Parent Project Muscular Dystrophy 2019
Gene Therapy: What we know today …

Practical Questions & Immune Response

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PGTC
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UFHealth
UNIVERSITY OF FLORIDA HEALTH

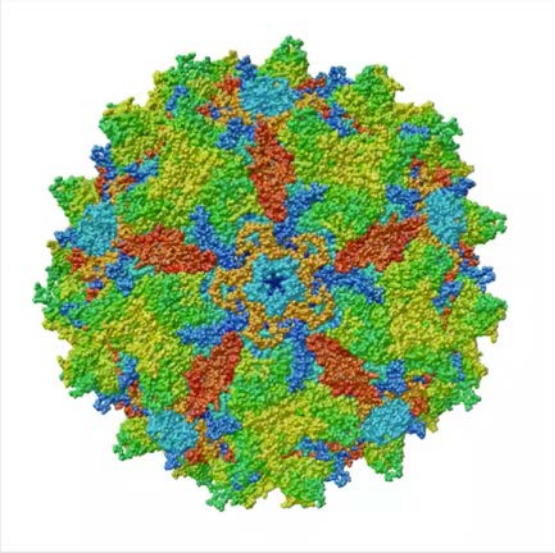
Disclosures

- Inventor of AAV technology related to neuromuscular disease and AAV production technology
- Member of Pfizer Rare Disease Unit - Therapeutic Area Scientific Advisory Committee
- Site investigator – Ignite DMD / SLDB, Sarepta, Roche, Italifarmicio, TRINDS,

Question #1

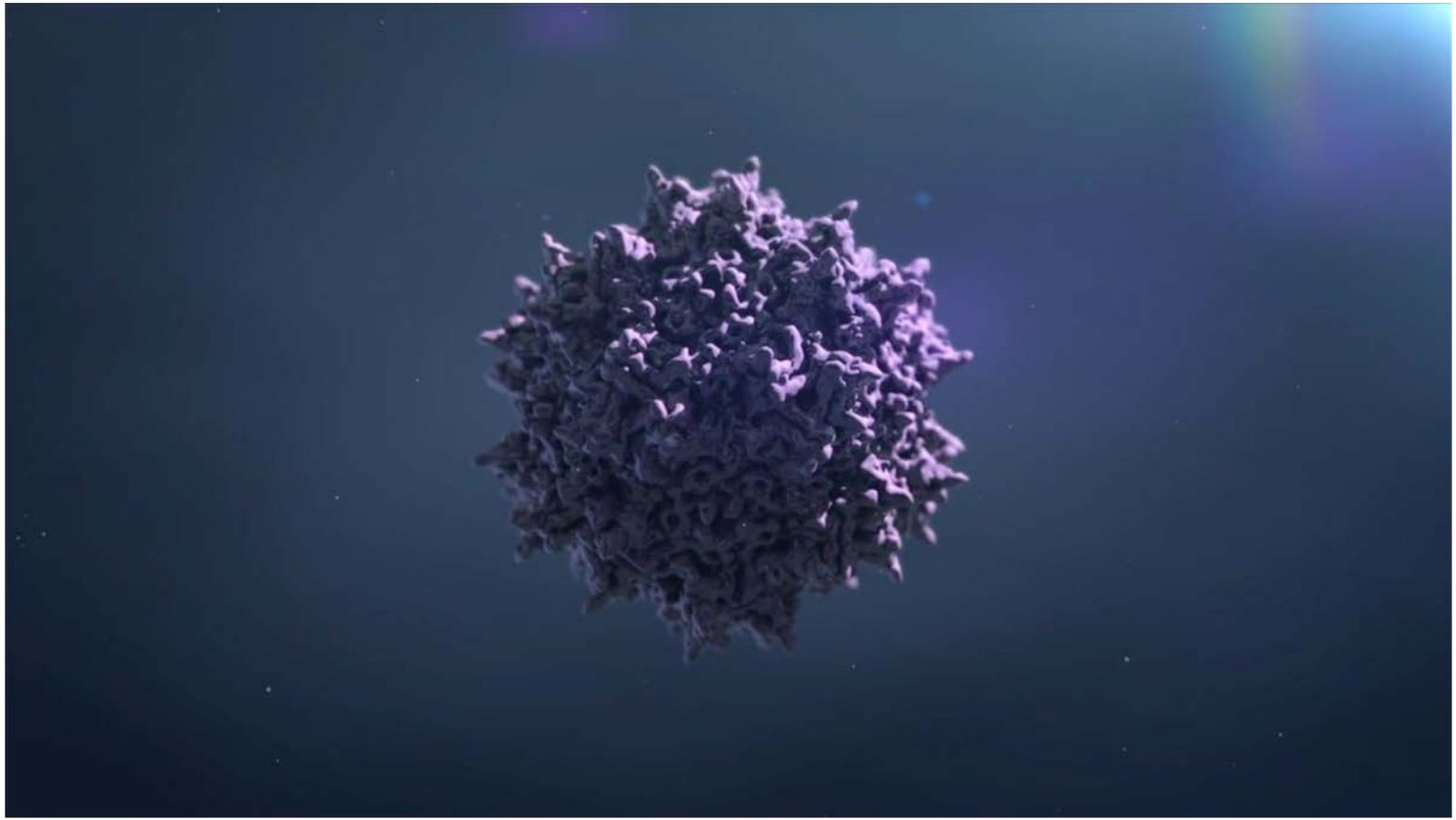
What is AAV and how are vectors made?

Adeno-Associated Virus (AAV) Vectors

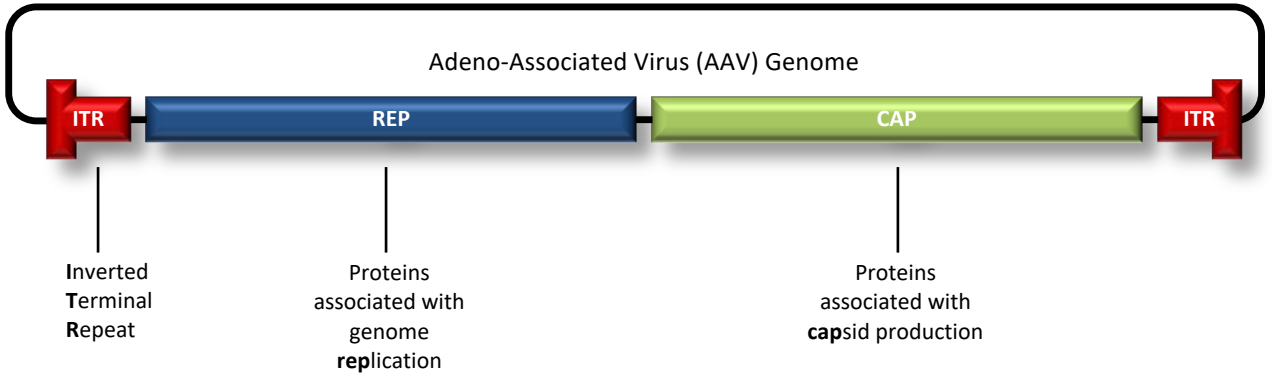


Courtesy of M. Agbandje-McKenna
University of Florida

- Inherently non-pathogenic, unique nature of high-dose therapy
- Many serotypes provide wide range of tissue tropism
- Persists long-term without integration
- Risk vs Benefit in favor of therapeutic benefit



