Duchenne and Becker muscular dystrophies

Kevin M. Flanigan, M.D.
The Research Institute at Nationwide Children’s Hospital
Columbus, Ohio
Duchenne Muscular Dystrophy

• Progressive skeletal muscle degeneration
  – Onset age 3-5:
    • Pelvic girdle weakness (difficulty arising/climbing stairs)
    • Gait abnormalities (toe walking)
    • Serum CK 50-100X normal

• Commonly cited incidence of 1:3500 live male births; closer to 1:5200

• MDA estimates up to 12,000 boys with DMD registered in clinics
• Loss of ambulation by age 12 years (range 7-12)

• Mean age at death around 19 years
  – Dilated cardiomyopathy
  – Ventilatory insufficiency

• Glucocorticoid corticosteroids
  – Prednisone 0.75 mg/kg/day
  – Deflazacort 0.9 mg/kg/day
  – AAN Practice Parameters; Cochrane review
  – Prolonged ambulation (up to 1-3 years)
  – Significant side effects

• Supportive Care
  – Nocturnal ventilatory support
  – Spinal surgery in appropriate candidates
RANDOMIZED, DOUBLE-BLIND SIX-MONTH TRIAL OF PREDNISONE IN DUCHENNE'S MUSCULAR DYSTROPHY


Figure 1. Change (Mean ± SEM) in the Score for Average Muscle Strength in the Placebo and Prednisone Groups after the Initiation of Their Regimens.

The solid line ("natural history") represents the values for change observed in 177 patients with Duchenne's muscular dystrophy who received no treatment.9,12
In a review of retrospective publications with long-term (>3 year) followup, corticosteroid treatment:

- Prolongs ambulation by 2 to 5 years
- Reduces the need for spinal stabilization surgery
- Improves cardiopulmonary function
- Delays the need for noninvasive nasal ventilation
- Increases survival and the quality of life of patients with Duchenne muscular dystrophy
Dystrophinopathies: Clinical diagnosis

Duchenne muscular dystrophy (DMD):
- Onset age 3-5
- Pelvic girdle weakness
- Tight heel cords
- CK 50-100X normal
- Loss of ambulation by age 12 (range 7-12)
- Death by age 20 (historically)

Becker muscular dystrophy (BMD):
- Classic definition: loss of ambulation > age 12
- Alternatively:
  - “intermediate muscular dystrophy” for loss of ambulation ages 12 through 15
  - BMD for loss of ambulation > age 15
- Limb-girdle syndromes in adulthood
- Muscle aches (myalgias)
- Isolated cardiomyopathy
Dystrophin Mutations

• Dystrophin gene (Xp21.1) is huge:
  – 2.4 million nucleotides
  – 79 exons and 8 promoters

• Large deletions (≥ 1 exon) account for ~65% of DMD/BMD patients

• ~5% have duplications
• ~15% of boys have nonsense mutations
• Remainder are frameshifting insertions/deletions, splice site mutations, missense mutations
Dystrophin mutations: Duchenne vs Becker

• **Size** of deletion does not correlate well with phenotype

• Best correlation is whether the deletion is “**in-frame**” or “**out-of-frame**”

• **In-frame deletions** are more likely to result in translation of a protein with partial function
  – (i.e., out-of-frame deletions are DMD ~90% of the time)
THE BIG RED DOG RAN AND SAT
TH EBI GRE DDO GRA NAN DSA T
Reading Frame

• THE BIG RED DOG RAN AND SAT
Reading Frame

- THE BIG RED DOG RAN AND SAT
- THE BIRD DREW ORANGE AND SAT
Reading Frame

- THE **BIG RED** DOG RAN AND SAT
- THE **BIG RED** DOG RAN AND SAT
- THE **BIG RED** DOG RAN AND SAT
Reading Frame

- THE BIG RED DOG RAN AND SAT
- THE BIR EDD OGR ANA NDS AT
  = Duchenne Muscular Dystrophy
- THE DOG RAN AND SAT
  = Becker Muscular Dystrophy
Dystrophin (427 kd)

GAPDH
Copy Number Testing (deletions, duplications, and related carrier states)

- Multiplex PCR
- Southern blot analysis
- Quantitative PCR (radiolabeled/fluorophore/dye concentration)
- Multiplex amplifiable probe hybridization
- Multiplex ligation-dependent probe amplification (MLPA)
- Comparative genomic hybridization (CGH)
MLPA

