Gene Therapy for Muscular Dystrophy

A Decade of Research and Challenges

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Objectives

- **Describe current status and future challenges for gene therapy for muscular dystrophy**
  - *Discuss the following points considering the clinical implications of each:*
  1. Choice of virus for gene delivery
  2. Methods of Gene Delivery
  3. The challenges posed by large size of the Dysferlin Gene
  4. Choices of potential genes to deliver
Choice of Virus

- Adenovirus
- Lentivirus
- Adeno-Associated virus (AAV)
Choice of Virus for Gene Replacement

- No Human disease
  - Reduced immune response
  - Reduced toxicity
  - Delivered to nucleus & no integration to genome

Adeno-associated virus
Small Capacity 4.7 kb
Dysferlin needs 6.2 kb

- Limitations
  - Small insert capacity
Preparing AAV as Gene Delivery Vehicle

Dysferlin Gene

Dysferlin Gene
Methods of Gene Delivery

- **Intramuscular**
  - Gene delivered directly to muscle. This method tests the validity of the gene and is the first safety test.
  - May be required by the FDA if first time in clinical trial.
First Gene Therapy Clinical Trial for LGMD type 2D
April 23, 2008
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LGMD2D Gene Therapy Trial (n = 6)  
Alpha-Sarcoglycan Deficiency

rAAV1.tMCK.hSCGA

Muscle Specific Promoter
Sustained Alpha-Sarcoglycan Gene Expression after Gene Transfer in Limb-Girdle Muscular Dystrophy, Type 2D

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Brian D. Coley, MD,4 Gloria Galloway, MD,1,2,3 Sarah Lewis, MD,3 Vinod Malik, MD,3
Chris Shilling, MD,3 Barry J. Byrne, MD,5,6 Thomas Conlon, MD,5,6
Katherine J. Campbell, MD,7 William G. Bremer, MD,7 Laura E. Taylor, MD,3
Kevin M. Flanigan, MD,1,3 Julie M. Gastier-Foster, PhD,8 Caroline Astbury, PhD,8
Janaiah Kota, MD,3 Zarife Sahenk, MD,1,2,3 Christopher M. Walker, MD,1,7
and K. Reed Clark, MD1,3

Objective: The aim of this study was to attain long-lasting alpha-sarcoglycan gene expression in limb-girdle muscular dystrophy, type 2D (LGMD2D) subjects mediated by adeno-associated virus (AAV) gene transfer under control of a muscle specific promoter (tMCK).

Methods: rAAV1.tMCK.hSGCA (3.25 × 1011 vector genomes) was delivered to the extensor digitorum brevis muscle of 3 subjects with documented SGCA mutations via a double-blind, randomized, placebo controlled trial. Control sides received saline. The blind was not broken until the study was completed at 6 months and all results were reported to the oversight committee.

Results: Persistent alpha-sarcoglycan gene expression was achieved for 6 months in 2 of 3 LGMD2D subjects. Markers for muscle fiber transduction other than alpha-sarcoglycan included expression of major histocompatibility complex I, increase in muscle fiber size, and restoration of the full sarcoglycan complex. Mononuclear inflammatory cells recruited to the site of gene transfer appeared to undergo programmed cell death, demonstrated by terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling and caspase-3 staining. A patient failing gene transfer demonstrated an early rise in neutralizing antibody titers and T-cell immunity to AAV, validated by enzyme-linked immunospot on the second day after gene injection. This was in clear distinction to other participants with satisfactory gene expression.

Interpretation: The findings of this gene replacement study in LGMD2D subjects have important implications not previously demonstrated in muscular dystrophy. Long-term, sustainable gene expression of alpha-sarcoglycan was observed following gene transfer mediated by AAV. The merit of a muscle-specific tMCK promoter, not previously used in a clinical trial, was evident, and the potential for reversal of disease was displayed.
Six LGMD2D Patients
Muscle biopsies at 3 and 6 months
Gene Expression levels reached near normal levels
• **Intravascular**
  Gene delivered directly to circulation
  – Currently using a method called **ISOLATED LIMB PERFUSION**
  – Will deliver virus to all muscles of the extremity
  – Currently Using this approach for **LGMD2D**
Study Initiated March 23, 2015
Efficient Gene Delivery to Muscle

Femoral Artery Vascular Delivery
ILP Safe and Effective
Intravenous Delivery
Immune Responses following Gene Delivery

- Patient's mutation and Gene must be a perfect match to prevent rejection

- If the patient has previously been exposed to AAV, the gene could be rejected
Dystrophin Immunity in Duchenne’s Muscular Dystrophy

Jerry R. Mendell, M.D., Katherine Campbell, B.S., Louise Rodino-Klapac, Ph.D., Zarife Sahenk, M.D., Ph.D., Chris Shilling, M.S., Sarah Lewis, Dawn Bowles, Ph.D., Steven Gray, Ph.D., Chengwen Li, Ph.D., Gloria Galloway, M.D., Vinod Malik, Ph.D., Brian Coley, M.D., K. Reed Clark, Ph.D., Juan Li, M.D., Xiao Xiao, Ph.D., Jade Samulski, M.P.M., Scott W. McPhee, Ph.D., R. Jude Samulski, Ph.D., and Christopher M. Walker, Ph.D.