Assembling the rAAV Cassette



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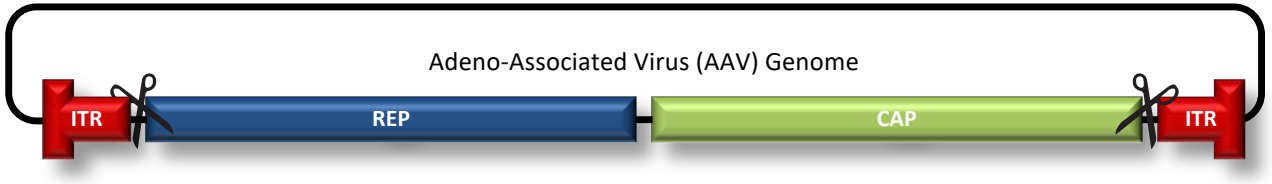
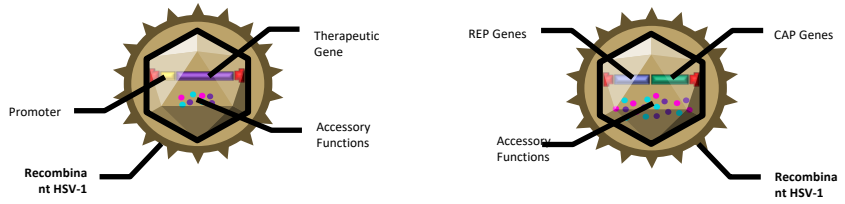
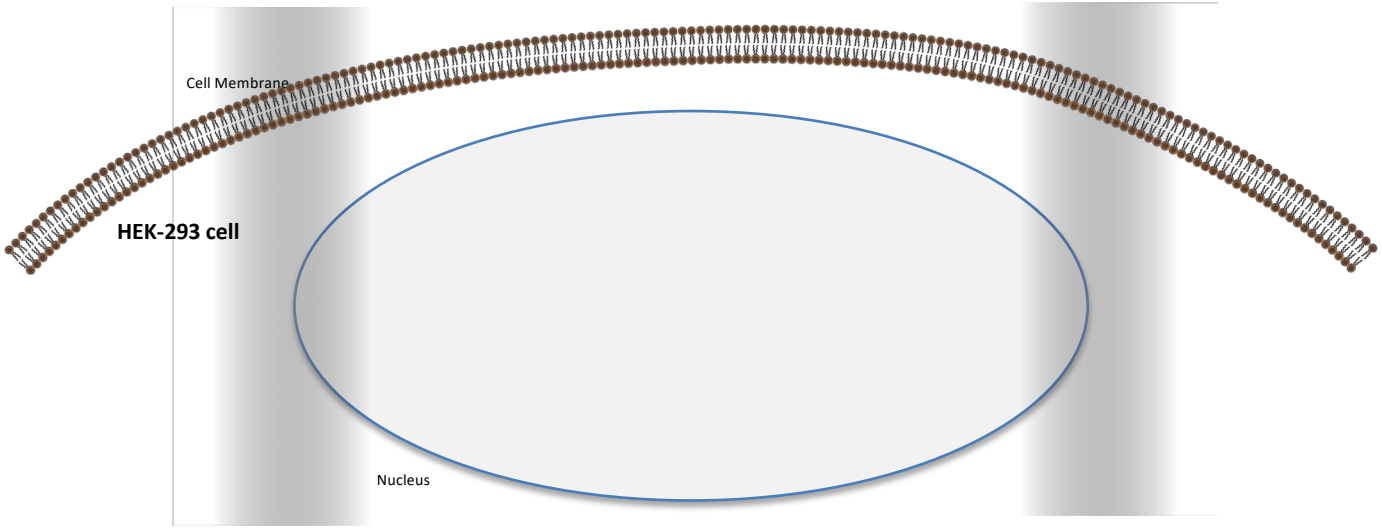