Sellner LN and Taylor GR. *Hum Mutat.* 2004;23(5):413-419.
MLPA Readout
Duplication: Exons 18-19
Deletion: Exon 45

43060
Deletion: Exons 8-9
Array Comparative Genomic Hybridization (Array-CGH)

Targeted CGH Dystrophin Array

## Distribution of mutations in an unselected cohort

(Dent et al; AJMG, 2005 Apr 30;134(3):295-8)

<table>
<thead>
<tr>
<th>Mutation Type</th>
<th>DMD</th>
<th>BMD</th>
<th>Carrier</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥1 exon deletion</td>
<td>32</td>
<td>13</td>
<td></td>
<td>45 (66%)</td>
</tr>
<tr>
<td>Premature Stop</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>9 (13%)</td>
</tr>
<tr>
<td>Missense</td>
<td>1</td>
<td>2</td>
<td></td>
<td>3 (4%)</td>
</tr>
<tr>
<td>Frameshift insertion or deletion</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>≥1 exon duplication</td>
<td>3</td>
<td>1</td>
<td></td>
<td>4 (6%)</td>
</tr>
<tr>
<td>No mutation detected</td>
<td>3</td>
<td>2</td>
<td></td>
<td>5 (7%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>45</td>
<td>21</td>
<td>2</td>
<td>68</td>
</tr>
</tbody>
</table>

Currently available methodology can detect 93%-96% of dystrophinopathy mutations from blood samples.

(Yan et al, Hum Mutat 2004; 23:203-204).
Mutations May Not Be Detectable by Genomic DNA Analysis

- Pseudoexon mutations
  - Deep intronic point mutations
  - Create splice donor or acceptor sites
  - Intronic DNA included as a “pseudoexon” in mRNA
  - Undetectable from blood

### Table 2. The Value of Mutational Reading Frame in Predicting a Phenotype of Duchenne Muscular Dystrophy

<table>
<thead>
<tr>
<th></th>
<th>DMD</th>
<th>I/BMD</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Exonic deletions only</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Truncating (out-of-frame)</td>
<td>254</td>
<td>32</td>
<td>88.8%</td>
<td>Positive predictive value</td>
</tr>
<tr>
<td>mutations</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-truncating (in-frame)</td>
<td>30</td>
<td>38</td>
<td>55.9%</td>
<td>Negative predictive value</td>
</tr>
<tr>
<td>mutations</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>89.4%</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specificity</td>
<td>—</td>
<td>54.3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All mutations&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Truncating mutations</td>
<td>519</td>
<td>79</td>
<td>86.8%</td>
<td>Positive predictive value</td>
</tr>
<tr>
<td>Non-truncating mutations</td>
<td>37</td>
<td>63</td>
<td>63.0%</td>
<td>Negative predictive value</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>93.3%</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specificity</td>
<td>—</td>
<td>44.4%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

45%-55% of BMD patients have out-of-frame mutations.
Nonsense Mutations Do Not Always Predict DMD

• Mutations predicted as nonsense mutations may instead affect exon splice regulatory signals\(^1,2\)
  – This results in exclusion of exons
  – The remaining mRNA may be in-frame

Distribution of BMD versus DMD nonsense mutations

In-frame exons (39) shaded  Out-of-frame (40) unshaded

- p.Trp3X

- DMD 176
- I/BMD 26

p = 0.004
BMD with nonsense mutations occurs preferentially in some exons more than others

i.e, Those with:

The weakest aggregate splice site signals of all \textit{DMD} exons

Weaker competing 3’-ss strengths than those exons that only have DMD mutations

Lower ESE densities than those exons that only have DMD mutations
Exon splice regulatory elements alterations occur within an exon definition context

described in A. Disset *et al.*, Human Molecular Genetics 2006
Nonsense-induced exon skipping

c.4240C>T
p.1414Gly>X
Modifiers of phenotype:

Modifiers at the *DMD* locus:

- Altered mRNA splicing (nonsense-associated BMD)

Other genetic modifiers
SPP1 genotype is a determinant of disease severity in Duchenne muscular dystrophy

- Cytokine involved in immune cell migration and survival
- Implicated in fibrosis through the TGF-beta pathway

**ABSTRACT**

**Objective:** Duchenne muscular dystrophy (DMD) is the most common single-gene lethal disorder. Substantial patient-patient variability in disease onset and progression and response to glucocorticoids is seen, suggesting genetic or environmental modifiers.

