Update on Molecular Therapy for Pediatric Neuromuscular Disease

Jerry R Mendell, MD
Linda Lowes, PhD, Lindsay Alfano, DPT, Kate Berry, PT
Center for Gene Therapy
Nationwide Children’s Hospital
Conflicts of Interest

- Professor in Department of Pediatrics of Ohio State University and employed by PAA of Dept of Pediatrics, Nationwide Children’s Hosp
- Serve as a consultant to Sarepta Therapeutics
- PI on clinical trials:
  - Avexis sponsored Gene Transfer Clinical Trial for SMA type 1
  - Sarepta sponsored Eteplirsen trials: DBRCT201/202, Open label 201,204, 301,
Exciting Results for 2015

Delivering the Survival Motor Neuron Gene (SMN) in AAV9 Phase I Clinical Trial in SMA type 1
# SMA Classification

<table>
<thead>
<tr>
<th>Type</th>
<th>Age Onset</th>
<th>Highest Function</th>
<th>Life Span</th>
<th>SMN Copies</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>Prenatal</td>
<td>Respiratory Support</td>
<td>&lt;3m</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>0-6m</td>
<td>Never Sit</td>
<td>&lt;2y</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>&lt;18m</td>
<td>Never Stand</td>
<td>&gt;2y</td>
<td>3,4</td>
</tr>
<tr>
<td>3</td>
<td>&gt;18m</td>
<td>Stand Alone</td>
<td>Adult</td>
<td>3,4</td>
</tr>
<tr>
<td>4</td>
<td>Adult</td>
<td>Stand Alone</td>
<td>Adult</td>
<td>4-8</td>
</tr>
</tbody>
</table>
Chr 5 harbors the SMN1 (Tel) and SMN2 (Cen)

SMN = Survival Motor Neuron Gene and Protein
Pre Clinical Data

- SMNΔ7 mouse model dies at 13-16 days

- Dose Ranging Gene Therapy Study at Post Natal Day 1 extended survival 1 year (vector scAAV9.CB.SMN)
TREATMENT TIME AND DOSE DEPENDENT

- A five-fold reduction in dose \((6.7 \times 10^{13} \text{ vg/kg})\) reduces survival to 35 days
- Treatment P10 had no effect
Hypothesis for Clinical Trial

- Early treatment prior to loss of motor neurons within the first six months of life will rescue motor neurons.

Taking this to clinical trial

- Phase I/IIA intravenous clinical gene transfer trial
- Enrollment of Nine (n=9) SMA type 1 patients with disease onset before 6 months
- Two Cohorts enrolled (FDA approved IND 15699)
  - Cohort 1 (n=3) $6.7 \times 10^{13} \text{ vg/kg}$
  - Cohort 2 (n=6) $2.0 \times 10^{14} \text{ vg/kg}$
SMA Type 1

- **Outcomes for Clinical Trial:**
  - **Primary Clinical Endpoint**
    - >50% Survival at 2 years of age
    - >16 hrs/day noninvasive ventilation for at least 2 weeks
  - **Primary Functional Endpoint**
    - CHOP INTEND (mean rate of decline is -1.27 points/yr)
### Table 1. Low Dose - SMA Cohort characteristics

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gender</th>
<th>Age at gene transfer</th>
<th>Current Age</th>
<th>scAAV.CB.SMN</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-01</td>
<td>Female</td>
<td>6 months</td>
<td>21 m</td>
<td></td>
</tr>
<tr>
<td>E-02</td>
<td>Female</td>
<td>6 months</td>
<td>16 m</td>
<td>6.7x10^{13} vg/kg</td>
</tr>
<tr>
<td>E-03</td>
<td>Male</td>
<td>7 months</td>
<td>18 m</td>
<td></td>
</tr>
</tbody>
</table>

**Mean AGE:** 18.3 m

**Mean CHOP INTEND:** +4.0 ± 2.0
Figure 1. Subject E-01, Liver enzymes and ELISpot

![Graph showing liver enzymes and ELISpot responses.