Assembling the rAAV Cassette

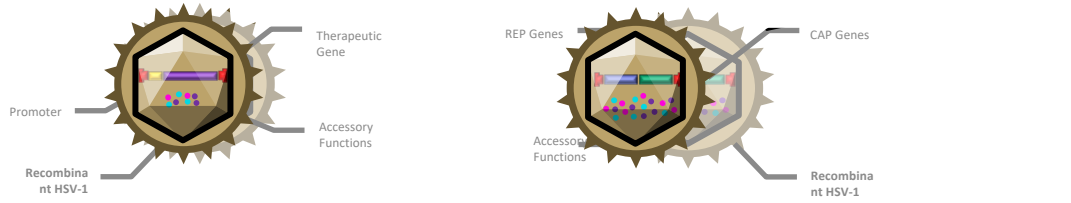
rAAV Manufacturing

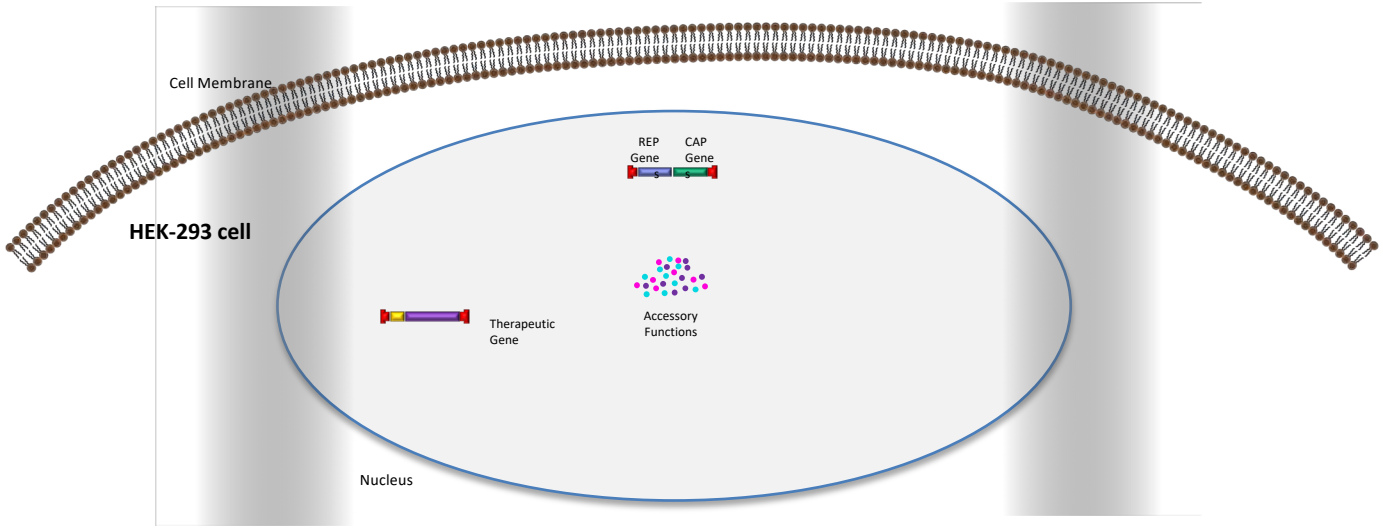
2-Component Infection





2-Component Infection





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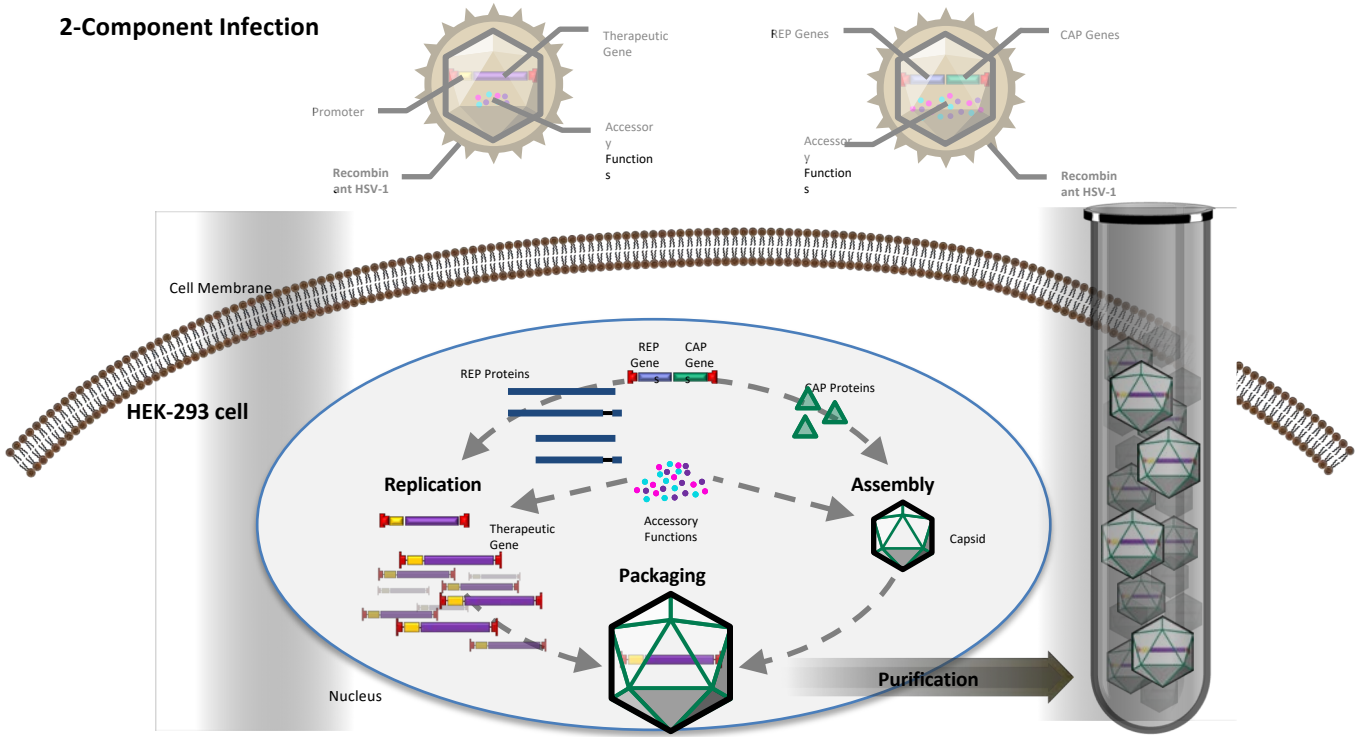
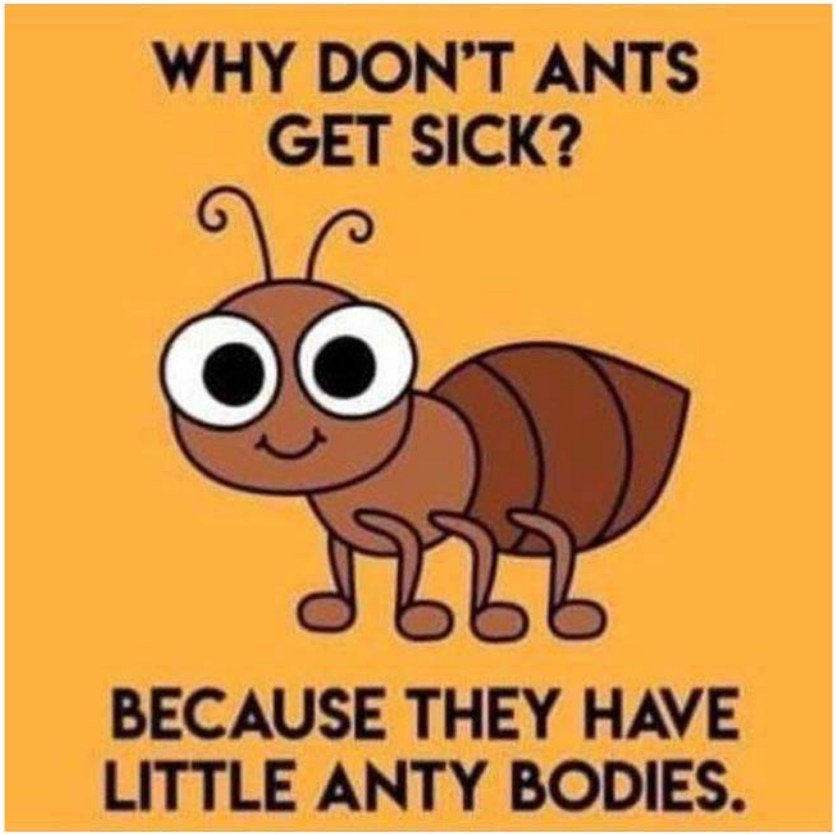
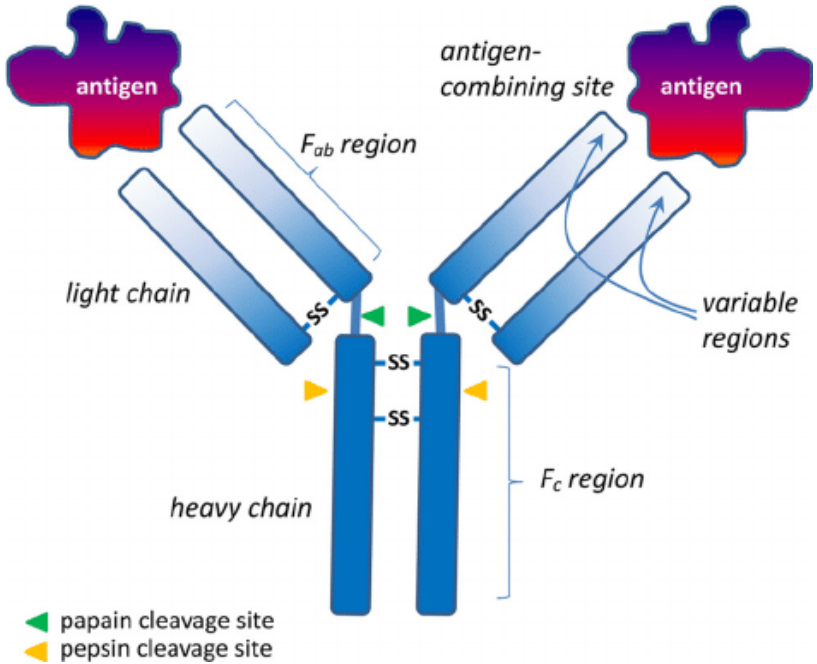


