Golodirsen Induces Exon Skipping Leading to Sarcolemmal Dystrophin Expression in Patients With Genetic Mutations Amenable to Exon 53 Skipping

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On Behalf of:
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Carrell-Krusen Neuromuscular Symposium, February 22–23, 2018; Dallas, TX
Duchenne Muscular Dystrophy (DMD)

- DMD is a rare, fatal, degenerative neuromuscular disease with X-linked recessive inheritance\(^1,2\)
- Due to mutations in the *DMD* gene, and most of these mutations disrupt the dystrophin mRNA reading frame and prevent production of functional dystrophin\(^3\)

mRNA, messenger ribonucleic acid.

Dystrophin Protein

- Dystrophin is a critical protein that functions to prevent muscle damage during eccentric contraction\(^1\)-\(^5\)
- Clinical effect of dystrophin loss is progressive muscle wasting and weakness, loss of function, and premature death\(^3\),\(^6\),\(^7\)

Disease Progression in DMD

5 TO 7 YEARS
- Motor delay
- Enlarged calves
- Toe walking
- Standing from supine, climbing stairs more difficult

8 TO 11 YEARS
- Increasing loss of walking ability
- Part-time wheelchair use

EARLY TEENS
- Loss of ambulation
- Full-time wheelchair use
- Increasing loss of upper limb function

TEENS
- Increasing respiratory impairment
- Ventilatory support often required
- Unable to perform activities of daily living

TEENS TO TWENTIES
- Increasing cardiac dysfunction
- Heart failure
- Death

PMOs Eteplirsen and Golodirsen

• Eteplirsen is approved by the US FDA for the treatment of DMD patients with confirmed mutations amenable to exon 51 skipping$^{1–6}$
  – Approximately 13% of DMD patients carry out-of-frame deletion mutations amenable to exon 51 skipping

• Golodirsen (formerly SRP-4053) binds and excludes exon 53 during dystrophin mRNA processing, allowing production of internally shortened dystrophin protein
  – Approximately 8% of DMD patients have mutations amenable to exon 53 skipping$^1$

• Additional exon targeting may address an unmet need for other patients

Phosphorodiamidate Morpholino Oligomers (PMOs)

- PMOs are a class of unique RNA therapeutics with an uncharged backbone that target endogenous nucleic acids through Watson-Crick base pairing\textsuperscript{1-4}
  - Sequences are designed complementary to the desired target

Sequence Specific Binding of PMOs to pre-mRNA

- In DMD, PMOs have been designed with a goal of skipping targeted exons to restore the dystrophin mRNA reading frame and allow production of internally shortened dystrophin protein.

2. Sarepta Therapeutics Data on File
The consortium is a broad group encompassing advocacy, research, healthcare providers and industry.

Francesco Muntoni is project lead (UCL, GOSH). Individual site leads are as follows:

- Institut de Myologie (Laurent Servais)
- University of Newcastle upon Tyne (Volker Straub)
- Università Cattolica del Sacro Cuore (Eugenio Mercuri)
- Royal Holloway & Bedford New College (George Dickson)

All stakeholders had a chance to contribute to trial design.
Study 4053-101: SKIP-NMD
An International Collaborative Study

Population:
• Age: 6-15 yrs
• 6MWT ≥250 m
• NSAA Total >17
  or Rise Time <7 sec

Key Endpoints (Part 2):
• 1°: 6MWT (Week 144), Western Blot (Week 48)
• PFTs
• Dystrophin Intensity, PDPF
• Exon Skipping

6MWT, 6-minute walk test; NSAA, North Star Ambulatory Assessment; PDPF, percent dystrophin-positive fibers; PFT, pulmonary function test.
Methods: Western Blot and RT-PCR

• RT-PCR completed to evaluate dystrophin exon 53 skipping
  – Semi-quantitative, end point method
  – Sanger DNA sequencing to confirm correct skipping

• Western blot: A validated quantitative assay assessed dystrophin protein levels

cDNA, complementary deoxyribonucleic acid; GCLP, Good Clinical Laboratory Practice; LLOQ, lower limit of quantification; ULOQ, upper limit of quantification.
Muscle Biopsies and Tissue Allocation

- Muscle biopsies:
  - Baseline & Week 48 (Part 2)
  - Contralateral biceps brachii
  - Standardized surgical procedure

- 2 sections of muscle (A + B) were excised during each surgery
  - Allocated and analyzed separately
  - Western blot: 4 replicates A+B, duplicate gels
  - Exon skipping: 8 replicates A+B, quadruplicate reactions
  - IHC: 4 replicates A+B, level 1 + 2 slides