SUMMARY

We report on delivery of a functional dystrophin transgene to skeletal muscle in six patients with Duchenne's muscular dystrophy. Dystrophin-specific T cells were detected after treatment, providing evidence of transgene expression even when the functional protein was not visualized in skeletal muscle. Circulating dystrophin-specific T cells were unexpectedly detected in two patients before vector treatment. Revertant dystrophin fibers, which expressed functional, truncated dystrophin from the deleted endogenous gene after spontaneous in-frame splicing, contained epitopes targeted by the autoreactive T cells. The potential for T-cell immunity to self and nonself dystrophin epitopes should be considered in designing and monitoring experimental therapies for this disease. (Funded by the Muscular Dystrophy Association and others; ClinicalTrials.gov number, NCT00428935.)
Subject 005

DMD Gene Therapy Trial

Missing portion of Gene from mutation

non-self

ABD H1 1 2

H3 22 23 24 H4

IFN-γ T cell assay

IFN-γ SFC/10^6 PBMC

Days Post Gene Transfer

Pre 15 30 60 90 120 180 365

0 50 100 150 200 250 300 350

DP1 DP2 DP3
Challenges Posed by Large Size of Gene for AAV Packaging

- Gene for transfer must be capable of fitting into AAV and if not Choices Include:
  - Reducing the size of the gene at the risk of not correcting the defect (dystrophin gene for DMD)
  - Packaging the gene in separate pieces more reliable for correcting defect
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The DYSF gene is located on the short (p) arm of chromosome 2 at position 13.3. (2p13.3)
AAV Choice of Virus for Gene Replacement

- Efficient for gene transfer
- “Episomal” - does not disturb other genes
- Long-term persistence with single injection

- AAV Size limitation - 4.7 kb
Package the Gene in Separate Pieces

Homologous Recombination

5' Vector

6.5 kb

3' Vector

DYSF
Dysferlin expression levels are maintained for at least 12 months.
MEMBRANE REPAIR

WT muscle fiber

129-Dysf KO

129-Dysf KO rAAV.Dysf
Gene Replacement for Dysferlin in LGMD2B

Looks Very Favorable

Will Move to Clinical Trial 2016
Follistatin Gene Therapy

Increasing Muscle Strength by Inhibition of Myostatin Pathway
Myostatin Gene Mutation

- Targeted disruption of the myostatin gene: increases muscle size and body weight

- “Mighty” Mouse (Mstn KO)
- Double-muscled cow (Mstn Het)
- Newborn with gene mutation

MYOSTATIN REGULATION OF MUSCLE SIZE

Circulating myostatin Propeptide Complex

INHIBIT BINDING
Follistatin
Circulating complex
Propeptide-myostatin

Propeptide cleavage

FOLLISTATIN

INJECT AAV INTO MUSCLE
FS344 Gene Transfer to Monkey
AAV1-FS Control

5 MO POST GENE TRANSFER

Control MCK-FS CMV-FS
Becker Muscular Dystrophy
Low Dose Follistatin 6e11 vg/kg

6MWT (meters)
Baseline Injection 30 days 60 days 90 days 6 months 1 year

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<td>BL</td>
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<td>550 416 466</td>
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<tr>
<td>12-mo change</td>
<td>+58 m +125 m +9 m</td>
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Becker Muscular Dystrophy
High Dose Follistatin 1.2e12 vg/k

![Graph showing 6MWT (meters) vs time (Baseline, Injection, 30 days, 60 days, 90 days, 6 months) for visits 04, 05, and 06.]

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<td>6-mo change</td>
<td>-14 m</td>
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6 Minute Walk Distance

6MWT in 6 Becker Patients

Statistically Significant 11.5% average improvement after 180 days
Path to Gene Therapy Trial

1. Establish unequivocal Proof of Principle

2. Discuss with FDA – Pre-IND Meeting
   - Plan Clinical Trial (age, inclusion criteria)
   - Plan Toxicology Study
     - Mice only or include non-human primates

3. Perform Toxicology study using same virus as for clinical trials

4. Present study to Recombinant DNA Advisory Committee (RAC)

5. Apply for approval to Internal Review Board

6. Final Step submitting the IND
Final Step Before Clinical Trial
Submit the IND

AAV1.CMV.Follistatin
IND
09-12-11
Nationwide Children’s Research Institute