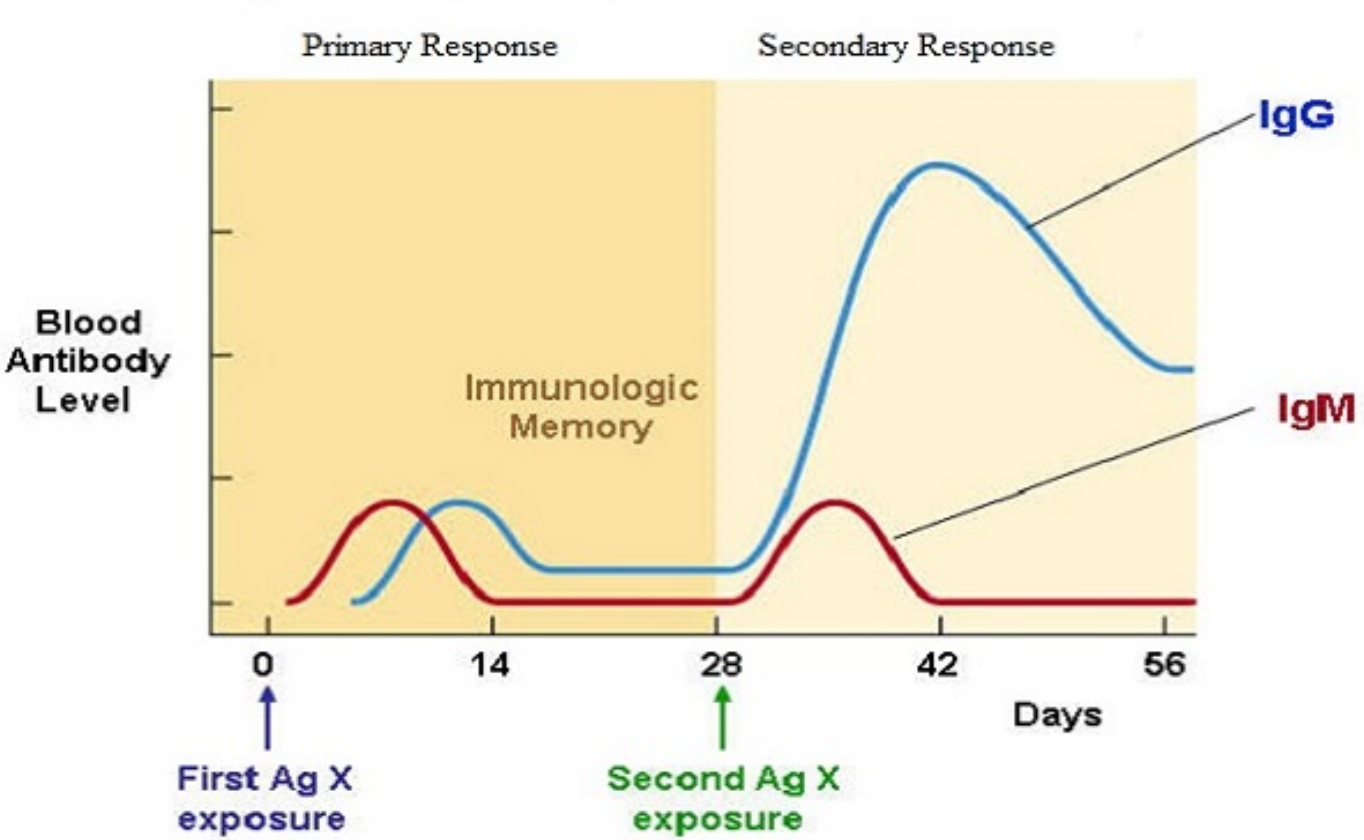
Question #2

What are AAV antibodies and why does that matter?

Question #2




antigen
antigen-
combining site
antigen
F_{ab} region
light chain
SS
SS
variable
regions
SS
SS
heavy chain
F_c region
papain cleavage site
pepsin cleavage site


Primary Response
Secondary Response
IgG
IgM
Blood Antibody Level
Immunologic Memory
0
14
28
42
56
Days
First Ag X exposure
Second Ag X exposure

Anti-AAV antibody testing: Neutralizing versus Total antibody

Neutralizing Ab assay:

- In vitro cell-based assay
- Identify binding to the capsid receptor epitope
- Sensitivity is influenced by lack of in vitro transduction
- Positive or negative effects by other serum proteins

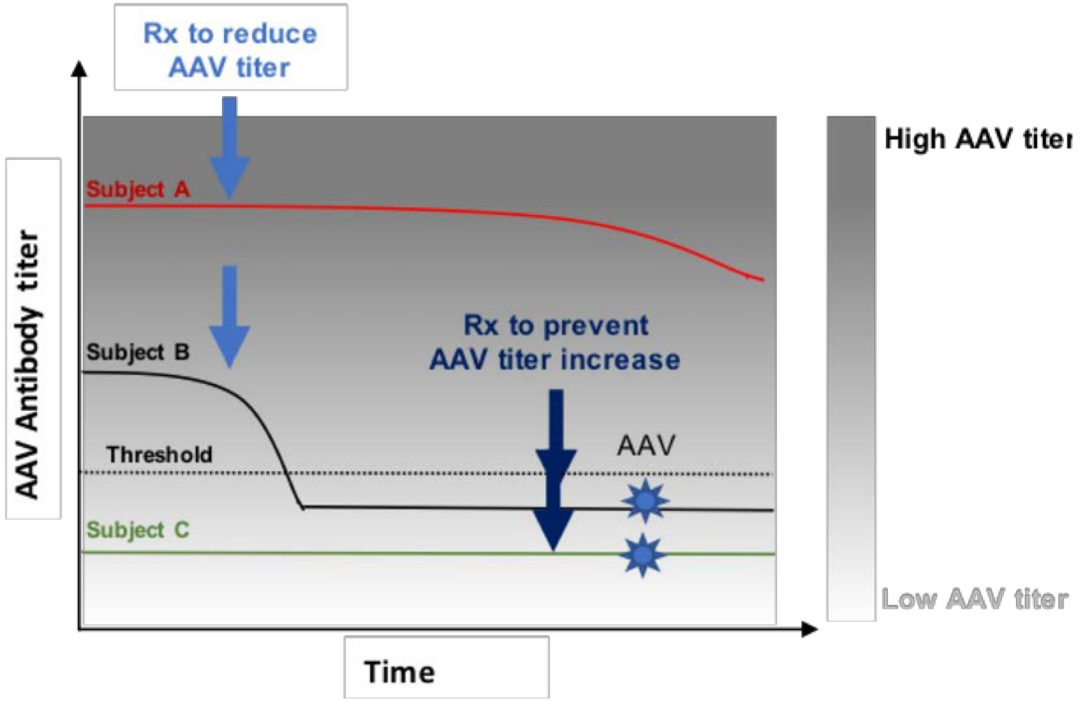
Total (binding) Ab assay:

- ELISA assay against intact AAV capsids
- Identifies all antibodies from a polyclonal response
- High reproducibility and sensitivity
- Sensitive at low dilution to predict pre-existing immunity

Management of environmentally-acquired preimmunity

- About **50-60%** of individuals are preimmuned to AAV
- Preimmunity of AAV is an exclusion criteria for most of studies

1) Is the threshold used in clinical trials appropriate?
2) What is the most effective immunomodulation regimen to decrease levels of preexisting AAV immunity?
3) What level of of preexisting antibody precludes treatment?



