Exon skipping therapy for Duchenne muscular dystrophy

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Dystrophin connects cell “skeleton” to connective tissue in muscle fibers.
Duchenne: dystrophin function lost
Becker: dystrophin partly functional

Connection is shorter, but functional
Dystrophin gene

Blueprint for dystrophin protein (genetic code)
Splicing

- Genetic code dystrophin is dispersed over “exons”
- Introns are located between exons and do not contain the genetic code
- Introns need to be removed before genetic code can be translated into protein
- This process is called splicing
Duchenne: genetic code disrupted
Exon 48-50 deletion

Genetic code disrupted

Protein translation stops prematurely

Dystrophin not functional
Becker: genetic code maintained
Exon 48-51 deletion

Genetic code maintained

Protein translation continues till end

Shorter but partially functional protein
Dystrophin gene

- Dystrophin gene contains genetic code for dystrophin protein
- Genetic code consists of “exons”
- Most Duchenne and Becker patients miss one or more exons (deletion)
- When exons “fit” the genetic code is maintained (Becker)
- When exons do not “fit” the genetic code is disrupted (Duchenne)
Exon skipping: restore genetic code
Test in cultured cells from a patient

Marker -AON +AON

Exon 47 Exon 51 Exon 52

Exon 47 Exon 52
Protein!

- AON
- 1 day
- 4 days
- 1 week

+ AON

Control (diluted)
Protein!
Pre-clinical results

- Exon skipping and dystrophin restoration confirmed in cultured cells from 15 Duchenne patients and mouse model for Duchenne
- Test in Duchenne patients
Clinical trial

"Innovative RNA-based Therapeutics acting at the cause of the disease"
Clinical study

- LUMC, Department of Neurology
- 4 Duchenne patients (8-14 year)
- 1 AON injection in shin muscle
- AONs: PRO051 (exon 51 skipping)
- Biopsy 4 weeks later
- Exon 51 skipping? Dystrophin restoration?
- Any side effects?
Pre-screening
AON injection
Exon 51 skipped
Pt.2
725 fibers

Pt.3
120 fibers

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Dystrophin levels

- After AON treatment, patients with more muscle fibers made more dystrophin protein
- AON targets dystrophin RNA
- Only muscle cells make dystrophin
- Fibrotic and adipose tissue replace muscle tissue in Duchenne patients
- These tissues do not make dystrophin
- Need muscle for the therapy to work!
Western Blot

### No. of Fibers

<table>
<thead>
<tr>
<th>Patient</th>
<th>Laminin α2</th>
<th>Dystrophin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>461</td>
<td>97</td>
</tr>
<tr>
<td>2</td>
<td>726</td>
<td>120</td>
</tr>
<tr>
<td>3</td>
<td>620</td>
<td>117</td>
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<tr>
<td>4</td>
<td>314</td>
<td>230</td>
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</table>

### Ratio of Dystrophin to Laminin α2

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>DMD</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>100</td>
<td>1</td>
<td>33</td>
<td>35</td>
<td>17</td>
<td>25</td>
</tr>
</tbody>
</table>

### Immunoblot Analysis

- **C**: Total protein 0.1
- **Pt.1**: Total protein 10
- **Pt.2**: Total protein 10
- **Pt.3**: Total protein 10
- **Pt.4**: Total protein 10

**DYS1**
Safety Assessment

- Retention of muscle strength
- Normal blood pressure and ECG
- Normal hematology and biochemistry parameters
- Normal Urine parameters
- No complement activation
- No serious (local) adverse effects
Towards clinical application

- Dose finding trial ongoing for PRO051 (Nathalie Goemans, Leuven)
- Preparing for phase I/II dose finding study with PRO044
- Preparing for longer phase II therapeutic trial with PRO051
- Optimizing AONs for other exons
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- Judith van Deutekom
Exon skipping with morpholinos

- Different AON chemistry
- Different “tool” but same principle
- Morpholinos developed by AVI-Biopharma
- Intramuscular study with morpholino AONs for exon 51 completed in UK (Muntoni, results pending)
- Systemic study with morpholino AON for exon 51 planned in UK (starts soon)
- Prepare for systemic study with modified morpholino for exon 50 in USA
Exon skipping with antisense genes

- Make a gene that produces antisense
- Deliver gene to muscle cells
- Permanent antisense production
- Antisense gene is small $\Rightarrow$ fits in AAV
- Nice results in mouse (Garcia, Genethon, Bozzoni, Italy)
- Similar problems as AAV microdystrophin therapy:
  - Immune response
  - Production
- Takes longer to develop
**Frequently asked questions**

Will exon skipping restore muscle that is already lost?

