Duchenne Muscular Dystrophy (DMD)

- An X-linked recessive inherited degenerative muscle disorder affecting about 1 in 3,500-6,000 live male births per year.
- Prevalence estimated from MDSTARnet 4-state population surveillance: 1.3 – 1.8 per 10,000 males ages 5-24 years (approximately 7500 – 9000 males in the US).
- Caused by mutations in the dystrophin gene, the largest gene in the human genome.
Muscle Structure - Dystrophin Protein
Where is dystrophin in the muscle fiber?

Normal:

DMD/ mdx:
Dystrophin Gene
2.5 million base pairs

Transcription and splicing

Translation

14 thousand base mRNA

3,600 amino-acids
427 kD

Amino-terminus (actin binding)
Spectrin-like rod domain
Cysteine-rich domain
Carboxy-terminus (membrane binding)
Dystrophin Mutation Classification

Pie chart representation of the mutations identified in N=256 study participants of the CINRG Duchenne Natural History Study
Diagnostic Strategy

- CK elevated
  - Yes: Further testing
  - No: not DMD

- Deletion mutation detected in WBCs
  - Yes: DMD
  - No: Further testing

- Small mutation detected in WBCs
  - Yes: DMD
  - No: Further testing

- Dystrophin protein absent on muscle biopsy (IHC/Western)
  - Yes: DMD
  - Partially: BMD (decreased dystrophin size and/or quantity)
  - No: not DMD/BMD
DMD Presentation

*Early presentation (up to age 2 years)*:
- Delayed walking
- High serum levels of CK
- Family history

*Late presentation (up to age 5 years)*:
- Frequent falls
- High serum levels of CK
- Difficulty in standing up, jumping, or climbing stairs
Clinical Course

- Proximal limb muscle weakness – legs earlier than arms
- Respiratory failure
- Cardiomyopathy

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>3-5</th>
<th>8-12</th>
<th>10+</th>
<th>20+</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>birth</td>
<td>diagnosis</td>
<td>loss of mobility</td>
<td>progressive onset of cardiorespiratory failure</td>
</tr>
</tbody>
</table>
Progression of Dystrophic Myopathy in DMD

- Defective Dystrophin Gene
  - Lack of Dystrophin
    - Damage to Individual Muscle Fibers
      - Death of Groups of Muscle Fibers
        - Satellite Cell Activation
        - Inflammation
          - Release of Cytokines (e.g. TGF-β)
          - **Fibrosis** (Formation of Scar Tissue)
        - Muscle Fiber Repair
DMD Pathology

- Inflammatory cell infiltration in the muscle
- Irreversible degeneration of muscle tissue
- Abnormal connective tissue proliferation
Effect of type of mutation on dystrophin quality and quantity

DMD

Loss of reading frame

In frame mutation

generates an internally deleted protein

BMD

Duchenne muscular dystrophy

Becker muscular dystrophy
Dystrophin cDNA Constructs for Gene Therapy

- Engineered internally-deleted, truncated dystrophin cDNA constructs
- Biological rationale for ‘exon skipping’ therapeutic
Centers for Disease Control (CDC)
DMD Care Considerations

• 2010 *Lancet Neurology*
• multidisciplinary model of care
• collaboration of multiple groups, 84 experts

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**Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and pharmacological and psychosocial management**
Katharine Bushby, Richard Finkel, David J Birnkrant, Laura E Case, Paula R Clemens, Linda Cripe, Ajay Kaul, Kathi Kinnett, Craig McDonald, Shree Pandya, James Poyssky, Frederic Shapiro, Jean Tomezsko, Carolyn Constantin, for the DMD Care Considerations Working Group

**Diagnosis and management of Duchenne muscular dystrophy, part 2: implementation of multidisciplinary care**
Katharine Bushby, Richard Finkel, David J Birnkrant, Laura E Case, Paula R Clemens, Linda Cripe, Ajay Kaul, Kathi Kinnett, Craig McDonald, Shree Pandya, James Poyssky, Frederic Shapiro, Jean Tomezsko, Carolyn Constantin, for the DMD Care Considerations Working Group*
DMD Management

- glucocorticoid treatment
- physical & occupational therapy
- assistive devices & wheelchairs
- monitor for scoliosis
- spine, contracture surgeries
- surveillance
- cough assist & ventilation devices
- surveillance
- heart medications to delay and treat cardiomyopathy
- support
- cognitive and behavioral interventions
Practice Parameter: Corticosteroid treatment of Duchenne dystrophy

Report of the Quality Standards Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society

R.T. Moxley III, MD; S. Ashwal, MD; S. Pandya, MS, PT; A. Connolly, MD; J. Florence, MHS, PT; K. Mathews, MD; L. Baumbach, MD; C. McDonald, MD; M. Sussman, MD; and C. Wade, PhD, PT, RN

Prednisolone

Deflazacort

- Delay loss of ambulation by up to 3 years
- Alter natural history of scoliosis development
- Delay loss of upper extremity function—able to self-feed longer
- Side-effects: behavioral, growth inhibition, delayed puberty
Glucocorticoid use has altered progression of DMD since the last comprehensive natural history studies.
Trial Readiness