Question #3

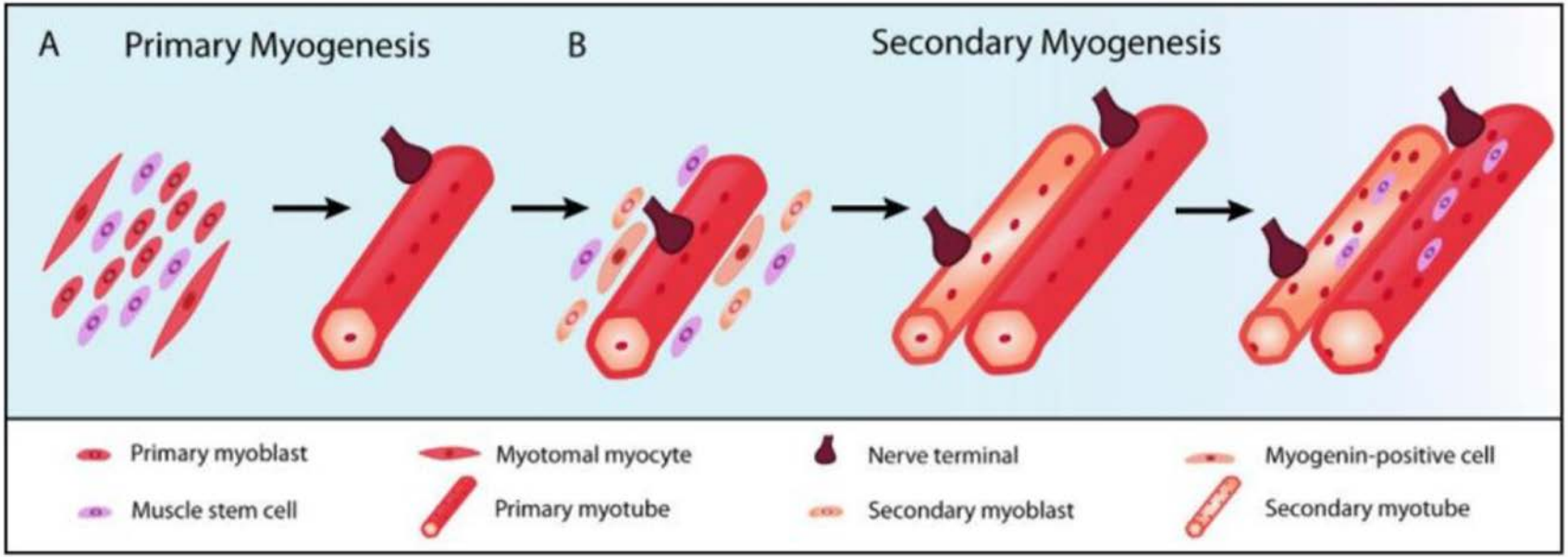
Is receiving DMD gene therapy durable for the life-span?

Question #3

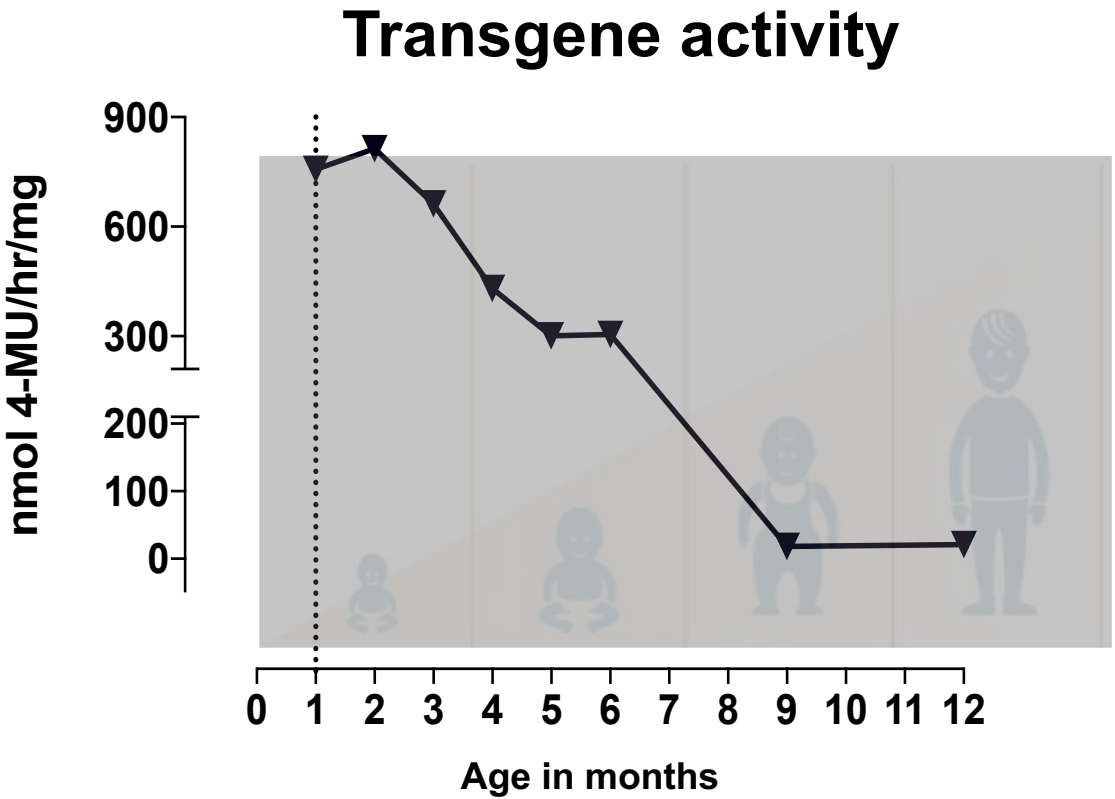
MAYBE … but probably NOT!

Early exposure = Less durable

Myoblasts are not exposed to AAV vectors



Reduced transgene expression in newborn primates over the first year due to growth



Systemic AAV9 in newborn NHPs

AAV Gene Therapy and Immunomodulation

Anti-AAV response is universal in gene therapy studies.

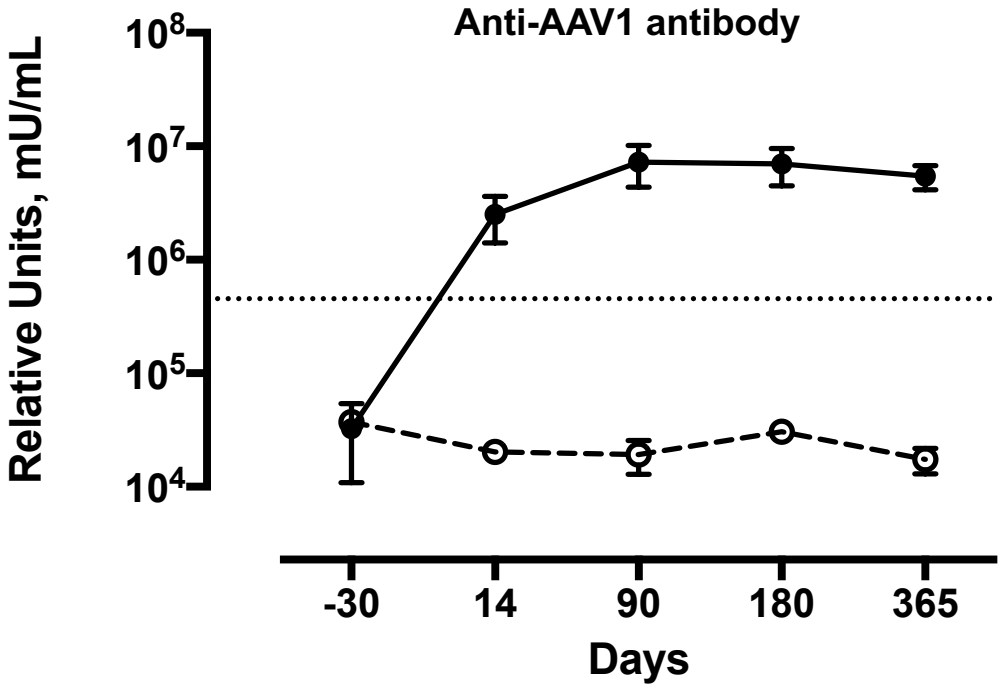
- Primary antibody formation effects vector clearance and efficacy.
- Repeat dosing must be considered in pediatric patients due to decline in genome copy number with somatic growth and muscle regeneration.

