A roadmap for bringing FKRP gene therapies into clinical use

Jeffrey S. Chamberlain, Ph.D.
McCaw Chair in Muscular Dystrophy
Wellstone Muscular Dystrophy Research Center - Seattle
Depts. of Neurology, Medicine and Biochemistry
University of Washington
Seattle, WA USA

Disclosures:  JSC is a member of the scientific advisory boards of Solid Biosciences, Ballard Biologics, AAVogen and Akashi Therapeutics
Gene therapy for muscular dystrophy

- Gene therapy approaches are showing promise for several neuromuscular disorders
  - Spinal muscular atrophy and Duchenne muscular dystrophy
- These encouraging results suggest that similar approaches might be successful for many other dystrophies
- Mutations in >20 different genes lead to ‘dystroglycanopathies’
  - Each of these leads to a failure to properly process a critical protein needed for maintaining healthy muscle cell walls (dystroglycan -Campbell lab)
- Gene therapy could restore normal protein production
  - Thus fixing the problem with altered dystroglycan function
Why gene therapy?

- Fix the actual *cause* of genetic disorders
- Potential for a permanent treatment
- Can be applied to muscles, and/or to muscle stem cells
- Many of the challenges in getting it to work are shared among different dystrophies
Gene Therapy for Dystroglycanopathies

- Among the most common dystroglycanopathies are those resulting from mutations in the FKRP gene
  - LGMD2i, MDC1C, WWS, MEB disorders
  - All result from defective production of the FKRP protein
- Can gene therapy be used to restore production of normal FKRP?
- Similar approaches may work for many or all dystroglycanopathies, and for many other types of muscular dystrophy

- What is needed to achieve these goals?
  - Examples from DMD and FKRP
Gene Therapy – different types

- **Gene replacement therapy:** deliver a new version of a gene to the target tissue (gene addition)

- **Gene editing:** directly modify a gene to fix or bypass a mutation
  - CRISPR/Cas9

- **Gene knockdown:** Shutdown the function of a mutant gene
  - Dominant disorders only- probably not applicable to dystroglycanopathies

- **RNA modification, e.g. ‘exon skipping’**
  - Sarepta’s drug for DMD- not applicable to FKRP
Potential for gene therapy supported by multiple recent successes in clinical trials:

- Hundreds of AAV gene therapy trials to date; Strong safety profile
- Two gene therapies recently approved by the FDA: a form of blindness; Car-T cells for leukemia
- Several more close to approval (e.g. hemophilia)
- 15 infants with spinal muscular atrophy successfully treated with gene therapy (Mendell/Kaspar/Avexis)
- Encouraging early data with Duchenne muscular dystrophy (DMD)
Gene Therapy for FKRP disorders

- **Goal:** Develop methods to *replace* or *repair* FKRP gene

- **Gene replacement:** AAV/FKRP

- **Gene editing:** CRISPR/Cas9
  - Must be adapted for each mutation
  - Best way to deliver remains uncertain (maybe AAV??)

- Gene replacement approaches – in human trials for several disorders

- Gene editing with CRISPR/Cas9 - future potential?
  - Not ready for prime time
Challenges for gene therapy of MDs

- How can you safely deliver a new gene to muscles (& brain?) bodywide?
  - Development of delivery VECTORS by manipulating viruses
  - Remove viral genes, replace with gene of interest (e.g. FKRP)

- Vectors derived from adeno-associated virus are promising
  - Some types enable systemic gene delivery via the bloodstream
  - AAV vectors have a small carrying capacity; gene size is important
Adeno-associated viral (AAV) vectors

**PROS:**
- Numerous ‘serotypes’, many target muscle (AAV6, 8 & 9; rh74)
- Relatively easy to produce; scalable to bioreactor production
- Can be used for bodywide gene delivery, especially to muscles
- Some types cross the blood-brain barrier

**CONS:**
- Small carrying capacity (problems with large genes)
- Generally poor results in stem cells
- Up to one-third of older patients may be immune to AAV
- Difficult to administer more than once
AAV vectors can deliver genes bodywide to muscles “systemic delivery”

- One IV injection of AAV/AP into adult mice; effect lasts more than 2 years

First identification of a way to deliver genes bodywide

Expression of micro-Dystrophin one year after AAV infusion into dystrophic mice (IV)

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Wildtype</th>
<th>Untreated</th>
<th>+AAV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forelimb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Masseter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diaphragm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quadriceps</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tibialis Ant.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastroc.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Treated gastrocnemius (lower leg muscle)

Gregorevic, Chamberlain et al: Nature Medicine, 2006
AAV-mediated gene therapy for DMD

- AAV-µDystrophin stops muscle loss, protects from exercise-injury and improves strength
- Efficient bodywide delivery has been achieved in mice and large animals
- No reduction in efficacy after at least 2.5 years
- High dose AAV delivery was safely achieved in humans (e.g. SMA- Nationwide Children’s)
- Clinical trials of AAV/µDys were started this year by 3 groups (DMD)
Clinical trials planned / in progress

- Solid Biosciences - Byrne et al (Chamberlain uDys); AAV9
- Nationwide Children’s – J Mendell et al (Sarepta) (Chamberlain uDys-1\textsuperscript{st} gen); AAV-rh74
- Pfizer – E. Smith et al (X. Xiao uDys); AAV9
- Genethon planning a trial in 1-2 years - Dickson et al (Chamberlain μDys 1\textsuperscript{st} gen; AAV8)

