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

Practical Questions & Immune Response

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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,
What is AAV and how are vectors made?
Adeno-Associated Virus (AAV) Vectors

- 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

Courtesy of M. Agbandje-McKenna
University of Florida
Assembling the rAAV Cassette

Adeno-Associated Virus (AAV) Genome

ITR

Inverted Terminal Repeat

REP

Proteins associated with genome replication

CAP

Proteins associated with capsid production
Assembling the rAAV Cassette

Adeno-Associated Virus (AAV) Genome
Assembling the rAAV Cassette

Recombinant AAV (rAAV) Cassette

- **Promoter**
- **Therapeutic Gene**
- **ITR**
rAAV Manufacturing
2-Component Infection

Therapeutic Gene

Promoter

Accessory Functions

Recombinant HSV-1

Therapeutic Gene

Accessory Functions

Recombinant HSV-1

Capsid

Packaging

Assembly

Replication

REP Proteins

CAP Proteins

Nucleus

HEK-293 cell

Cell Membrane

Purification
What are AAV antibodies and why does that matter?
Question #2

WHY DON'T ANTS GET SICK?

BECAUSE THEY HAVE LITTLE ANTY BODIES.
The graph illustrates the primary and secondary immune responses.

**Primary Response**
- **Blood Antibody Level**
- **Immunologic Memory**

**Secondary Response**
- **IgG**
- **IgM**

Key Events:
- **First Ag X exposure**
- **Second Ag X exposure**
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 preexisting antibody precludes treatment?
Question #3

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

**Transgene activity**

Systemic AAV9 in newborn NHPs.
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

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

![Graph showing antibody levels over time with two groups: one with no immune modulation and the other with Rituximab + Sirolimus. The graph indicates lower antibody levels in the group treated with Rituximab + Sirolimus compared to the control group.](image-url)
No antibodies vs the transgene or capsid after repeated AAV-GAA with immunosuppression

<table>
<thead>
<tr>
<th>Excipient</th>
<th>AAV9-CMV-GFP</th>
<th>AAV9-DES-GAA once + Immunomodulation</th>
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**Antibodies vs. GAA**

- **Excipient**
  - AAV9-CMV-GFP: no immunomodulation
  - AAV9-DES-hGAA: once + Immunosuppression
  - AAV9-DES-hGAA: twice + Immunosuppression
  - AAV9-DES-hGAA: once no immunomodulation

**Antibodies vs. AAV9**

- **Excipient**
  - AAV9-CMV-GFP
  - AAV9-DES-hGAA

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- **Antibodies vs. AAV9**
  - **Excipient**
    - AAV9-CMV-GFP
    - AAV9-DES-hGAA
Immunomodulation increases transgene expression after single and repeat AAV-GAA dosing in NHP

GAA activity

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GAA activity (nmol/hr/mg protein)
Human data: preliminary results

Antibody against AAV9
- IgM IM AAV9 + Rx
- IgG IM AAV9 + Rx
- IgG IM AAV1 No Rx

Antibody against GAA
- IM AAV9 + Rx
- IM AAV1 No RX
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

Anti-AAV9 IgG

- Negative control
- Immunomodulation
- AAV9-µDys, 3x10^{13} vg/kg
- AAV9-µDys, 2x10^{14} vg/kg
- AAV9-µDy twice
- AAV9-µDys twice with immunomodulation
Can existing anti-AAV be reduced to allow for entry into a gene therapy study?
YES!
Pre-immunity Study

- **Week 1**: Preimmunization: AAV9-Empties (6x10^{11} vg/kg)
- **Week 4**: Anti-AAV9 antibody levels
- **Week 8**: • Anti-AAV9 antibody levels
  • B and T cell in PBMCs
  • Second Exposure: AAV9-hFXNco
  • Anti-AAV9 antibodies
  • Viral genomes
  • RNA expression
  • B and T cells in spleen
  • Histology
- **Week 12**
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 not affect antibody titers
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
• 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.
• 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:
Manuela Corti, PT, PhD
Melissa Elder, MD
Barbara Smith, PT, PhD
Samantha Norman, MPH

UF Toxicology core:
Kirsten Coleman, MBA

UF Preclinical team:
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Janet Benson, PhD
Gensheng Wang, PhD

UC Davis:
Alice Tarantal, PhD

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Nina Raben, PhD

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