The Approach

- B-cell depletion with rituximab & sirolimus prior to AAV exposure will successfully block immune responses to the AAV capsid and transgene

- The strategy could allow for incremental or repeat administration of a vector of the same AAV serotype

B-cell depletion prevents the development of antibodies against AAV1



No immune modulation
N=6

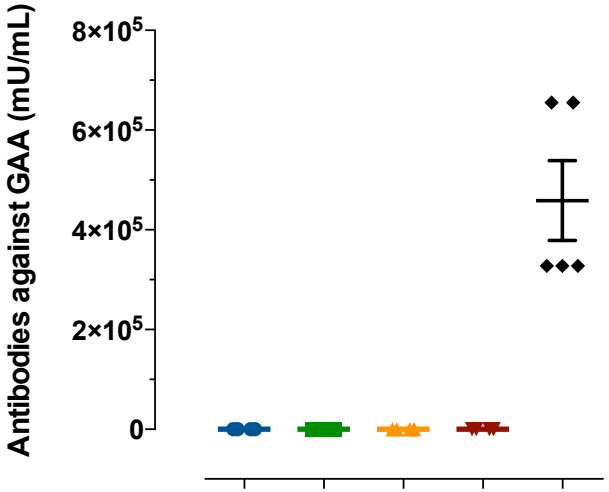
Rituximab + Sirolimus
N=3

Corti et al., 2017 and 2014; Elder et al. 2013

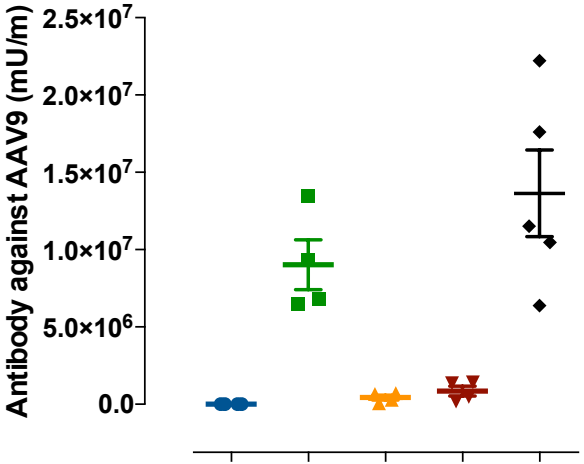
No antibodies vs the transgene or capsid after repeated AAV-GAA with immunosuppression

Excipient
AAV9-CMV-GFP
AAV9-DES-GAA once + Immunomodulation
AAV9-DES-GAA twice + Immunomodulation
AAV9-DES-GAA

