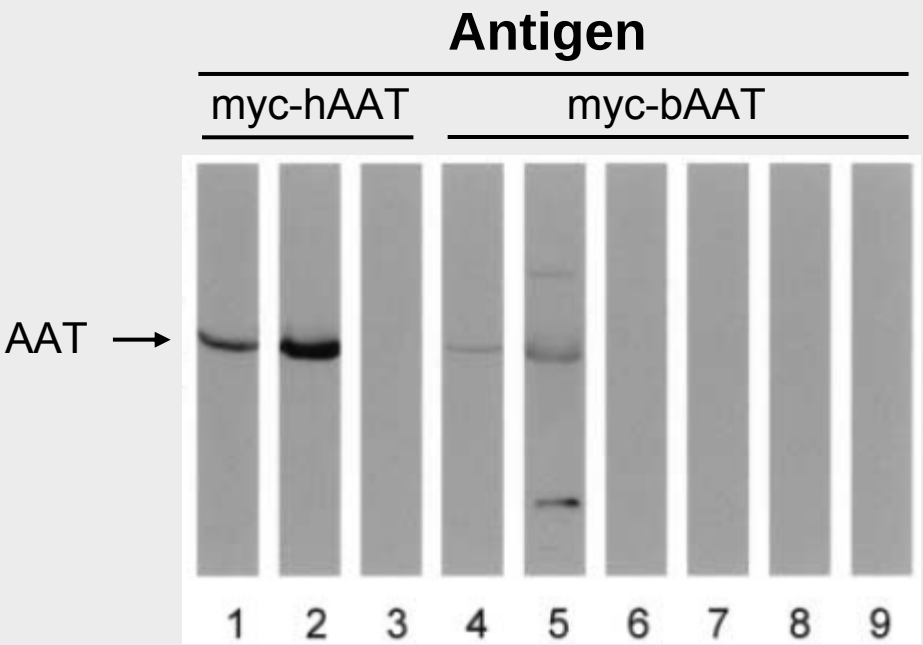
ELISA assay, 1:100 dilution of serum from C57BL/6 mice injected with rAAV-CB-hAAT vector packaged in different AAV capsids



Data from Lu et. al, 2006

Immunogenicity of AAT in Baboons

- myc-tagged human AAT but not baboon AAT induces antibody response in baboons



Antibody Probes

Lane 2	baboon post rAAV-hAAT
Lane 6,7,8	baboon post rAAV-bAAT
Lanes 1,4,5	mouse anti-myc
Lanes 3,9	negative control

Bridging Toxicology Design

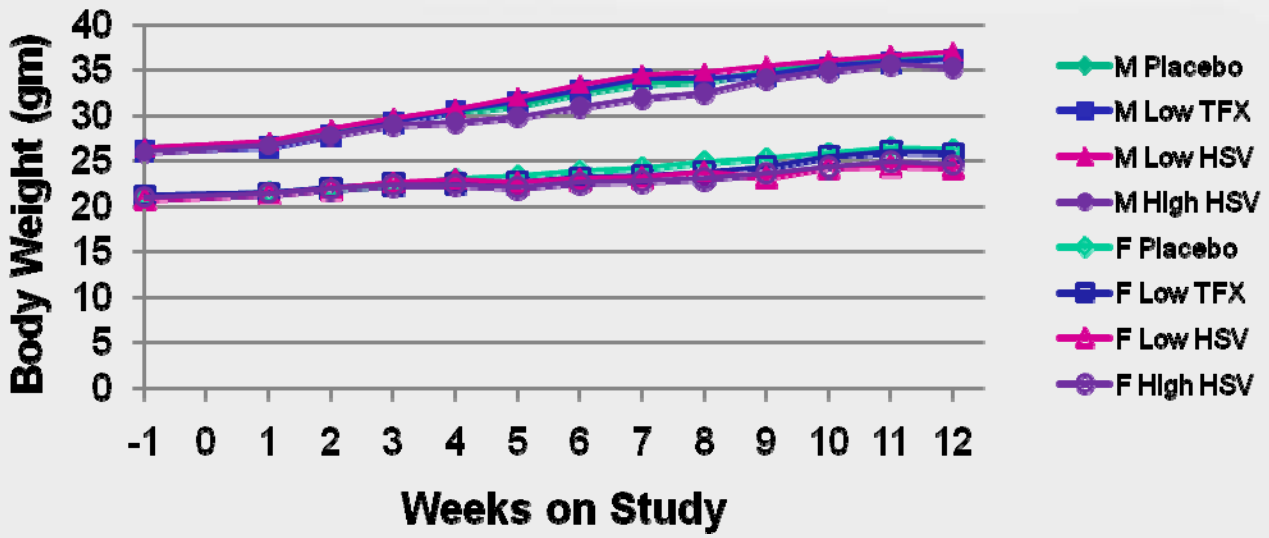
- Four groups of C57BL/6 mice
  - Placebo
  - TFX-made vector 3 x 10^{11} vg (1.2 x 10^{13} vg/kg)
  - HSV-made vector 3 x 10^{11} vg (1.2 x 10^{13} vg/kg)
  - HSV-made vector 2 x 10^{12} vg (8.0 x 10^{13} vg/kg)
- Injection Day 0, sacrifice Days 21, 60, 90

Bridging Tox Study Results

- Findings similar to previous toxicology studies
  - Only finding was chronic inflammation with myofiber degeneration at injection site
  - Generally minimal to mild intensity
  - No changes in CK, other laboratory or clinical parameters
  - Frequency of injection site observations:
    High HSV > Low HSV > Low TFX > Placebo
    - Consistent with expected increased hAAT expression

