Understanding the Areas of Duchenne Research and Therapies

Melissa Spencer, Ph.D.
Professor of Neurology and Neuromuscular Program Director
Co-Director, Center for Duchenne Muscular Dystrophy at UCLA
A main goal of PPMD’s DuchenneConnect registry is to connect registrants with actively recruiting clinical trials and research studies.

EXAMPLES INCLUDE:

- Coenzyme Q10 and Lisinopril
- Dystrophin Restoration/Replacement
- Cardiac
- Blood Flow
- Mitochondria
- Inflammation & Fibrosis
- Calcium Regulation
- Muscle Growth and Protection

[Calcium Homeostasis]
[Exon-Skipping]
[Follistatin Upregulation]
[Myostatin Inhibition]
[Stem Cells]
No protein produced

Dystrophin

No protein produced
The absence of dystrophin leads to a fragile muscle cell.
Chromosomes are made of tightly wound DNA

All of our genes are coded in the DNA
The DNA is made into a xerox copy (RNA) that leaves the nucleus.

Made into RNA (xerox copy)

Made into protein
The DMD gene holds the code for making dystrophin protein in muscle and brain.
Therapeutic Approaches for Duchenne

- Exon-Skipping
- Gene Therapy
- CRISPR/Cas9
- Stop-Codon Readthrough

Dystrophin Restoration/Replacement
Therapeutic Approaches for Duchenne

- Exon-Skipping
- Gene Therapy
- CRISPR/Cas9
- Stop-Codon Readthrough

Dystrophin Restoration/Replacement

DMD Gene → DMD Gene in AAV → Deliver to all muscles in body
By taking out parts of the dystrophin gene that aren’t needed, it can be put into an AAV virus for delivery throughout the body.

Dystrophin 14 kb

Mini-Dystrophin 4.4 kb

Micro-Dystrophin
By taking out parts of the dystrophin gene that aren’t needed, it can be put into an AAV virus for delivery throughout the body.
Micro/Mini Dystrophin Gene Therapy

- Replacement of the gene and delivery with a virus.
- Not dependent on type of patient mutation—will work for all patients.
- Prior exposure to AAV may prevent this therapy from being administered.
- Not permanent, but may last as long as 10 years.
Therapeutic Approaches for Duchenne

- Exon-Skipping
- Gene Therapy
- CRISPR/Cas9
- Stop-Codon Readthrough

Dystrophin Restoration /Replacement

Treating Duchenne
The DNA is made into a xerox copy that leaves the nucleus.

Made into RNA (xerox copy)

This copy is the target for exon skipping.
Pieces of the DMD gene fit together like puzzle pieces
One piece=One exon

Figure 2. The exons of the DMD gene.

Pieces of the DMD gene fit together like puzzle pieces
One piece = One exon

Figure 2. The exons of the DMD gene.
Pieces of the DMD gene fit together like puzzle pieces
One piece=One exon

Figure 2. The exons of the DMD gene.
Pieces of the DMD gene fit together like puzzle pieces
One piece=One exon

Figure 2. The exons of the DMD gene.
Exon Skipping

- This therapy is applicable for patient mutations who can restore the reading frame with single exon skips;
- Accelerated FDA approval for skipping exon 51;
- Other exons in the pipeline........
Exon Skipping Pipeline

<table>
<thead>
<tr>
<th>Exon</th>
<th>Discovery/Preclinical</th>
<th>Phase I</th>
<th>Phase II</th>
<th>Phase III</th>
<th>Commercial</th>
<th>% of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 51</td>
<td>Blue</td>
<td></td>
<td></td>
<td></td>
<td>Blue</td>
<td>13%</td>
</tr>
<tr>
<td>Exon 53</td>
<td>Blue</td>
<td></td>
<td></td>
<td>Blue</td>
<td></td>
<td>8%</td>
</tr>
<tr>
<td>Exon 45</td>
<td>Blue</td>
<td></td>
<td></td>
<td>Blue</td>
<td></td>
<td>8%</td>
</tr>
<tr>
<td>Exon 52</td>
<td>Blue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4%</td>
</tr>
</tbody>
</table>

Sarepta

Wave

Exon 51

Exon 45, 53, 44

NS Pharma

Exon 53

% of Patients

13%

8%

8%

4%
Therapeutic Approaches for Duchenne

- Exon-Skipping
- Gene Therapy
- CRISPR/Cas9
- Stop-Codon Readthrough

Dystrophin Restoration
/Replacement

Treating Duchenne
Every gene has a “start” and a “stop” code
Some mutations can create a premature “stop”
The mRNA (xerox copy) is made into protein at the ribosome.

The "stop" codon tells the ribosome that it reached the end.

Premature stop codon
Translarna (Ataluren) causes stop-codon suppression
Stop Codon Suppression

• Applicable to approximately 10% of patients
• PTC Therapeutics—Drug name Ataluren or Translarna
• Ad Com is scheduled for September 28th
Therapeutic Approaches for Duchenne

- Exon-Skipping
- Gene Therapy
- CRISPR/Cas9
- Stop-Codon Readthrough

Dystrophin Restoration/Replacement

Treating Duchenne
CRISPR/Cas9 gene editing

Flag = guide RNA

Scissors = Cas9, cuts DNA

CRISPR/Cas9 cuts out a piece of DNA and the two ends come together to restore the reading frame
Exon 45-55 deletion leads to a milder disease course

Figure 2. The exons of the DMD gene.