All are using bodywide delivery of an AAV vector to patients

- Early focus is on safety, and production of dystrophin
- Unclear on how long it will take to observe a \textit{functional} benefit
Preliminary results from DMD gene therapy trials

- Sarepta: data from 3 patients announced late June 2018
  - 1 biopsy from each: widespread micro-dystrophin observed in the gastrocnemius muscle
  - ~35-55% of normal dystrophin levels (2 patients)
  - Serum CK levels reduced ~85% (3 patients)
Preliminary results from DMD gene therapy trials

- Solid Biosciences and Pfizer have not yet announced results
  - Parents report *anecdotal* improvement, but not a reliable result
  - Reporting data on Facebook/Twitter?
- Data reported so far matches data from large animal studies

Future:
- At least 12 more patients to be studied over the next year
- Future results will include strength measurements
- Testing in older patients
- *Continued positive results will simplify gene therapy for other muscular dystrophies*
Gene therapy for FKRP mutations

- Consortium formed to plan trials in March 2018
- Initial meeting - U Mass
  - Qi Lu, Isabelle Richard, Katherine Matthews, Kathryn Wagner, Francesco Muntoni, Jeff Chamberlain, Volker Straub, Brenda Wong, Terry Flotte, Jean-Pierre Laurent, Kelly Brazzo, Herb Stevenson

- Possibility of two trials (France, USA)
- Details of vector/gene/dosing being explored
- Immediate goal to begin screening patients for antibodies (immunity) to AAV vectors
Vector development and testing for gene therapy with AAV-FKRP in mouse models

Several groups have designed AAV vectors to make FKRP

*Current studies are asking:*

- Which type of AAV is best
- Best promoter to use (on/off switch)?
- Design of FKRP gene?
- How much FKRP is needed
- Does the stage of disease (& age) affect therapy?

*AAV vector:*

```
ITR  Promoter            FKRP gene  stop  ITR
```
AAV-FKRP gene delivery improves muscle structure in a mouse model for LGMD2i

A

<table>
<thead>
<tr>
<th></th>
<th>PBS injected muscles</th>
<th>AAV-FKRP injected muscles</th>
</tr>
</thead>
<tbody>
<tr>
<td>FKRP</td>
<td><img src="image" alt="Western blot - FKRP production" /></td>
<td></td>
</tr>
<tr>
<td>GAPDH</td>
<td><img src="image" alt="Western blot - FKRP production" /></td>
<td></td>
</tr>
</tbody>
</table>

B

WT | FKRP-L276I | FKRP-L276I AAV-FKRP

Muscle structure

C

WT | FKRP-L276I | FKRP-L276I AAV-FKRP

Muscle damage

- Data from Isabelle Richard’s lab; Genethon (France)
AAV-FKRP gene delivery improves muscle function at a variety of mouse ages (MDC1C model)

- Qi Lu lab, Mol Ther Meth Clin Dev 2017

Red = glycosylated alpha-dystroglycan (shows restored function of FKRP)
Production of FKRP using AAV-FKRP

- promoter: muscle-restricted promoter/enhancer (on/off switch)
- Same basic vector backbone as in Solid Biosciences DMD trial

![AAV vector diagram]

**C2C12s transduction - untagged rAAV6-CK8-FKRP**

Muscle cells in a petri dish

**Western blots for FKRP protein:**

Intramuscular injection (1e11vg) - untagged rAAV6-CK8-FKRP

Whole mouse

Jane Seto & J Chamberlain, unpublished
AAV-FKRP gene delivery improves muscle function *(MDC1C mouse model)*

Dan Rodgers & J Chamberlain, unpublished
Gene therapy with AAV-FKRP shows significant promise in mouse models - what is needed to begin clinical trials?

- Re-visit issue of how much FKRP is needed and if too much is bad (e.g. Tucker & Lu, 2018)
- Choose ‘best’ version of AAV-FKRP vector
  - on/off switch; version of FKRP gene; type of AAV
- Safety studies in large animal models
  - Rat? Monkey?; choose dose
  - Seeking guidance from the FDA
- Identify contractor to produce clinical grade AAV
- Recruit patients
- Begin phase I/II clinical trials
Patients and FKRP gene therapies

- What age/stage of disease to enroll?
- LGMD2i or MDC1C?
- Maintain and update patient databases
- Do we have adequate assays/biomarkers to measure outcome? How long will it take to see an effect?

- Screen for AAV neutralizing antibodies
- Develop informed consent protocols/forms
- IRB and FDA approval
- Enroll patients
Future prospects for FKRP Gene

- Basic science, proof of principle is in place
- SMA & DMD trials provide hope for efficacy and safety
- Several issues still need to be resolved
  - Dose, final vector design, etc
  - Biomarkers and outcome measures needed
- Major efforts now shifting to manufacturing, regulatory approval, trial design

- Gene therapy for many other dystrophies can also move towards the clinic

**However, development of these therapies is still early and highly experimental!**
  - Let’s not abandon alternatives
Thank you for coming to the Iowa Wellstone Center 2018 Dystroglycanopathy Patient and Family Conference!

The Chamberlain lab is supported by:
The LGMD2i fund
The Muscular Dystrophy Association, and
The National Institutes of Health