Results Through Day 90

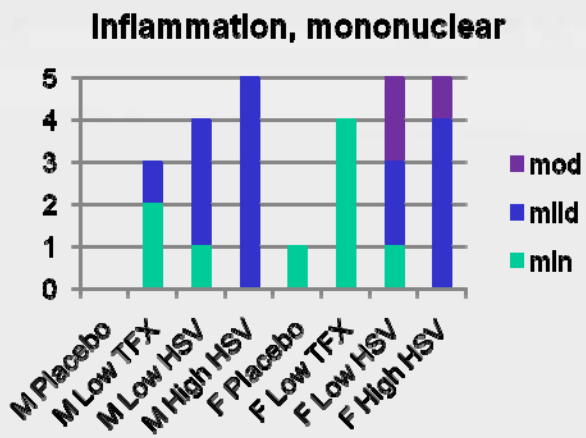
- No effect on clinical observations or body weight

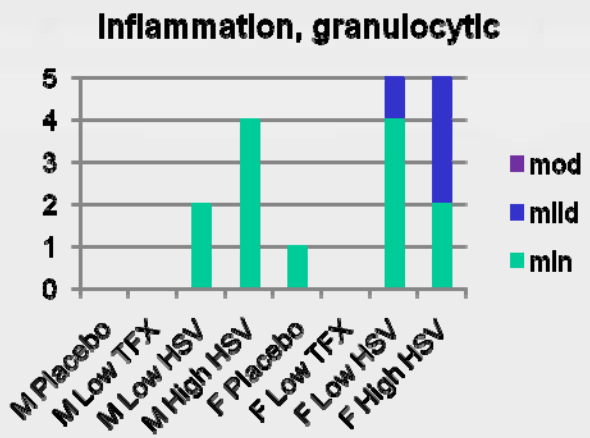


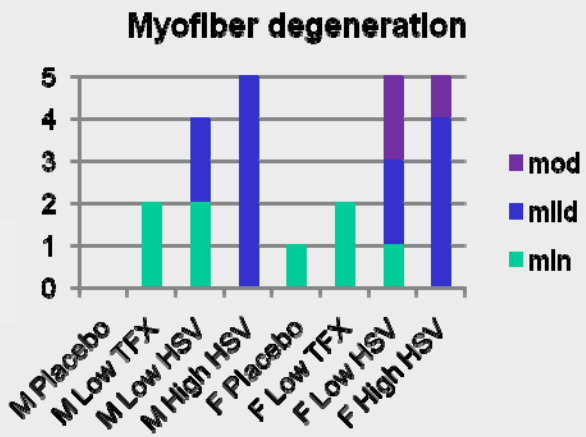
- No effect on hematology, CK or other clinical chemistries
- No gross pathology findings

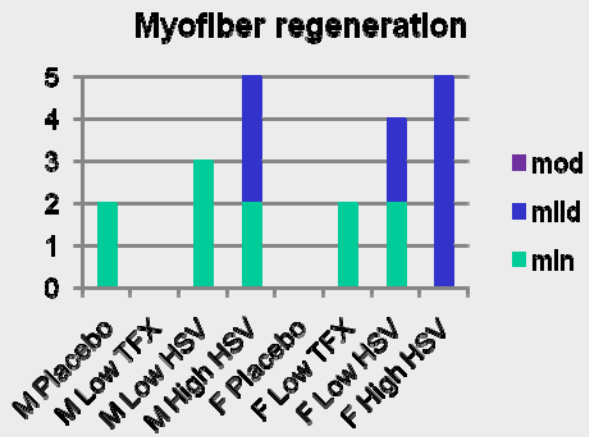
Histology Day 21 (Gastrocnemius)

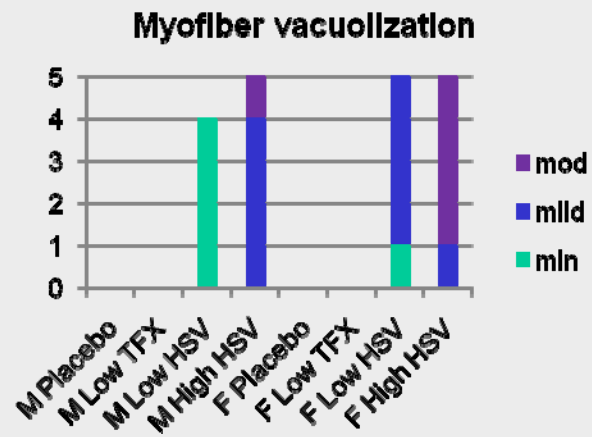
Y axis = number of animals (n=5 per group)



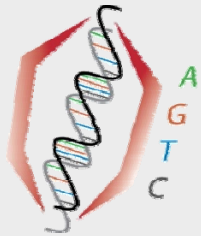






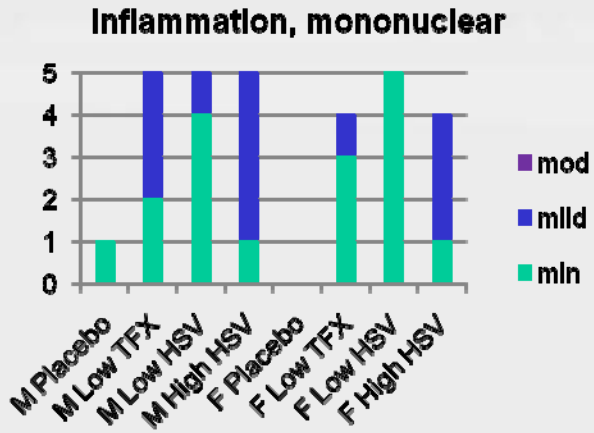


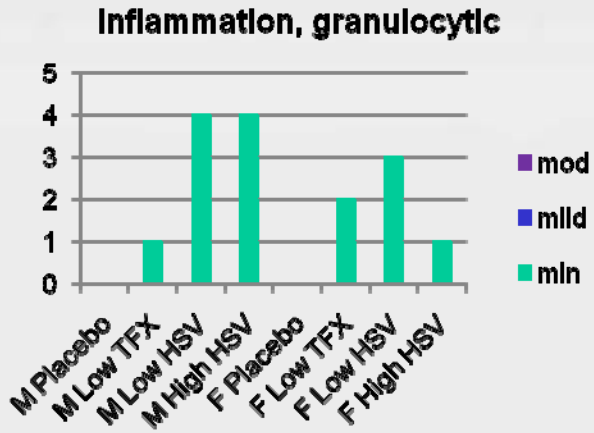
Low = 1.2 x 10^{13} vg/kg, High = 8 x 10^{13} vg/kg