- Neuromuscular clinical research multi-center networks—eg. Cooperative International Neuromuscular Research Group (CINRG)
- Characterization of homogeneity of study population—eg. effect of SNPs on inclusion criteria/sample size for clinical trials
- Outcome measure and biomarker development: Specific issues for DMD:
  - Spectrum of a progressive, multi-system disability over the life-span
  - Impact of musculo-skeletal growth and development
  - Impact of medical management, especially glucocorticoids
- Novel therapeutics
- Partnerships and mutual understanding: academic—industry—foundation/advocacy—regulatory
New Approaches to Therapy

• Mutation-specific therapies
  – Nonsense mutation suppression

• Mutation-independent therapies
  – Utrophin up-regulation \((\text{pre-clinical development})\)
  – Modification of dystrophic muscle phenotype \((\text{eg. Improve muscle energetics; inhibit NF-\(\kappa\)B; etc.})\)
Nonsense Mutation Read-through Human Clinical Trials

- Results of gentamycin mixed in 3 different small trials. Mixture of gentamycin isomers causes batch variability.

- PTC124: small molecule identified by PTC Therapeutics through a high through-put screen to discover small molecules that regulate translation. Granted orphan drug status to test for CF and DMD.

- PTC124: Phase 2a open label, dose ranging clinical trial with 28d treatment. Across all dose levels there was an improvement in 47% of subjects (n=38) in dystrophin expression by muscle biopsy. Serum CK values were lowered by treatment.

- PTC124 renamed Ataluran: A 174-participant, blinded, randomized phase 2b study; primary outcome of 30 meter improvement in the 6MWT. Met end-point for low-dose (10-, 10-, 20-mg/kg) therapy.

- Phase 3 study of Ataluran in nonsense mutation-mediated DMD initiated. Plan 1:1 randomization of approx. 220 participants to low-dose therapy or placebo for 48-week trial with primary outcome of 6MWT.
Anti-sense Approach to Out-of-frame Mutations

**Diagram (a):**
- Deletion of exons 45-54
- Out-of-frame dystrophin transcript missing exons 45-54
- Truncated dystrophin
- DMD

**Diagram (b):**
- 44AON1
- In-frame dystrophin transcript missing exons 44-54
- BMD-type dystrophin
- BMD-like phenotype

*Nature Reviews | Genetics*
Antisense drug – synthetic oligomers

- Phosphodiester DNA
- 2’O-methyl-modified ribose
- Morpholino (PMO)
### Table 3 | Overview of therapeutic exon skipping for a series of DMD-causing deletions

<table>
<thead>
<tr>
<th>Skippable exon</th>
<th>Therapeutic for DMD deletions (exons)</th>
<th>Percentage of deletions in LDMD database</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>3–7, 3–19, 3–21</td>
<td>2.9</td>
</tr>
<tr>
<td>8</td>
<td>3–7, 4–7, 5–7, 6–7</td>
<td>4.5</td>
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<tr>
<td>17</td>
<td>12–16, 18–33, 18–41, 18–44</td>
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<td>43</td>
<td>44, 44–47, 44–49, 44–51</td>
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<td>44</td>
<td>14–43, 19–43, 30–43, 35–43, 36–43, 40–43, 42–43, 45, 45–54</td>
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<tr>
<td>46</td>
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<td>50</td>
<td>51, 51–53, 51–55</td>
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<tr>
<td>52</td>
<td>51, 53, 53–55</td>
<td>4.0</td>
</tr>
<tr>
<td>55</td>
<td>45–54, 48–54</td>
<td>1.8</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>12 AONs</strong></td>
<td><strong>73.5</strong></td>
</tr>
</tbody>
</table>

This series of Duchenne muscular dystrophy (DMD)-causing deletions were reported in the Leiden DMD (LDMD) database. Antisense oligonucleotide (AON)-induced skipping of just 1 of the 12 skippable exons listed would (theoretically) restore the reading frame in a series of DMD patients that were affected by different deletions.
Exon 51 Skipping Human Trials

- Phase I trial with PRO051 (2-OMe) skeletal muscle injection. Dystrophin expression detected.

- Phase I/II trial with PRO051 subcutaneous injection. Dystrophin expression detected.

- Phase I/II trial with AVI-4658 (morpholino) skeletal muscle injection. Dystrophin expression detected at higher dose.

- Phase Ib/II with systemic injection of AVI-4658. Weekly injection for 6 weeks. Dystrophin expression detected.

- Phase IIb randomized, double-blind, placebo-controlled systemic injection of Eteplirsen (12 participants age 7-13 years treated once weekly with 30 or 50 mg/kg IV for 24 weeks). Met primary end-point: achieved 22.5% dystrophin-positive fibers compared to no increase in the placebo group (p≤0.002).
Acknowledgments

• CINRG clinical trials network
  – Avital Cnaan, Coordinating Center Director
  – Eric Hoffman, Scientific Director
  – Program management, data management and statistician staff
  – Researchers at 26 clinical study sites worldwide

• Translational and basic science collaborators at Children’s National Medical Center and Carolinas Medical Center

• Funding partners: NIAMS, Department of Defense, MDA, FED, PPMD