- **T-cell response to AAV9 capsid**
- **ALT**

**Days Post Gene Transfer**

- Days 30, 60, 90, 120, 150, 180, 240, 300, 360

Liver enzymes and ELISpot responses post-gene transfer.
Results of Pre-Treatment with Prednisolone 1mg/kg
## Intermediate Dose Cohort

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gender</th>
<th>Age Gene Transfer</th>
<th>Current Age</th>
<th>Intermediate Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-04</td>
<td>F</td>
<td>6 m</td>
<td>14 m</td>
<td></td>
</tr>
<tr>
<td>E-05</td>
<td>M</td>
<td>4.5 m</td>
<td>11 m</td>
<td></td>
</tr>
<tr>
<td>E-06</td>
<td>F</td>
<td>2 m</td>
<td>8 m</td>
<td></td>
</tr>
<tr>
<td>E-07</td>
<td>F</td>
<td>4 m</td>
<td>7 m</td>
<td></td>
</tr>
<tr>
<td>E-08</td>
<td>F</td>
<td>8 m</td>
<td>11 m</td>
<td></td>
</tr>
<tr>
<td>E-09</td>
<td>F</td>
<td>5 m</td>
<td>8 m</td>
<td></td>
</tr>
</tbody>
</table>

**Mean AGE**

\[10.3 \pm 2.9 \text{ m}\]

**Mean CHOP INTEND**

\[15.5 \pm 6.3\]
Recent enrolled within 3m Dx
Chronic enrolled more than 3m after Dx

- mean rate of decline -1.27 points/yr)
## Natural History (Finkel Neurology 2014)

<table>
<thead>
<tr>
<th></th>
<th>Sx younger 3m</th>
<th>Sx older 3 m</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Enrolled within 3m of Dx</td>
<td>Enrolled 3m after Dx</td>
</tr>
<tr>
<td><strong>Non-Inv 16h</strong></td>
<td>25% (3.5m)</td>
<td>80% (13.5m)</td>
</tr>
<tr>
<td><strong>CHOP INTEND Rate of Δ</strong></td>
<td>-1.83 points per year</td>
<td>- 0.83 points per year</td>
</tr>
</tbody>
</table>
SMA Type 1 Gene Transfer at 2 months
Age of Video 8 mo = 6 mo post gene transfer
SMA Type 1 treated at 2 mo and picture 6 mo post GT
Summary

• Gene Delivery for SMA is safe and effective
  – Low dose cohort $6.7 \times 10^{13}$ vg/kg survived to 18.3 months with CHOP INTEND 4.0 ± 2.0
  – Intermediate Dose Cohort $2 \times 10^{14}$ vg/kg survived to 10.3 months
  – Every SMA child enrolled in Gene Therapy Study improved with dose-dependent response

• Liver enzymes most significant gene related adverse event; suppressed by prednisolone
This is what it is all About!!!!

Treating kids with Neuromuscular Disease
Exon Skipping Shows Promise for DMD

Results of 3.2 years Phase IIB study using Eteplirsen
Exon-Skipping APPROACH

ETEPLIRSEN skipping exon 51 in patient with gene mutation of deletion of exons 49-50

SKIPPING EXON 51 ENABLES PRODUCTION OF FUNCTIONAL DYSTROPHIN PROTEIN (targets dystrophin region where skipping 51 corrects 13% DMD mutations)
- **Phosphorothioate linkage** between Ribose sugars and the addition of the alkyl group (2' OmePS AON) targeting sequences at pre-mRNA level & blocking exon splicing
- **Morpholino Oligomer with Phosphorodiamidate linker**, RNase H independent targets sequences at pre-mRNA level & promotes exon skipping
  - Charge neutral and clears kidney without binding to epithelium
ETEPLIRSEN PHASE IIb STUDY DESIGN

STUDY 201: RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED
STUDY 202: OPEN-LABEL, LONG-TERM SAFETY AND EFFICACY

Study 201: Double-blinded, Placebo-controlled Phase IIb

- 30 mg/kg/wk: n=4
- 50 mg/kg/wk: n=4
- Placebo: n=4

Study 202

- Open-label, Long-term Safety and Efficacy Study

- 30 mg/kg/wk: n=4
- 50 mg/kg/wk: n=2

*Placebo-controlled group rolled over onto open-label eteplirsen.