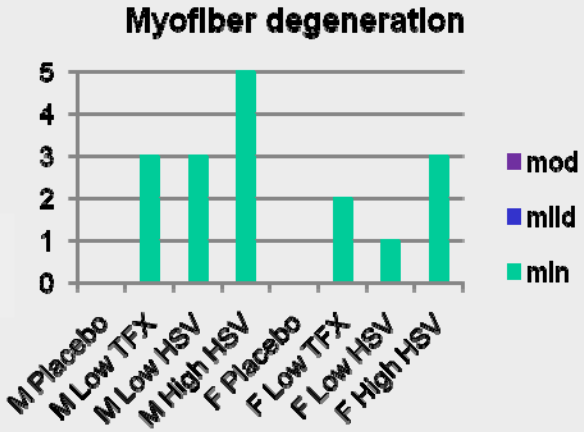


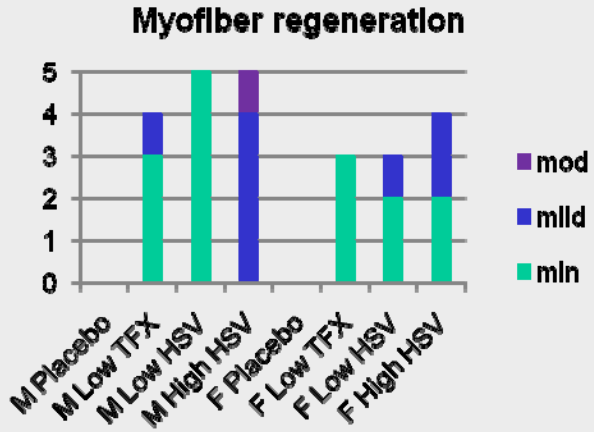
Histology Day 60 (Gastrocnemius)

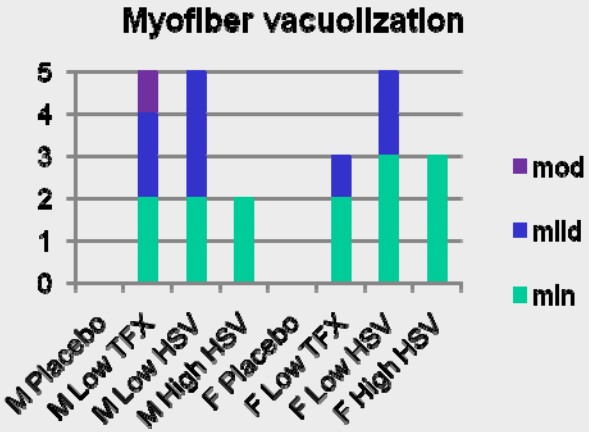
Y axis = number of animals (n=5 per group)











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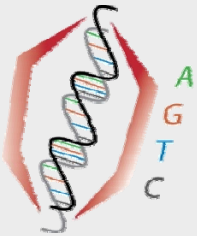
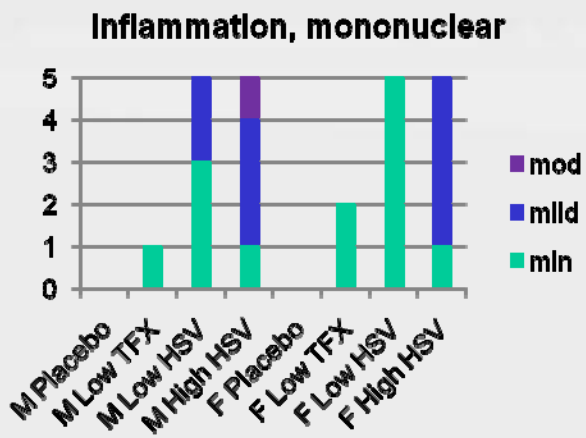
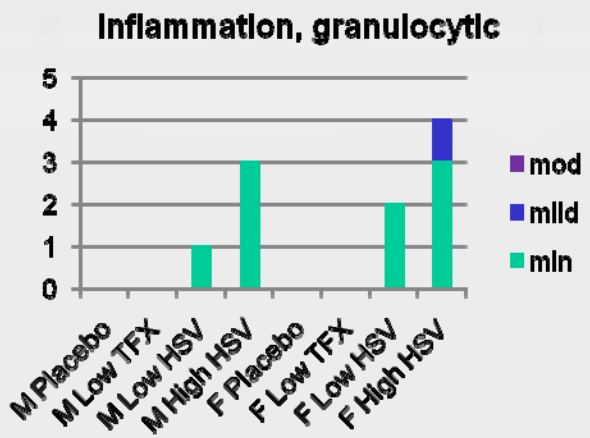
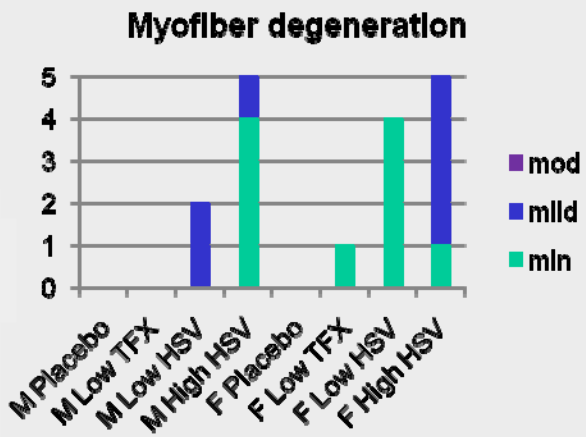