**Methods:** Two DMD cohorts were used as test and validation groups to define genetic modifiers: a Padova longitudinal cohort (n = 106) and the Cooperative International Neuromuscular Research Group (CINRG) cross-sectional natural history cohort (n = 156). Single nucleotide polymorphisms to be genotyped were selected from mRNA profiling in patients with severe vs mild DMD, and genome-wide association studies in metabolism and polymorphisms influencing muscle phenotypes in normal volunteers were studied.

**Results:** Effects on both disease progression and response to glucocorticoids were observed with polymorphism rs28357094 in the gene promoter of SPP1 (osteopontin). The G allele (dominant model; 35% of subjects) was associated with more rapid progression (Padova cohort log rank p = 0.003), and 12%-19% less grip strength (CINRG cohort p = 0.0003).

**Conclusions:** Osteopontin genotype is a genetic modifier of disease severity in Duchenne dystrophy. Inclusion of genotype data as a covariate or in inclusion criteria in DMD clinical trials would reduce intersubject variance, and increase sensitivity of the trials, particularly in older subjects.

*Neurology* 2011;76:219-226
Figure 2: SPP1 genotype is associated with decreased strength in steroid-treated patients with Duchenne muscular dystrophy.

\[ p = 0.0017 \]

Figure 3: SPP1 genotype is associated with greater severity of progression in Duchenne muscular dystrophy (DMD).

The proportion of patients with DMD in the Padova cohort remaining ambulatory at the specific age noted is shown (n = 106). The GT/GG genotype is associated with more rapid progression.

Pegoraro et al, 2011
Latent TGFβ binding protein 4 (LTBP4) is a member of the Fibrillin superfamily.
An insertion/deletion in LTBP4 modifies muscular dystrophy

Heydemann et al. 2009
LTBPs regulate TGFβ availability

SECRETING CELL

LTBP

TGFB

proteolysis

extracellular matrix

Large latent complex

LTBP + TGFB

RECEIVING CELL

SMAD-P

TGFβ receptor

Gene expression
Variability of the LTBP4 proline rich region across species

Ceco, Heydemann
Single nucleotide polymorphisms in human $\textit{LTBP4}$
Does *LTBP4* influence DMD phenotype?

- Use loss of ambulation as a dichotomous outcome

- To take an unbiased approach, we included all subjects who had a recorded loss of ambulation before age 20

- N = 254 subjects
  - 244 (96%) were catalogued as DMD
  - 8 = IMD (lost ambulation between age 12 and 15)
  - 2 = BMD (lost ambulation after age 15 but before age 20)
The $LTBP4$ “IAAM” haplotype predicts prolonged ambulation in DMD.
Prolonged ambulation with \textit{LTBP4} “IAAM” is not explained by DMD mutation.
Steroid use with \textit{LTBP4} “IAAM” predicts prolonged ambulation in DMD
Steroid use with *LTBP4* “IAAM” predicts prolonged ambulation in DMD

![Graph showing the relationship between steroid use and age at ambulatory loss.]
Haplotype analysis of nonsynonymous *LTBP4* variants associated with age of ambulatory loss

Mean age of ambulatory loss for “IAAM” versus other haplotypes:
- In steroid-treated patients: 12.5 ± 3.3 years vs. 10.7 ± 2.1 years
- In steroid-naïve patients: 11.2 ± 2.7 vs. 9.8 ± 2.0 years

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>All (n=254)</th>
<th>Steroid treated (n=137)</th>
<th>Steroid naive (n=102)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Global P^a = 0.002</td>
<td>Global P^a = 0.013</td>
<td>Global P^a = 0.12</td>
</tr>
<tr>
<td>VTTT</td>
<td>freq 0.53</td>
<td>score -1.51</td>
<td>p-val 0.1</td>
</tr>
<tr>
<td>IAAM</td>
<td>freq 0.31</td>
<td>score 3.43</td>
<td>6 x 10^-4</td>
</tr>
</tbody>
</table>
"LTBP4 "IAAM" fibroblasts have reduced TGFβ signaling"
LTBP4 conclusions

- The *LTBP4* IAAM haplotype is associated with decreased TGFb signaling.