- Muscle biopsy: baseline
  - Screening/Eligibility
- Muscle biopsy: 12 weeks
  - Randomized
- Muscle biopsy: 24 weeks
- Muscle biopsy: 48 weeks
- Muscle biopsy: 48 weeks
- SRP-4658 (Eteplirsen)
- Placebo

12 Weeks 24 48 weeks Ongoing Extension
Three happy boys receiving weekly IVs
Not a single side effect!!
6MWT change from baseline to week 48:
Data from 6 evaluable patients treated from start of study

Note: Statistical analysis based on Intent-To-Treat Population using ANCOVA test
6MWT CHANGE FROM BASELINE TO WEEK 120:
DATA BASED ON MAXIMUM 6MWT SCORE WHEN TEST WAS REPEATED

Note: Statistical analysis based on modified Intent-To-Treat Population using MMRM Test
3.2 years of continuous Eteplirsen Treatment

- After 3.2 years of therapy the mean age of the boys in the continuous eteplirsen arm was 12.4 years (median age 12.5)
- Baseline ambulation for the continuous eteplirsen treatment cohort (MITT) was 399.7 meters and the placebo rollover cohort was 394.5 meters
- After 168 weeks of continuous eteplirsen treatment the MITT cohort (N=6) walked an average of 323 meters
Eteplirsen Compared to Natural History Controls

Baseline Age: Exon 51 Amenable = 9.5 y.o., eteplirsen-treated = 9.4 y.o.

**p<0.01; † Difference in mean change from baseline

=151m†  

N=13
Eteplirsen MEP / MIP reduced 5% vs Untreated MEP/ MIP reduced 11.5%

FVC 9.4% treated VS untreated 14.3%
ETEPLIRSEN SAFETY PROFILE

SAFETY PROFILE OF ETEPLIRSEN FOR LONG TERM USE

- No clinically significant treatment-related adverse events observed through 168 weeks
  - One treatment-unrelated serious adverse event: distal femur fracture
  - Two instances of changes to coagulation due to thrombosis in device: port not flushed adequately of heparin
  - Reported cases of transient urine protein elevation resolved without intervention and resulted in no clinical symptoms or other laboratory kidney marker changes

- No clinically significant treatment-related changes detected on any monitored safety laboratory parameter
  - Liver-specific enzymes, kidney function, coagulation profiles, or platelet counts

- No hospitalizations, discontinuations, or treatment interruptions

- Well tolerated with > 1890 doses (~49 patient years) administered in studies 201/202
  - No subject missed more than two consecutive doses
    - Missed doses primarily due to vacation and/or summer camp
  - PBO/Delayed-Tx cohort (n=4) completed on average 142.3 out of 144 possible doses
  - Eteplirsen cohorts (n=8) completed on average 165.9 out of 168 possible doses

- No signs or symptoms of immune activation, including lack of infusion reactions, lack of treatment related hypersensitivity, and no flu-like symptoms
  - Only one instance of injection site pain reported over greater than three years of weekly infusions
  - No reported incidents of erythema, induration or discoloration at injection sites
Impact of Exon Skipping

- What if we start treatment at birth or shortly thereafter?