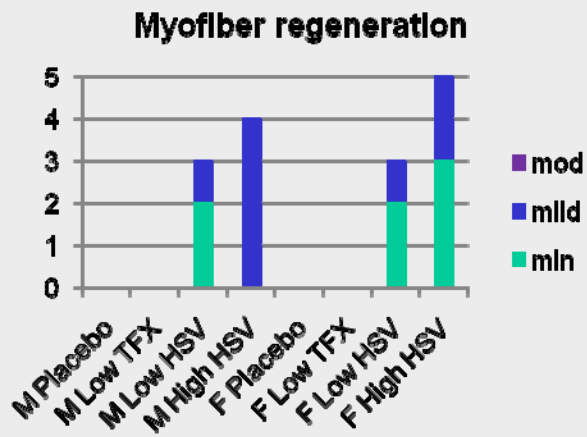
Histology Day 21 (Quadriceps)

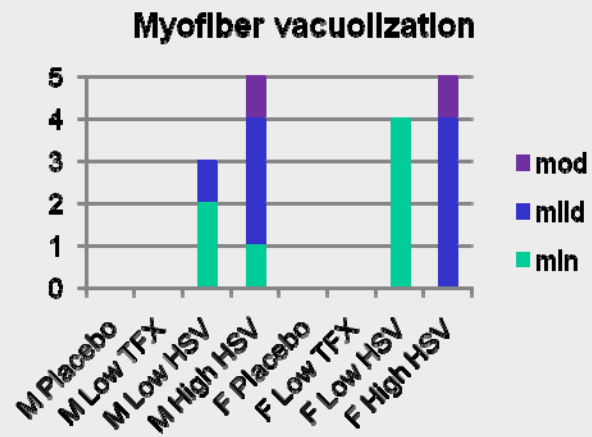
Y axis = number of animals (n=5 per group)











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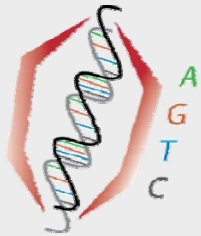
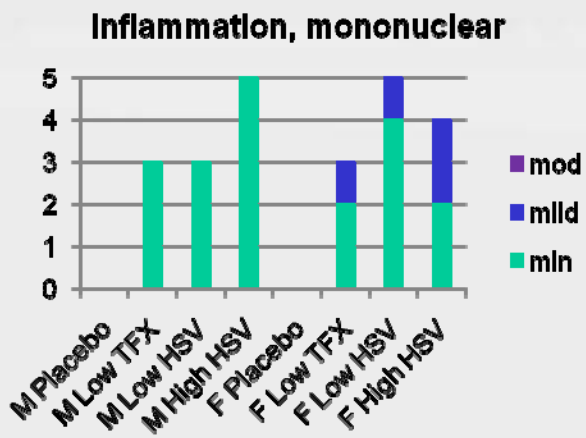
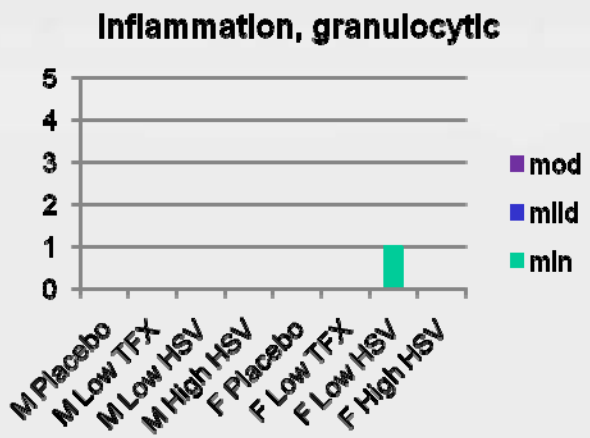
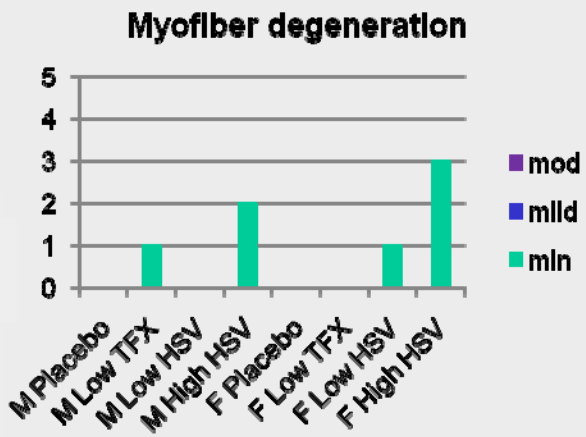


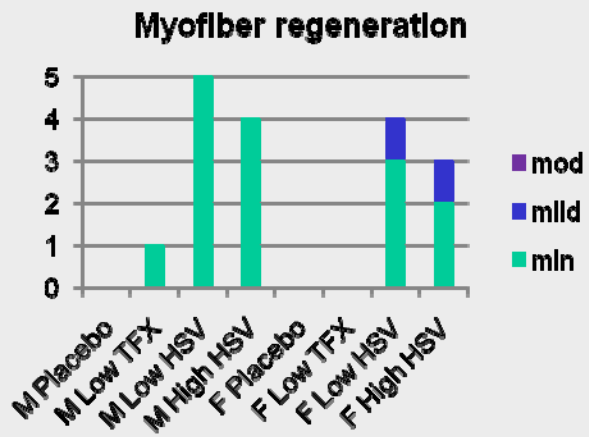
Histology Day 60 (Quadriceps)