- *LTBP4* genotype effect is:
  - seen independent of the primary mutation (truncating or not)
  - seen in both glucocorticoid treated and naïve DMD subjects.

- Stratification for *LTBP4* genotype should be considered for clinical trials (or predefined post-hoc analysis)

- LTBP4 presents a novel therapeutic target.
Potential Therapies in Trials

- Stop Codon Readthrough
- Exon Skipping
- Gene Transfer
Potential Therapies in Trials

- Stop Codon Readthrough
- Exon Skipping
- Gene Transfer
6MWT NATURAL HISTORY

BOYS >7 YEARS EXPERIENCE PROGRESSIVE AMBULATORY DECLINE OVER 1-3 YEARS

*Note. Natural history studies imputed zero values for patients who lost ambulation during follow-up periods.

6MWT, 6-minute walk test; BL, baseline.
Premature stop codon mutations

Premature translation termination vs. Full-length dystrophin
Aminoglycoside antibiotics restore dystrophin function to skeletal muscles of mdx mice.
Gentamicin-Induced Readthrough of Stop Codons in Duchenne Muscular Dystrophy

Vinod Malik, PhD,1 Louise R. Rodino-Klapac, PhD,1 Laurence Viollet, PhD,1 Cheryl Wall, RN,3 Wendy King, PT,3 Roula Al-Dahhak, MD,1 Sarah Lewis,1 Christopher J. Shilling, MS,1 Janaiah Kota, PhD,1 Carmen Serrano-Munuera, MD,3 John Hayes, PhD,2 John D. Mahan, MD,2 Katherine J. Campbell,4 Brenda Banwell, MD,5 Majed Dasouki, MD,6,7 Victoria Watts,6,7 Kumaraswamy Sivakumar, MD,8 Ricardo Bien-Willner,8 Kevin M. Flanigan, MD,1,2,3,9 Zarife Sahenk, MD, PhD,1,2,3 Richard J. Barohn, MD,6,7 Christopher M. Walker, PhD,2,3,4 and Jerry R. Mendell, MD1,2,3

Gentamicin 7.5 mg/kg infusion:
Fourteen doses, with biopsy at six months

Phase 2a Study of Ataluren-Mediated Dystrophin Production in Patients with Nonsense Mutation Duchenne Muscular Dystrophy

Figure 3. Percentage Change From Pretreatment in Dystrophin:Spectrin Ratio.
doi:10.1371/journal.pone.0081302.g003
ATALUREN TREATMENT OF PATIENTS WITH NONSENSE MUTATION DYSTROPHINOPATHY

FIGURE 1. Change in 6MWD. Mean changes in the ataluren 10, 10, 20 mg/kg and placebo arms were −12.86 and −44.14 meters, respectively, resulting in a difference of 31.28 meters.

Potential Therapies in Current Trials

- Stop Codon Readthrough
- Exon Skipping
- Gene Transfer
Antisense oligonucleotides can induce exon skipping

Out-of-frame deletion (DMD) pre-mRNA:
THE BID OGR ANA NDS AT

In-frame deletion (BMD) mRNA:
THE DOG RAN AND SAT

Antisense oligomer
Antisense oligonucleotide backbones

Safety and efficacy of drisapersen for the treatment of Duchenne muscular dystrophy (DEMAND II): an exploratory, randomised, placebo-controlled phase 2 study

Thomas Voit, Haluk Topaloglu, Volker Straub, Francesco Muntoni, Nicolas Deconinck, Giles Campion, Sjef De Kimpe, Michelle Eagle, Michela Guglieri, Steve Hood, Lia Liefaard, Afrodite Lourbakos, Allison Morgan, Joanna Naklefny, Naashika Quarcoo, Valeria Ricotti, Katie Rolfe, Laurent Servais, Claire Wardell, Rosamund Wilson, Padraig Wright, John E Kraus

Table 2: Change from baseline in 6-min walk distance (intention-to-treat population, observed case data)