CRISPR/Cas9

- Can permanently remove 1 or more exons. Will be able to be applied to a large number of mutations.
- Multiple strategies can be used to approach editing of the DMD gene.
- Furthest from trials.
- Issue is delivery and potential off target effects.
- Viral delivery may be challenging.
Different gene therapy strategies target different stages of making dystrophin and can be permanent or temporary.

- **DMD gene**
- **Dystrophin mRNA**
- **Dystrophin protein**

**GENE THERAPY**
- semi-permanent
- CRISPR-permanent

**EXON SKIPPING**
- temporary

**READ-THROUGH**
- temporary
Therapeutic Approaches for Duchenne

- Treating Duchenne
  - Muscle Growth and Protection
    - Myostatin Inhibition
    - Follistatin Upregulation via Gene Therapy
    - Selective Androgen Receptor Modulators
    - Utrophin Upregulation
      - Utrophin upregulation
      - Summit PLC-SMT 1100
Utrophin can functionally replace dystrophin
Utrophin upregulation

- Increase a protein that can functionally compensate for the loss of dystrophin.
- Temporary (need to keep taking the drug).
- Will work for all patients regardless of mutation.
Other ways to functionally compensate for dystrophin

- **Upregulation of compensatory proteins (integrin α7)**
  - Dean Burkin

- **Upregulate Sarcospan**
  - Rachelle Crosbie-Watson

- **Membrane stabilizers (polaxamer)**
  - Joe Metzger
Therapeutic Approaches for Duchenne

Dystrophin Restoration/Replacement

Treating Duchenne

Muscle Growth and Protection

Stem Cells
Skeletal muscle has endogenous “stem cells”

Muscle stem cells

Peripherally located myonuclei

Myofiber

Activate Endogenous stem cells

Replace muscle with stem cells
Therapeutic Approaches for Duchenne

- Dystrophin Restoration/Replacement
- Treating Duchenne
- Muscle Growth and Protection
- Stem Cells
Engraftment of cells to skeletal muscle

H-Lamin A/C, H-Spectrin, H-Dystrophin
Laminin, DAPI
Therapeutic Approaches for Duchenne

- Treating Duchenne
  - Muscle Growth and Protection
    - Myostatin Inhibition
    - Follistatin Upregulation via Gene Therapy
    - Selective Androgen Receptor Modulators
- Examples Include:
  - SRP-4045 and SRP-4053 (ESSENCE)
  - Tamoxifen
  - Myoblast Transplantation
  - Follistatin Gene Transfer
  - RycalARM210
  - Vamorlone (VBP15)
  - HT-100
  - FG-3019
Myostatin blockers
Pfizer
Bristol-Myers-Squibb
Follistatin overexpression (Mendell)

Myostatin blockade

• This therapy is applicable for all patients, regardless of mutation.
• Leads to increased muscle mass, but does not restore dystrophin protein.
• Also acts as an anti-fibrotic.
Therapeutic Approaches for Duchenne

- Steroid Replacement
- Anti-Fibrotics

Inflammation & Fibrosis

Treating Duchenne
Dystrophic muscle fibers become damaged with use.

Non-Duchenne muscle cell

Immune cells enter the muscle to help the muscle to repair

Duchenne muscle cell

Chronic immune cell entry (inflammation) impedes repair and leads to fibrosis.
Fibrosis in DMD
Therapeutic Approaches for Duchenne

Vamorolone-ReveraGen

HT-100-Akashi
FG-0319-Fibrogen
LTBP4 antibody-SOLID

Steroid Replacement

Anti-Fibrotics

Inflammation & Fibrosis

Prednisone
Deflazacort/Emflaza-PTC Therapeutics
NFKB inhibitors (edosolonexant-Catabasis)
Osteopontin inhibitors (PTC-pre-clinical)

Treating Duchenne
Therapeutic Approaches for Duchenne
Targeting the Mitochondria

MUSCLE FIBER

- Nucleus
- Sarcolemma
- Mitochondria
- Myofibril

Raxone (Santhera)
MTB1 (Mitobridge)
Co-Q 10
Green Tea Extract
Therapeutic Approaches for Duchenne

Exon-Skipping
Gene Therapy
CRISPR/Cas9
Stop-Codon Readthrough

Steroid Replacement
Anti-Fibrotics

Dystrophin Restoration/Replacement

Inflammation & Fibrosis
Calcium Regulation
Ryanodine Receptors
Calcium Homeostasis

Cardiac
Blood Flow
Mitochondria

Muscle Growth and Protection

Myostatin Inhibition
Follistatin Upregulation via Gene Therapy
Selective Androgen Receptor Modulators
Utrophin Upregulation

Stem Cells
Traditional Cardiac Drugs

nNOS Upregulation
Mitochondrial Biogenesis
Mitochondrial Enhancers
Thank you for your attention