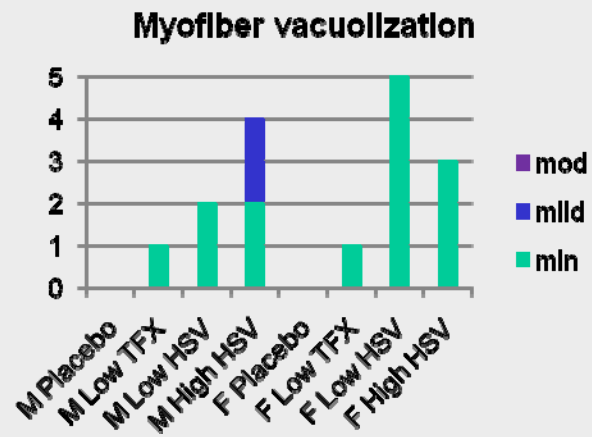
Y axis = number of animals (n=5 per group)











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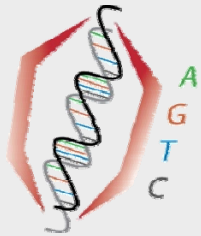
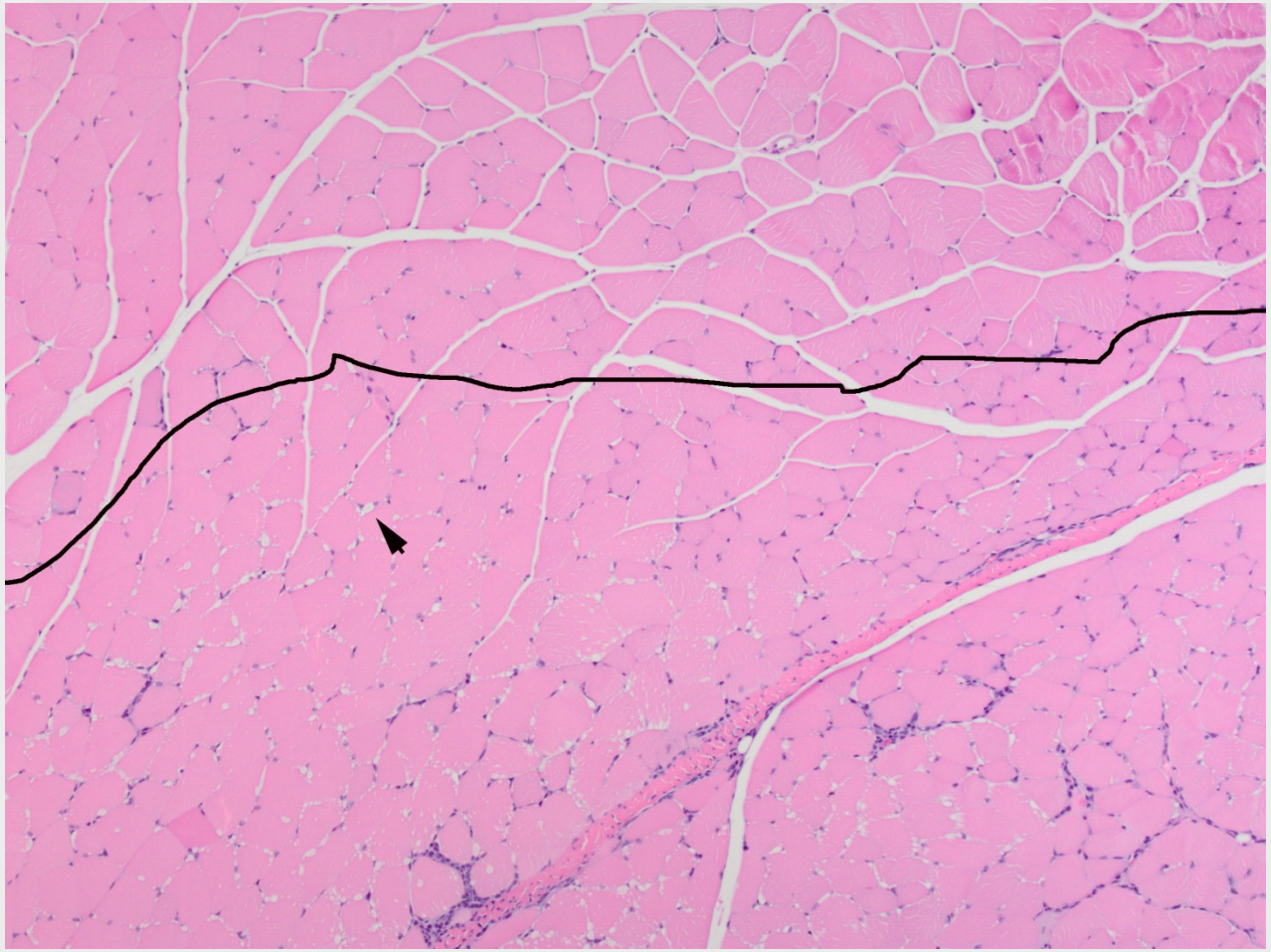