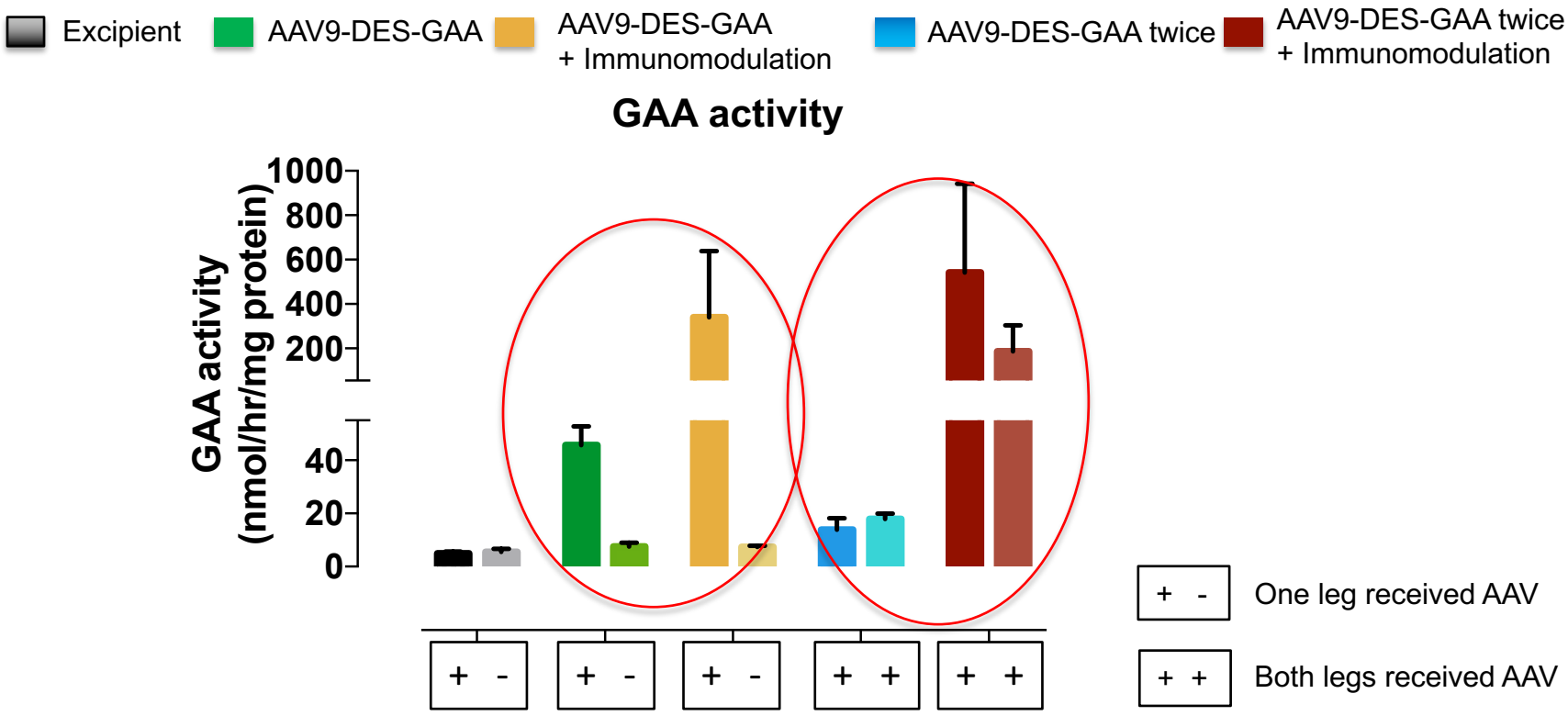
Antibodies vs. GAA



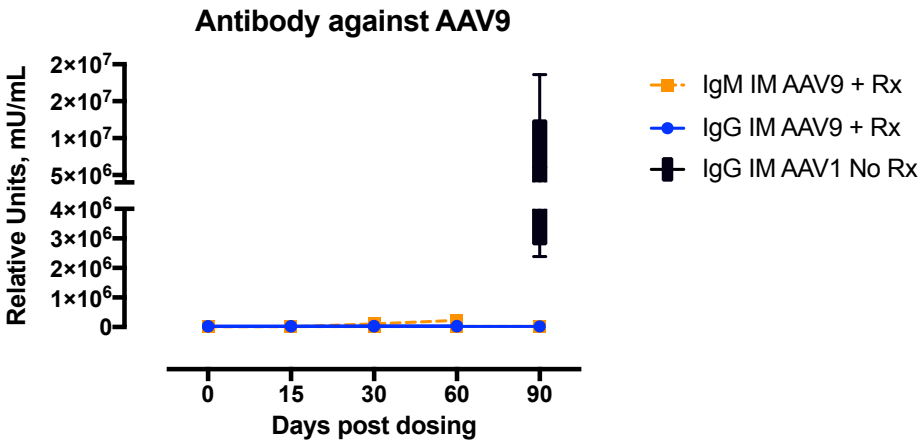
Antibodies vs. AAV9

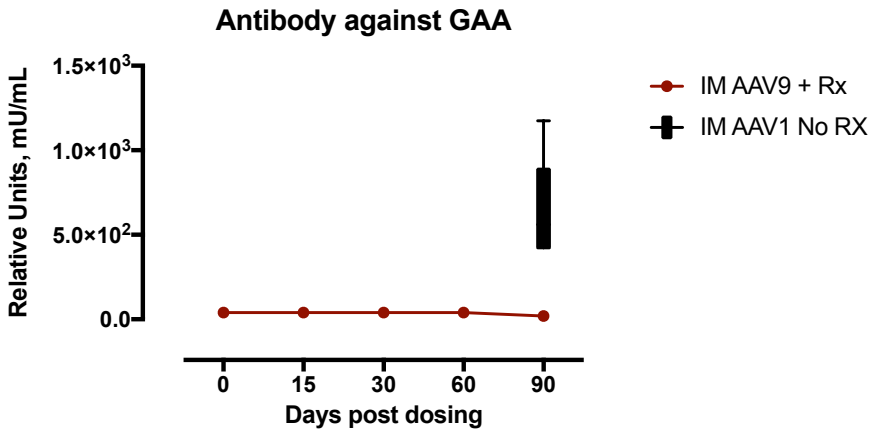


Immunomodulation increases transgene expression after single and repeat AAV-GAA dosing in NHP



Human data: preliminary results





Relevance to DMD Gene Therapy

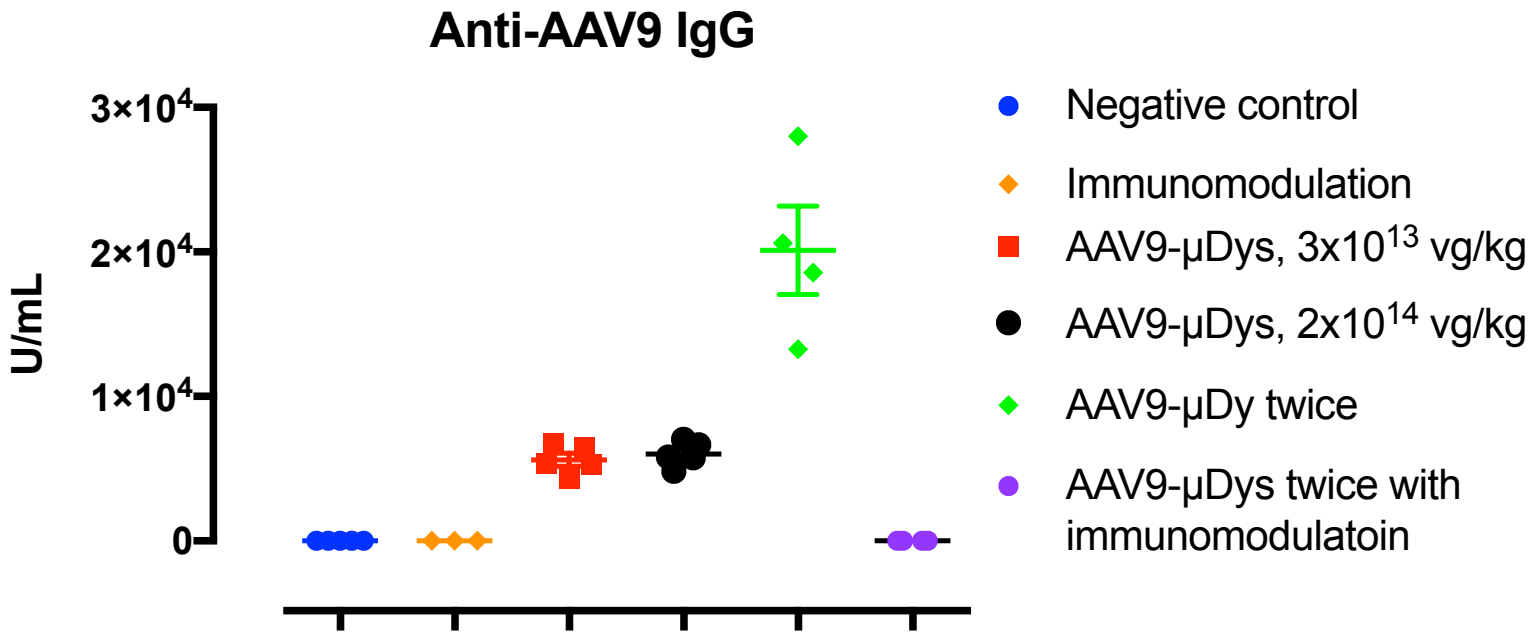
1. Management of anti-capsid antibody formation is important in both primary and secondary responses.