No, it will reduce the speed at which still existing muscle function is lost. In all likelihood it will not bring back lost muscle (although it is possible to combine exon skipping with drugs to enhance muscle mass (e.g. myostatin inhibitors). Thus it is important to start treatment early.
Frequently asked questions

Is it possible to treat patients who are already in a wheelchair?

Yes. It is not anticipated that these patients will start walking, but exon skipping may slow down loss of muscle function in the arms.
Frequently asked questions

How applicable is exon skipping?

Overview of exons applicable to largest groups of patients

<table>
<thead>
<tr>
<th>Exon</th>
<th>All mutations</th>
<th>Deletions</th>
<th>Duplications</th>
<th>Small mutations</th>
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</thead>
<tbody>
<tr>
<td>51</td>
<td>13.0%</td>
<td>19.1%</td>
<td>0.3%</td>
<td>3.0%</td>
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<tr>
<td>45</td>
<td>8.1%</td>
<td>11.8%</td>
<td>0.2%</td>
<td>2.2%</td>
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<tr>
<td>53</td>
<td>7.7%</td>
<td>11.4%</td>
<td>0.1%</td>
<td>1.5%</td>
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<tr>
<td>44</td>
<td>6.2%</td>
<td>8.85</td>
<td>0.4%</td>
<td>2.7%</td>
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<tr>
<td>46</td>
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<td>6.2%</td>
<td>0.2%</td>
<td>1.6%</td>
</tr>
<tr>
<td>52</td>
<td>4.1%</td>
<td>5.7%</td>
<td>0.5%</td>
<td>2.3%</td>
</tr>
<tr>
<td>50</td>
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<td>0.2%</td>
<td>1.9%</td>
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<tr>
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<td>3.8%</td>
<td>5.3%</td>
<td>0.2%</td>
<td>2.6%</td>
</tr>
<tr>
<td>6&amp;7</td>
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<td>3.6%</td>
<td>0.1%</td>
<td>6.3%</td>
</tr>
<tr>
<td>8</td>
<td>2.3%</td>
<td>2.3%</td>
<td>0%</td>
<td>8.0%</td>
</tr>
</tbody>
</table>

10 exons applicable to >40% of all patients
How can my son participate in a clinical trial?

The first clinical trials involve a small number of patients that live close to the trial center. Patients are recruited through these centers. There is no “list” at AVI/Prosensa from which patients are selected. Patients can only be recruited through their physicians! For larger future trials patients may be selected from international mutation databases (e.g. TREAT-NMD database).
Frequently asked questions

Some NBs for exon skip trials

- Approach is mutation specific, so not all patients are eligible!
- Initial trials are done to confirm that the drug is safe, later trials to confirm that the drug is effective. We hope the drug has a therapeutic effect, but this is not certain yet.

*A trial drug is NOT a therapy!*
Frequently asked questions

Some NBs for exon skip trials

• A trial drug is NOT a therapy!
• There is no guarantee that patients will benefit from participating in the trial (in fact: early phases often only involve local treatment and/or are very short, so it is unlikely patients benefit from these trials)
• Trials cost a lot of money and are not without risk! Therefore drugs are first tested in a small number of patients
Frequently asked questions

My son’s mutations cannot be restored by exon 51 skipping. Can he use exon 51 AONs anyway?

Example: deletion exon 45
Exon 51 skipping disrupts the genetic code at an additional location
Skipping exon 44 or 46 would restore the genetic code (43 fits to 46 and 44 fits to 47)
Frequently asked questions

Which is better? Morpholino or 2OMePS chemistry?

AONs are new types of drugs and not much is known about their long term effects. It may be that one chemistry works better for some exons and the other for other exons, or that one chemistry is toxic after long term use. For now both chemistries are developed in parallel.
How long will it take before AONs are available for clinical application?

Clinical trials take a lot of time. The AONs for exon 51 are furthest in development, but it will take at least till 2011 before these AONs are available (if they are confirmed to be therapeutic!). Hopefully AONs for other exons (e.g. 44, 53, 45 and 50) will follow soon after.