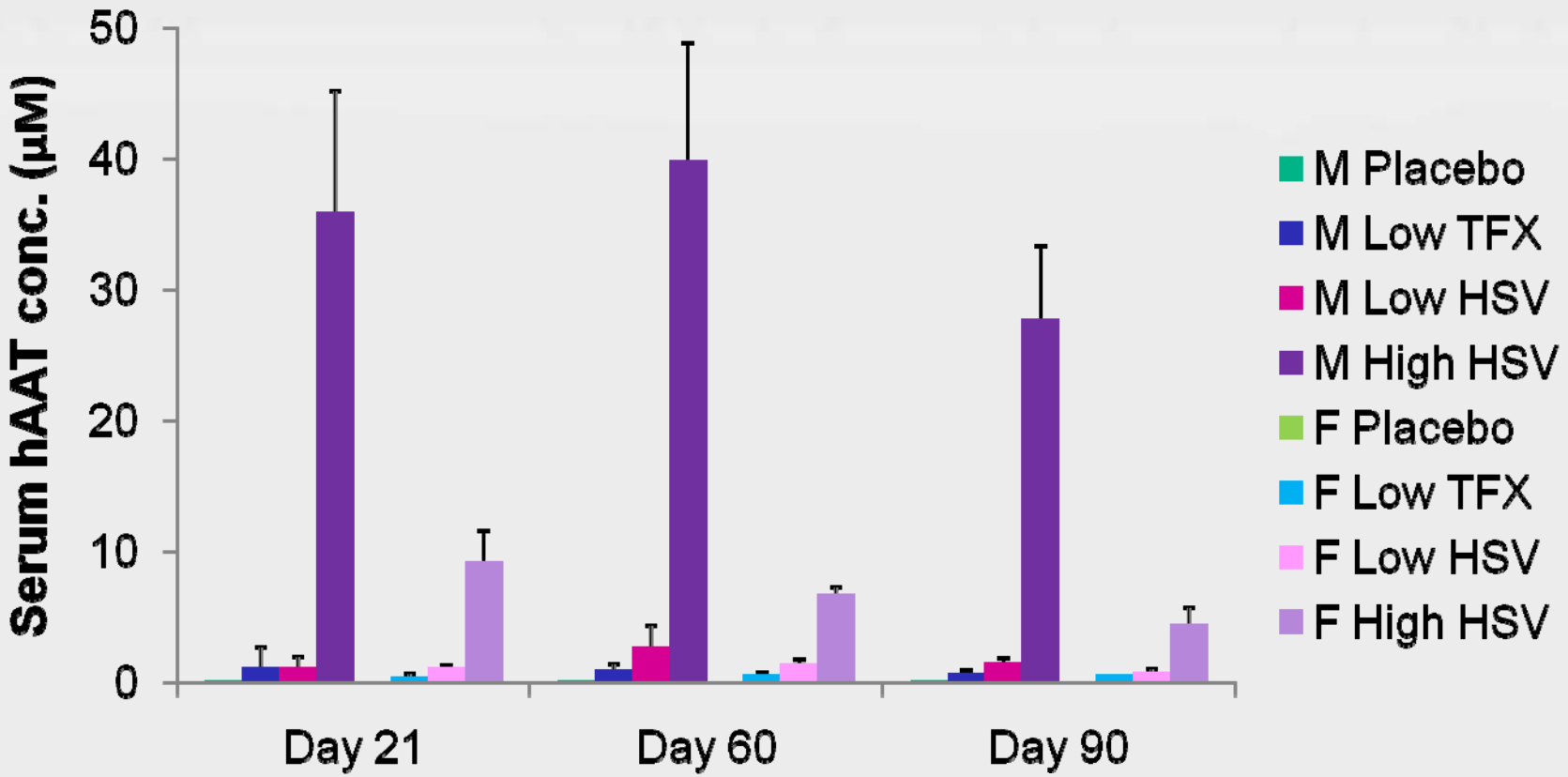
Example of Mild Inflammation

Normal area

Area of mild inflammation

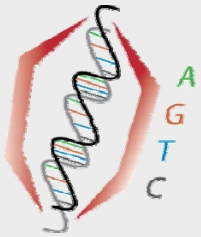


Serum hAAT Concentration



Toxicology Conclusions

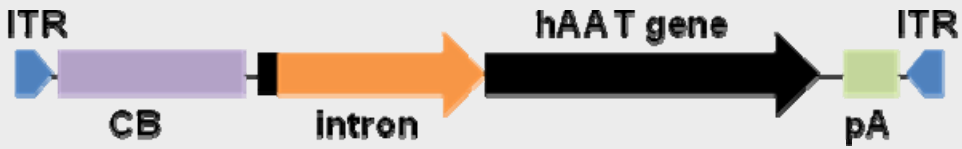
- Only finding was chronic inflammation with myofiber degeneration at injection site
- Dose-dependent
- Consistent with response to human AAT expressed at injection site



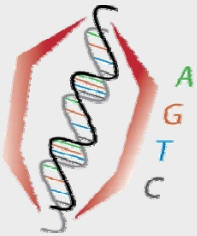
Design of Phase 2 Study

Study Overview

- Phase 2 trial of rAAV1-CB-hAAT
  - non-replicating, *rep/cap*-deleted, recombinant adeno-associated virus (rAAV) vector based on AAV serotype 1 and containing cDNA encoding the human alpha-1 antitrypsin (hAAT) protein

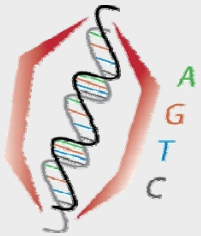


- Dose escalation safety and efficacy evaluation
  - 9 patients with alpha-1 antitrypsin deficiency
    - 3 cohorts of 3 patients each
    - Increasing dose levels between cohorts



Study Population

- Patients with alpha-1 antitrypsin deficiency
- 18 to 75 years old
- FEV1 >25% of predicted
- Weight ≤90 kg



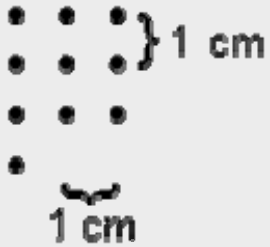
Study Design

Group	Vector dose (vg/kg)	Total dose (vg per subject) [a]	Vector concentration (vg/mL) [a]	Injection number x volume
1	6.0×10^{11}	5.4×10^{13}	4.00×10^{12}	10 x 1.35 mL
2	1.9×10^{12}	1.7×10^{14}	3.96×10^{12}	32 x 1.35 mL
3	6.0×10^{12}	5.4×10^{14}	4.00×10^{12}	100 x 1.35 mL