IHC, immunohistochemistry; OCT, optimal cutting temperature; RT-PCR, reverse transcription polymerase chain reaction.
# Results: Patient Demographics and Baseline Disease Characteristics

<table>
<thead>
<tr>
<th>Baseline Characteristic</th>
<th>Mean (SD) N=25 Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>8.2 (2.2)</td>
</tr>
<tr>
<td>Height, cm</td>
<td>120.1 (10.4)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>28.2 (9.1)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>19.1 (3.7)</td>
</tr>
<tr>
<td>6MWT distance, m</td>
<td>403.7 (56.7)</td>
</tr>
<tr>
<td>Time since DMD diagnosis, months</td>
<td>55.2 (24.9)</td>
</tr>
<tr>
<td>Duration of corticosteroid use, months</td>
<td>34.6 (24.7)</td>
</tr>
</tbody>
</table>

Note: Values shown are from the N=25 patients who were treated and received a muscle biopsy.

BMI, body mass index.
Results: RT-PCR for Exon 53 Skipping

100% exon skipping response rate

- All 25 patients displayed an increase in the exon 53 skipped band \((P<0.001)\) over baseline levels at Week 48

<table>
<thead>
<tr>
<th>Mean % Exon Skipping</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BL</strong></td>
</tr>
<tr>
<td>2.6</td>
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</tbody>
</table>

Sample Result
Del 49-52
15.42% skip
Results: RT-PCR for Exon 53 Skipping

- Range of individual mean percent increase from baseline: 2.50% to 37.32%
Results: Western Blot for Dystrophin

Significant increase in dystrophin protein observed from baseline to Week 48 in golodirsen treated patients

<table>
<thead>
<tr>
<th>Mean % Normal Dystrophin</th>
<th>BL</th>
<th>Wk48</th>
<th>Δ</th>
<th>P</th>
<th>Fold↑</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1</td>
<td>1.0</td>
<td>+0.9</td>
<td>&lt;0.001</td>
<td>10.7</td>
</tr>
</tbody>
</table>

SD, standard deviation.

*above 4% upper limit of quantitation
Results: Western Blot for Dystrophin Protein

Western Blot, Mean Dystrophin % Normal

- Range of dystrophin in individual patient biopsies: 0.09%–4.30% of normal
- Baseline biopsies (N=24) uniformly expressed very low levels of dystrophin
  - Only one had dystrophin above BLOQ (0.31%)
- Significant positive correlation between exon skipping and de novo dystrophin protein expression observed
  - Spearman-r correlation coefficient, 0.500; \( P=0.011 \)
Results: IHC for Dystrophin Localization

- Dystrophin localization to the sarcolemma clearly demonstrated
  - Evidence that the dystrophin is functional and present throughout the muscle fiber
  - Whole-slide scan images at baseline and Week 48 from 1 patient is shown as an example

Note: Indirect immunofluorescence staining of tissue cryosections was performed using MANDYS106 and anti-laminin 2 alpha antibodies
Conclusions

• Treatment with golodirsen resulted in increases in dystrophin
  – 1° biological endpoint achieved: Statistically significant mean increase in dystrophin observed (p<0.001)
  – ↑ exon 53 skipping observed in all patients
  – Dystrophin correctly localized to sarcolemma
  – Exon skipping significantly correlated to dystrophin expression

• Golodirsen is the second PMO to demonstrate increased dystrophin expression and sarcolemmal membrane localization through exon skipping
  – These findings further validate the potential of the PMO platform in DMD

• Using rigorous methods to measure dystrophin expression should facilitate the evaluation of dystrophin restorative therapies
Acknowledgments

- **University of Iowa**: Steven A. Moore, Melissa Jans, and Terese Nelson
- **University College London**: Francesco Muntoni, Caroline A. Sewry, Darren Chambers, Valentina Sardone, Rahul Phadke, Adam Jones, Silvia Torelli, Jenny Morgan, and Lucy Feng
- **Royal Holloway—University of London**: Linda Popplewell, Anita Le Heron, and George Dickson
- **Sarepta Therapeutics, Inc.**: Diane E. Frank, Frederick J. Schnell, Cody Akana, Joanna Cataldo, Michelle O’Connor, Jay S. Charleston, Saleh El Husayni, Kati Vu, Madeline Schaub, Max Stewart, Cody A. Desjardins, Genevieve Laforet, Uditha DeAlwis, and Cas Donoghue
- **Flagship Biosciences, Inc.**: AJ Milici, Kristin Wilson, Crystal Faelan, G. David Young, and Holger Lange
- Biopsy processing: Mauro Monforte (Rome), Maud Chapart and Stéphane Vasseur (Paris), and Richard Charlton (Newcastle)
- All participants in the **SKIP-NMD Consortium**