- NBS Studies from Ohio show that CKs elevated at birth <2000 U/L

- Gross, fine and composite motor scores are delayed from early infancy (0-3 years of age)
Strategies for Restoring BMD/DMD Function

Follistatin Gene Therapy
The Clinical Problem

- Quadriceps muscle weakness
  - Becker muscular dystrophy
- Frequent falls
  - Limb fractures
  - Loss of ambulation
- Improving quadriceps muscle strength could result in a "clinically meaningful outcome"
MYOSTATIN REGULATION OF MUSCLE SIZE
Follistatin Peptide Blockade

Circulating complex
Propeptide-myostatin

Propeptide cleavage

FOLLISTATIN

Activin IIB Receptor

AAV1-FOLLISTATIN

INJECT AAV INTO MUSCLE
Preparation for Clinical Trial
In Non-Human Primate
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)

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

- 04
- 06
- 05

PRE FOLLISTATIN | POST FOLLISTATIN
E4-073 | E5-877 | E6-009 | E4-073 | E5-877 | E6-009

Control

DNA gel electrophoresis
Low Dose Follistatin  
6e11 vg/kg

<table>
<thead>
<tr>
<th></th>
<th>Distance 6MWT</th>
<th>Distance 6MWT</th>
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<tbody>
<tr>
<td>Screen</td>
<td>492</td>
<td>291</td>
<td>457</td>
</tr>
<tr>
<td>30 Day</td>
<td>491</td>
<td>314</td>
<td>464</td>
</tr>
<tr>
<td>60 Day</td>
<td>511</td>
<td>329</td>
<td>468</td>
</tr>
<tr>
<td>90 Day</td>
<td>525</td>
<td>386</td>
<td>455</td>
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<tr>
<td>180 Day</td>
<td>550</td>
<td>401</td>
<td>470</td>
</tr>
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<td>1 year</td>
<td>550</td>
<td>416</td>
<td>466</td>
</tr>
<tr>
<td>TOTAL</td>
<td>+58 meters</td>
<td>+125 meters</td>
<td>+9 meters</td>
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</table>
Becker Muscular Dystrophy
High Dose Follistatin 1.2e12 vg/kg

6MWT (meters)

Screen 1  30 days  60 days  90 days  180 days

- 07
- 08
- 09
## High Dose Follistatin
### 1.2e12 vg/kg

<table>
<thead>
<tr>
<th></th>
<th>Distance 6MWT</th>
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<tbody>
<tr>
<td>Screen</td>
<td>439</td>
<td>515</td>
<td>452</td>
</tr>
<tr>
<td>30 Day</td>
<td>437</td>
<td>574</td>
<td>477</td>
</tr>
<tr>
<td>60 Day</td>
<td>427</td>
<td>570</td>
<td>469</td>
</tr>
<tr>
<td>90 Day</td>
<td>434</td>
<td>600</td>
<td>475</td>
</tr>
<tr>
<td>180 Day</td>
<td>425</td>
<td>623</td>
<td>481</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>-14 meters</strong></td>
<td><strong>+108 meters</strong></td>
<td><strong>+29 meters</strong></td>
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Correlative histology and 6 MWT

Patient 08

<table>
<thead>
<tr>
<th>Visit</th>
<th>6MWD</th>
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<tbody>
<tr>
<td>BL</td>
<td>515</td>
</tr>
<tr>
<td>D30</td>
<td>574</td>
</tr>
<tr>
<td>D60</td>
<td>570</td>
</tr>
<tr>
<td>D90</td>
<td>600</td>
</tr>
<tr>
<td>D180</td>
<td>623</td>
</tr>
<tr>
<td>6mo Δ</td>
<td>+108m</td>
</tr>
</tbody>
</table>
DMD FS344 Gene Therapy Trial

• FDA approved DMD gene therapy study based on safety profile

• Multiple site FS344 gene transfer by Intramuscular injections to target specific muscle groups
  2.4e12 vg/Kg

• Remote or regional effects increase the chance for improvement
Thanks!!

Research Institute Labs NCH
Christopher Walker
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Kevin Flanigan
Linda Lowes
Lindsay Alfano
Xiomara Rosales
Vinod Malik
Sarah Lewis
Linda Cripe
Katie Church
MAKING GREAT PROGRESS IN TREATMENT OF DMD!

THANK YOU AND QUESTIONS