2. Address pre-immunity in older DMD population.

3. Enable early treatment (esp. with NBS).

4. Potential for incremental dosing to reach desired effect.

Confirmed findings in mdx mice DMD



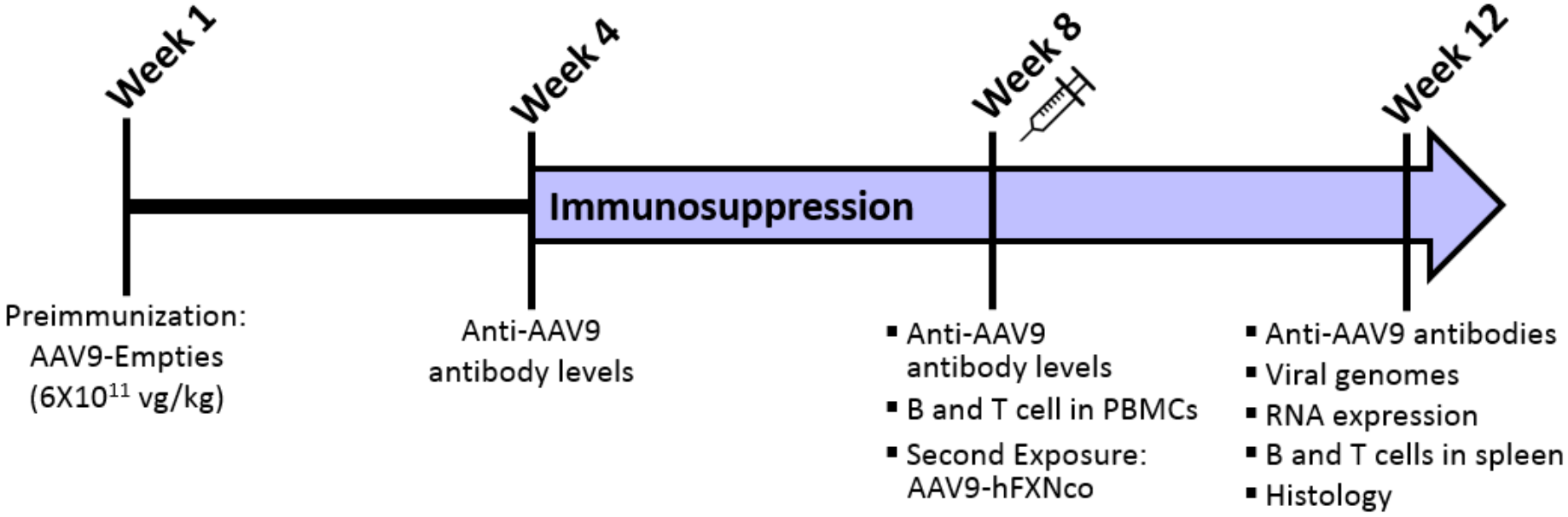
Question #4

Can existing anti-AAV be reduced to allow for entry into a gene therapy study?

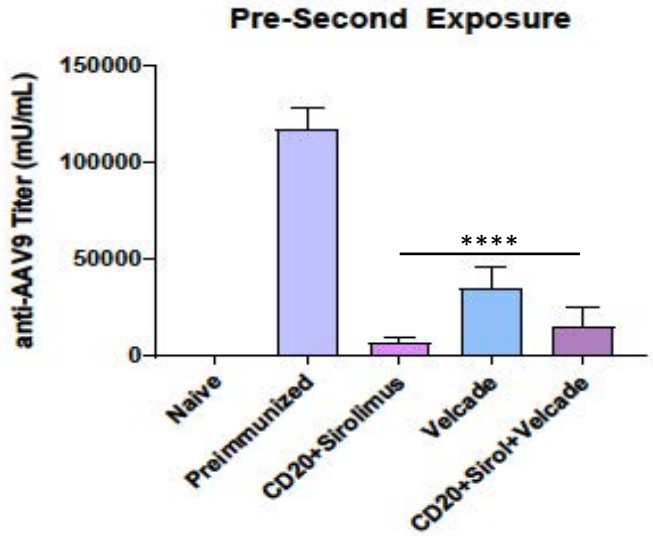
Question #4

YES!

Pre-immunity Study



Immunosuppression reduced anti-AAV9 titers



All immunomodulation groups were below inclusion criteria cutoff after 4 weeks of treatment

- Preimmune ~117 U/ml
- CD20+Sirol ~7 U /mL
- Velcade ~25 U/mL
- CD20+Sirol+Velcade ~15 U/mL

Steroid treatment does <u>not</u> affect antibody titers

Question #5

What are the side effects of gene therapy?

Side Effects to Consider

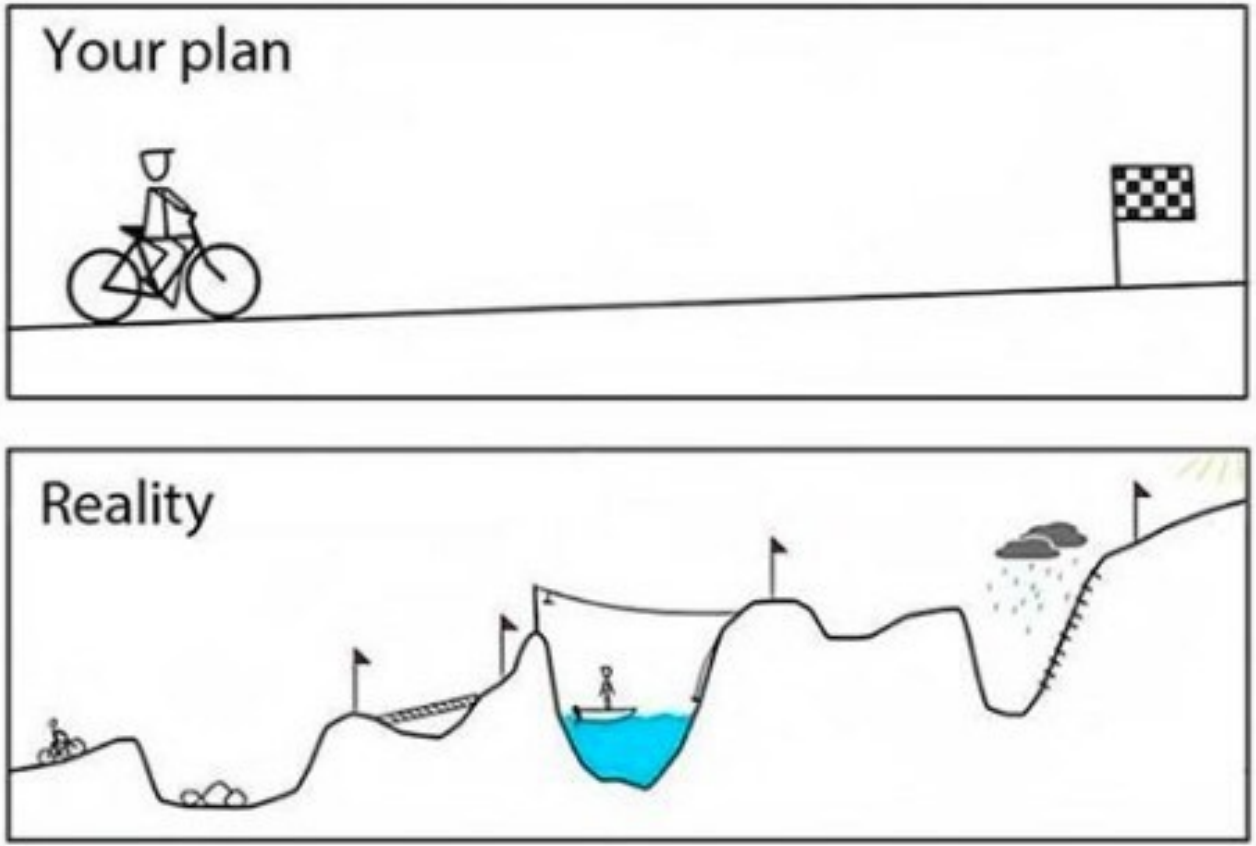
1) Fever
2) Nausea
3) Direct effect on blood count
4) Liver inflammation
5) Generalized systemic immune response
6) Late effects are undetermined

Take Home …

- AAV can be made in sufficient quantity and quality for registration studies – commercial supply is an ongoing challenge.
- Prevention is required to block antibodies to AAV.
- Early exposure = Less durable.
  BUT … Primary immune response to AAV can be blocked.

Take Home …

- Pre-existing Ab can be treated to allow for AAV gene therapy.
- AAV gene therapy is associated with side-effects/risk that must be justified with long-term benefit.


Your plan
Reality

Thanks to …

Clinical and Lab Team

Patients (Will Barkowski, artist)

Thanks to …

UF Clinical team:
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Melissa Elder, MD
Barbara Smith, PT, PhD
Samantha Norman, MPH

UF Toxicology core:
Kirsten Coleman, MBA

UF Preclinical team:
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Brian Cleaver, PhD

Lovelance Institute:
Janet Benson, PhD
Gensheng Wang, PhD

UC Davis:
Alice Tarantal, PhD

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Nina Raben, PhD

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