[a] Total vector dose and vector concentration for a 90 kg subject

Injection Procedures

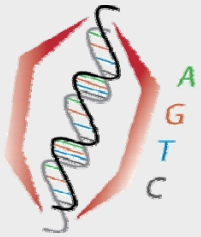
- IM injections in fixed volume of 1.35 mL
- Administration similar to Phase 1
  - Similar grid pattern
  - Same injection density
    - 9 injections per 4 cm^2
  - Slightly higher volume
    - 1.35 mL vs. 1.1 mL per injection



- Optional use of conscious sedation
  - Determined by preference of each subject

Outcome Measures

- Safety
  - Adverse events, CBC, clinical chemistry, creatine kinase
  - Immune responses to AAT
  - Muscle biopsy
    - Histology, cellular phenotype, vector quantification
- Efficacy
  - Changes in serum AAT
    - Total and vector-expressed (M-specific)
- Other
  - Vector DNA in blood & semen
  - Immune responses to AAV

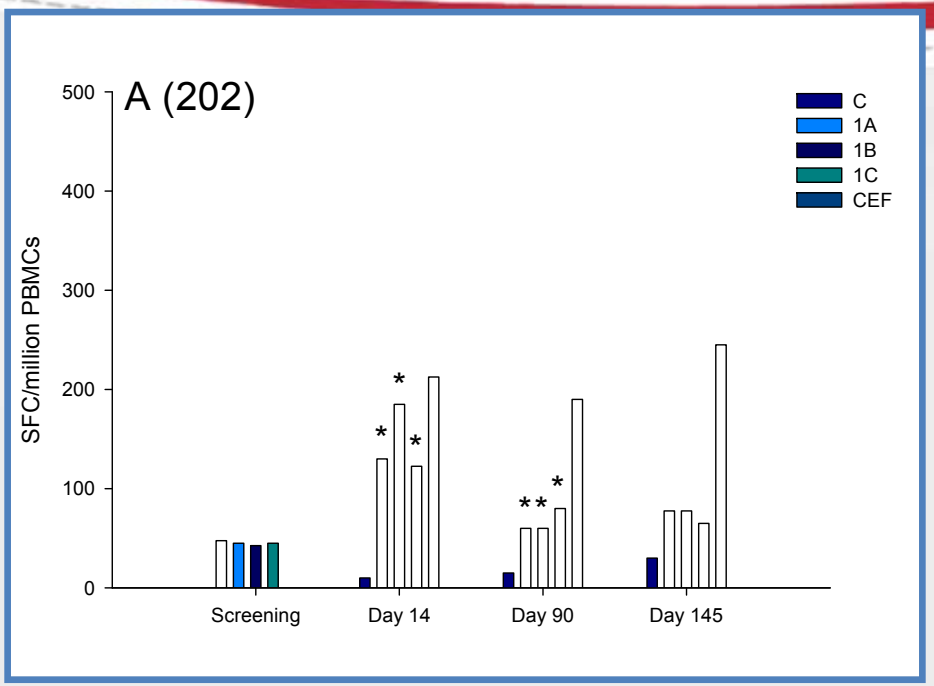


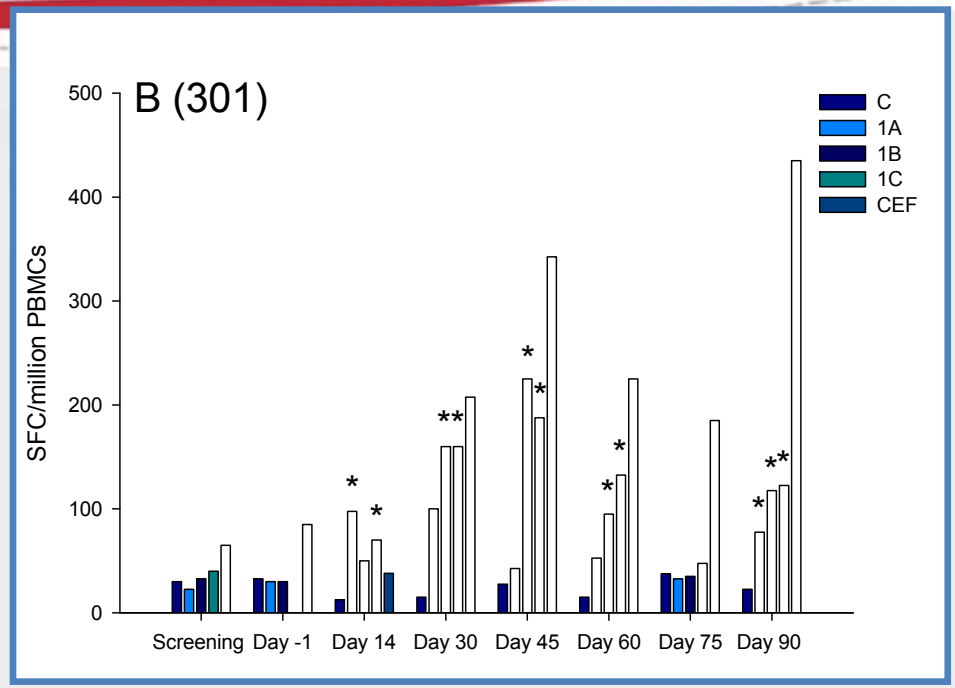
DSMB Review

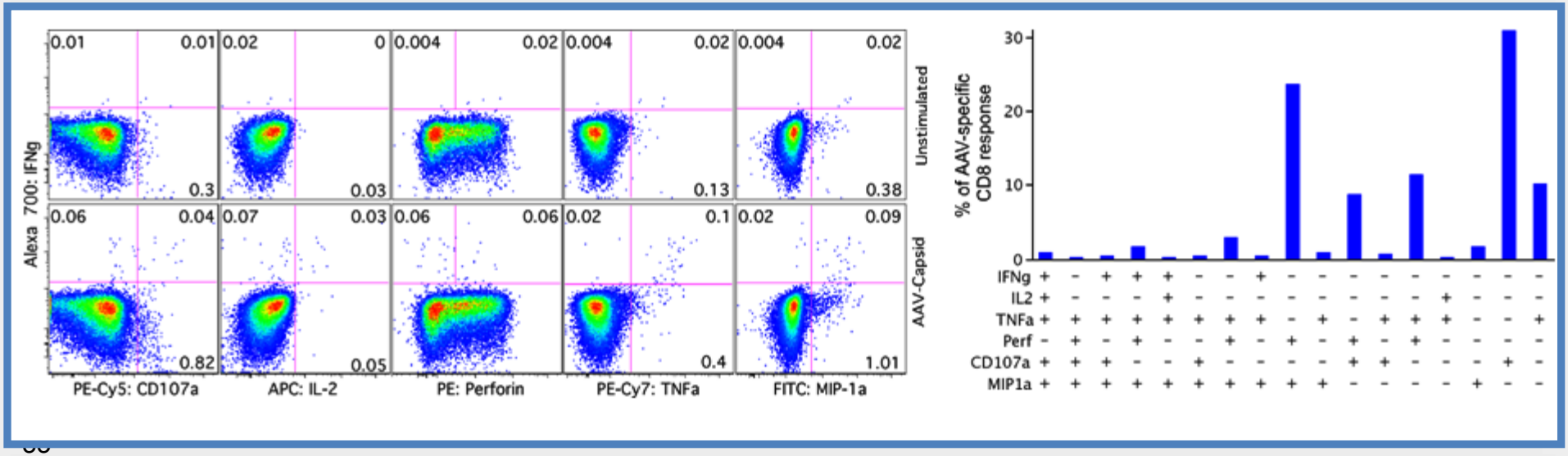
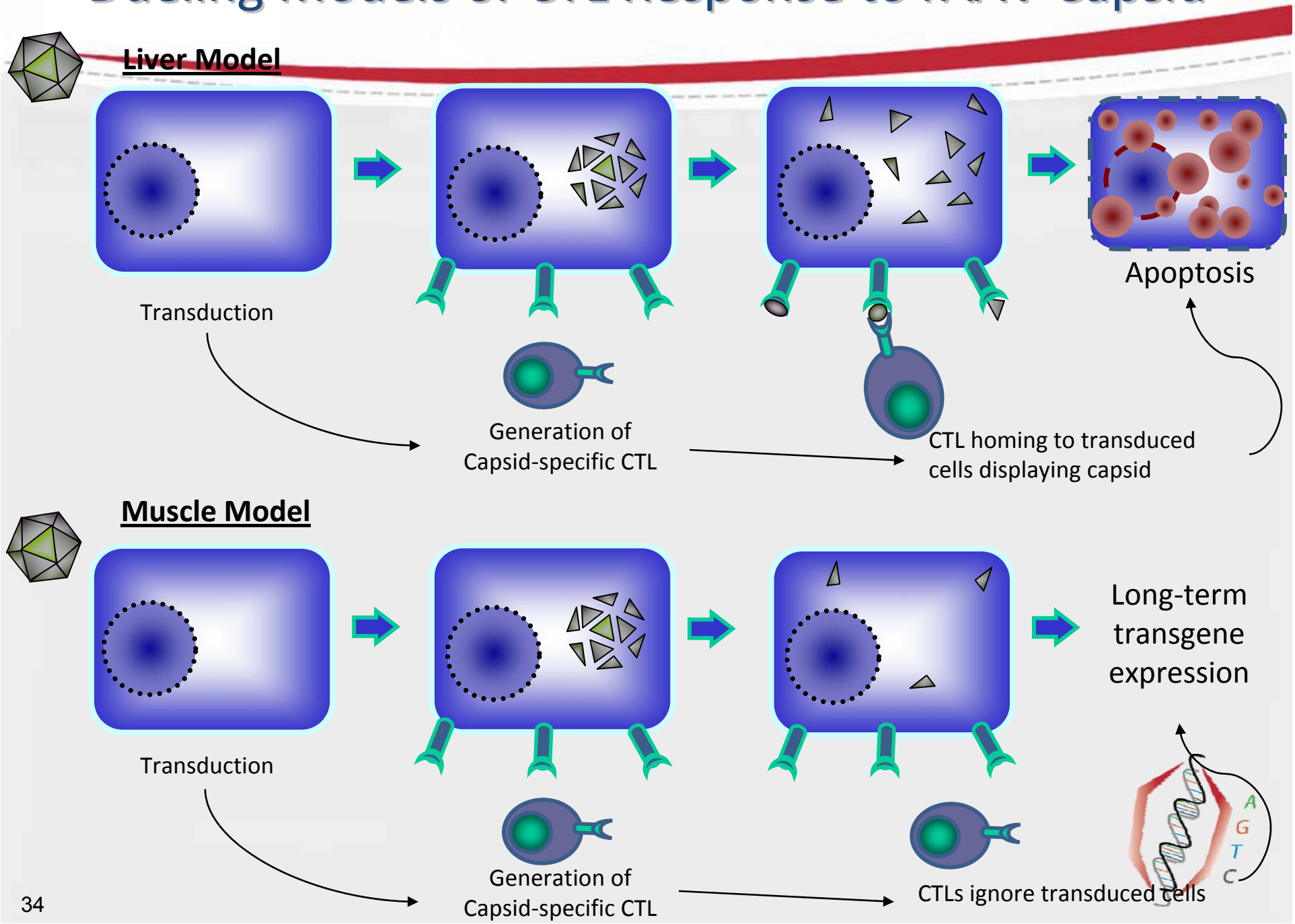
- NHLBI DSMB review on at least 2 occasions
  - After first 3 patients complete Day 14 visit
  - After second 3 patients complete Day 14 visit
  - Occurrence of dose-limiting toxicity
    - Any adverse event that is Grade 3 or greater and is possibly, probably or definitely related to the study agent

Responses to RAC Review

Immune Responses to rAAV1







Dueling Models of CTL Response to rAAV Capsid