<table>
<thead>
<tr>
<th></th>
<th>Placebo (combined; n=18)</th>
<th>Drisapersen 6 mg/kg continuous (n=18)</th>
<th>Drisapersen 6 mg/kg intermittent (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of patients</td>
<td>18</td>
<td>18</td>
<td>17</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>403.18 (45.13)</td>
<td>427.61 (70.05)</td>
<td>394.57 (66.95)</td>
</tr>
<tr>
<td><strong>Week 25 (primary endpoint)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of patients</td>
<td>16</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>Adjusted mean change (SE)</td>
<td>-3.6 (9.7)</td>
<td>31.5 (9.8)</td>
<td>-0.1 (10.3)</td>
</tr>
<tr>
<td>Adjusted mean difference vs placebo (95% CI)</td>
<td>35.09 (7.59 to 62.60)</td>
<td>3.51 (-24.34 to 31.35)</td>
<td></td>
</tr>
<tr>
<td>p value</td>
<td>0.014</td>
<td>0.801</td>
<td></td>
</tr>
<tr>
<td><strong>Week 49 (secondary endpoint)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of patients</td>
<td>17</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td>Adjusted mean change (SE)</td>
<td>-24.7 (12.8)</td>
<td>11.2 (12.6)</td>
<td>2.4 (13.6)</td>
</tr>
<tr>
<td>Adjusted mean difference vs placebo (95% CI)</td>
<td>35.84 (-0.11 to 71.78)</td>
<td>27.08 (-9.83 to 63.99)</td>
<td></td>
</tr>
<tr>
<td>p value</td>
<td>0.051</td>
<td>0.147</td>
<td></td>
</tr>
</tbody>
</table>

Missing data for the 6-min walk distance (6MWD) in the primary analysis was a result of tight visit windows—i.e., when a visit was attended 4 or more days early or late.
# Eteplirsen for the Treatment of Duchenne Muscular Dystrophy

Jerry R. Mendell, MD,1,2,3,4 Louise R. Rodino-Klapac, PhD,1,4 Zariife Sahenk, MD, PhD,1,2,3,4 Kandice Roush, RN,5 Loren Bird, RN,5 Linda P. Lowes, PhD,4 Lindsay Alfano, PT,6 Ann Maria Gomez, MD,1,4 Sarah Lewis, HT, ASCP,1,4 Janaiah Kota, PhD,1,4 Vinod Malik, PhD,1,4 Kim Shontz, BA, MS,1,4 Christopher M. Walker, PhD,1,4,6 Kevin M. Flanigan, MD,1,2,3,4 Marco Corridore, MD,7 John R. Kean, MD,4,7 Hugh D. Allen, MD,1,4 Chris Shilling, MS,1,3,4 Kathleen R. Melia, PhD,8 Peter Sazani, PhD,8 Jay B. Saoud, PhD,8 Edward M. Kaye, MD,8 and the Eteplirsen Study Group

## Study 201: Double-Blinded, Placebo-Controlled Phase IIB Study

<table>
<thead>
<tr>
<th>Patient</th>
<th>30 MG/KG</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-Tx</td>
<td>24 wks of Tx</td>
<td>48 wks of Tx</td>
</tr>
<tr>
<td>02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>09</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## Study 202: Open-Label, Long-Term Safety and Efficacy Study

<table>
<thead>
<tr>
<th>Patient</th>
<th>50 MG/KG</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-Tx</td>
<td>12 wks of Tx</td>
<td>48 wks of Tx</td>
</tr>
<tr>
<td>03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>04</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Placebo Control Group Rolled Over to Open-Label Eteplirsen*

**Legend:**
- **Muscle Biopsy: Baseline**
- **Muscle Biopsy: 12 weeks**
- **Muscle Biopsy: 24 weeks**
- **Muscle Biopsy: 48 weeks**

Mendell et al, Annals of Neurology 2013
Eteplirsen for the Treatment of Duchenne Muscular Dystrophy

Jerry R. Mendell, MD,¹,²,³,⁴ Louise R. Rodino-Klapac, PhD,¹,⁴ Zarife Sahenk, MD, PhD,¹,²,³,⁴ Kandice Roush, RN,⁵ Loren Bird, RN,⁵ Linda P. Lowes, PhD,⁴ Lindsay Alfano, PT,⁵ Ann Maria Gomez, MD,¹,⁴ Sarah Lewis, HT, ASCP,¹,⁴ Janaiah Kota, PhD,¹,⁴ Vinod Malik, PhD,¹,⁴ Kim Shontz, BA, MS,¹,⁴ Christopher M. Walker, PhD,¹,⁴,⁶ Kevin M. Flanagan, MD,¹,²,³,⁴ Marco Corridore, MD,⁷ John R. Kean, MD,⁴,⁷ Hugh D. Allen, MD,¹,⁴ Chris Shilling, MS,¹,³,⁴ Kathleen R. Melia, PhD,⁸ Peter Sazani, PhD,⁸ Jay B. Saoud, PhD,⁸ Edward M. Kaye, MD,⁸ and the Eteplirsen Study Group

CHANGE FROM BASELINE (METERS)

- Placebo/Delayed Tx (N=4)
- Eteplirsen for 48 Weeks (N=6)

*p ≤ 0.001

Mendell et al, Annals of Neurology 2013
LESS THAN 80 METERS LOST OVER 3.2 YEARS IN CONTINUOUS ETEPLIRSEN COHORT (mITT; n=6)

LESS THAN 80 METERS LOST OVER 2.5 YEARS IN PLACEBO/DELAYED ETEPLIRSEN COHORT FROM LAST TIME POINT BEFORE DYSTROPHIN CONFIRMED (n=4)

- After general stability on the 6MWT through 120 weeks, similar declines of walking distance were observed from week 120 through week 144 with 61 meter and 63 meter declines in the continuous eteplirsen and delayed eteplirsen treatment groups, respectively.
- All patients showed declines in walking distance from week 144 to 168, including one patient in each arm that declined by more than 75 meters (highest 6MWT performer in continuous group; lowest 6MWT performer in delayed group).
- After 3.2 years of therapy the mean age of the boys in the continuous eteplirsen arm (mITT) was 12.6 years (median age 12.9).
- After 168 weeks of continuous eteplirsen treatment the mITT cohort (n=6) walked an average of 323 meters.

Note: Statistical analysis based on modified Intent-To-Treat (mITT, n=10, excludes two patients who experienced rapid decline and lost ambulation early in the study) Population using MMRM Test
PATIENTS MAINTAINED AVERAGE 6MWT DISTANCE OF MORE THAN 300 METERS THROUGH 168 WEEKS IN CONTINUOUS ETEPLIRSEN COHORT (mITT n=6)

PLACEBO-DELAYED PATIENTS DECLINED AT SIMILAR RATE AFTER DYSTROPHIN CONFIRMED

Age at Baseline (yrs):
Mean 9.1
Median 9.3

Age at WK 168 (yrs):
Mean 12.4
Median 12.5

Note: Statistical analysis based on modified Intent-To-Treat (mITT, n=10, excludes two patients who experienced rapid decline and lost ambulation early in the study) Population using MMRM Test
Potential Therapies in Trials

- Stop Codon Readthrough
- Exon Skipping
- Gene Transfer
Emerging viral gene therapy approaches

• Myostatin inhibition (follistatin)
• Gene replacement
  – Microdystrophin
• Expression of other genes (Galgt2)
Myostatin and other negative regulators inhibit the growth of muscle tissue

Source: Acceleron Pharma
McPherron et al., Nature 1997

Scheulke et al., NEJM 2004
Loss of myostatin attenuates severity of muscular dystrophy in mdx mice
• Wyeth sponsored 11 Center Trial (10 USA; 1 GB) Using MYO-029 antibody to myostatin
  – No Clinical Benefit
  – Muscle histology showed a trend toward increased muscle fiber size
  – Demonstrated safety of systemic delivery of a myostatin inhibitor in a clinical trial
GENE THERAPY

Follistatin Gene Delivery Enhances Muscle Growth and Strength in Nonhuman Primates

Janaiah Kota,¹ Chalonda R. Handy,¹,² Amanda M. Haidet,¹,² Chrystal L. Montgomery,¹
Amy Eagle,¹ Louise R. Rodino-Klapac,¹ Danielle Tucker,¹ Christopher J. Shilling,¹
Walter R. Therlfall,³ Christopher M. Walker,¹,² Steven E. Weisbrode,³ Paul M. L. Janssen,²
K. Reed Clark,¹,² Zarife Sahenk,¹,² Jerry R. Mendell,¹,²* Brian K. Kaspar¹,²*

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Becker Muscular Dystrophy
Low Dose Follistatin $6 \times 10^{11}$ vg/kg

6MWT (meters)

- 300
- 325
- 350
- 375
- 400
- 425
- 450
- 475
- 500
- 525
- 550
- 575
- 600

- Screening
- 30 days
- 60 days
- 90 days
- 180 days
- 1 year

- 04
- 06
- 05

PRE FOLLISTATIN
E4-073
E5-877
E6-009
POST FOLLISTATIN
E4-073
E5-877
E6-009
Becker Muscular Dystrophy
High Dose Follistatin 1.2e12 vg/kg
## Change in 6 minute walk test in BMD patients

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Change in Distance (meters)</th>
</tr>
</thead>
<tbody>
<tr>
<td>04</td>
<td>+55</td>
</tr>
<tr>
<td>05</td>
<td>+125</td>
</tr>
<tr>
<td>06</td>
<td>+9</td>
</tr>
<tr>
<td>07</td>
<td>-14</td>
</tr>
<tr>
<td>08</td>
<td>+108</td>
</tr>
<tr>
<td>09</td>
<td>+29</td>
</tr>
</tbody>
</table>

Gene transfer trial of DMD subjects (IM, lower extremities) is imminent.
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.

*DMD* gene transfer (biceps) with an AAV2.5.CMV.minidystrophrin vector
Full-length dystrophin: 11 kb (427 kDa)

Becker minidystrophin: 6.3 kb (229 kDa)

Minidystrophin: 3.6 kb (~140 kDa)
Biopsied at 6 weeks (subjects #1, 3, 4, 6) or at 3 months (subjects #2, 5).

- No significant dystrophin expression.
- Development of T-cell immunity to the transgene (dystrophin) in 2/6 subjects.

<table>
<thead>
<tr>
<th>Vector Dose</th>
<th>Patient</th>
<th>Age</th>
<th>Exon Deletion</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2.0 \times 10^{10} \text{ vg/kg})</td>
<td>1</td>
<td>8</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>9</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>9</td>
<td>46–50</td>
</tr>
<tr>
<td>(1.0 \times 10^{11} \text{ vg/kg})</td>
<td>4</td>
<td>5</td>
<td>49–54</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>11</td>
<td>3–17</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>9</td>
<td>46–52</td>
</tr>
</tbody>
</table>
Improved microdystrophphin vector

\[ \text{rAAV(rh.74).MCK.uDys} \quad \text{Harper et al. (2002) Nature Med. 3:253-61} \]

- IND and IRB approval
- Initial intramuscular studies starting
Surrogate gene transfer: GALGT2


\[ rAAV(\text{rh.74}).\text{MCK.GALGT2} \]
Dystrophin associated glycoprotein (DAG) complexes in skeletal muscle

**Extrasynaptic**

**Synaptic**

\[ \text{Galgt2} \]
Dystrophin-deficient mdx mice

Extrasynaptic mdx

Synaptic Galgt2
Galgt2 transgenic mdx mice:
1. Upregulation of the Synaptic DAG Complex
2. No Development of Muscle Pathology

Lack of muscle damage in Galgt2 transgenic (CT) mdx mice
A

![Graph showing force as a function of eccentric contraction cycles](image)

**Force (Fraction of First Contraction)**

Eccentric Contraction Cycles

- **Galgt2 (1x10^11 vg)**
- **Micro-dys (1x10^11 vg)**
- **WT C57Bl/10**
- **mdx**

***P<0.001

B

![Bar chart showing specific force](image)

- **WT**
- **Galgt2**
- **Micro-dys**
- **mdx**

*P<0.05

C

- **EDL**
  - rAAV8-MCK-Galgt2 (human)
  - Mock-infected

- **TA**
  - rAAV8-MCK-Galgt2 (human)
  - Mock-infected

anti-CT carbohydrate

anti-dystrophin

D

- **EDL**
  - rAAV8-MCK-Micro-dys (human)
  - Mock-infected

- **TA**
  - rAAV8-MCK-Micro-dys (human)
  - Mock-infected

Percentage of Myofibers Overexpressing Transgene (Extensor Digitorum Longus)
Galgt2 shows broad therapeutic potential for treating multiple muscular dystrophies

1. Galgt2 is therapeutic in multiple forms of muscular dystrophy
2. IND approval obtained
3. Initial IM safety studies are pending IRB approval
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