Less is more: therapeutic exon skipping for Duchenne muscular dystrophy

Duchenne muscular dystrophy (DMD) is a lethal X-linked progressive muscle-wasting disease caused by premature truncation of the translation of DMD mRNA into dystrophin. Owing to improved respiratory support and treatment with steroids, patients can now survive into early adulthood, which is one or two decades longer than they could survive without such interventions. Despite much research worldwide, no disease-modifying treatment is yet available; however, promising results are being achieved with antisense oligonucleotides. Antisense oligonucleotides are modified DNA or RNA analogues that hybridise with a target DNA or RNA sequence, and they can be designed to prevent specific exons of DMD from being spliced into the mRNA. This so-called exon skipping can reframe the disrupted open reading frame of DMD to produce a shorter but functional protein (figure), such as the protein produced in individuals with the milder form of the disease, Becker muscular dystrophy. This less is more approach has been developed over the past decade in patient-derived cell cultures and in the mdx mouse model.

Modifications to the chemistry of antisense oligonucleotides are necessary to increase their half life and prevent the cleavage of RNA–RNA hybrids by RNase H, which is the goal of antisense-mediated knockdown of gene function but is unwanted for antisense-mediated modulation of splicing. The most commonly used chemistries for exon-skipping antisense oligonucleotides are 2′-O-methyl RNA with a negatively charged phosphorothioate backbone (2′OMePS) and uncharged phosphorodiamidate morpholino oligomers (PMO), because both are stable and non-toxic. In 2007, an intramuscular proof-of-concept clinical study was reported by van Deutekom and co-authors. They used PRO051, a 2′OMePS RNA that targets exon 51. Skipping of exon 51 corrects several mutations in the DMD deletion “hotspot”, which amounts to about 13% of the mutations that cause DMD. In the September issue of The Lancet Neurology, Kinali and co-authors’ report the results of a similar proof-of-concept study with intramuscular AVI-4658, a PMO that also targets exon 51. Although there are similarities and differences between the two studies, the main combined messages are that there are no drug-related adverse effects and that both backbone chemistries have about equal effectiveness. These findings are valuable because they imply that there is more freedom in the choice of chemistry than has been known so far. However, although nucleic acid-based drugs, such as PMO or 2′OMePS, are promising because of their apparent low toxicity, and they have not yet been tested over the prolonged period of administration required for treating childhood disease.

The table compares two studies. van Deutekom and co-authors injected a 2×1 cm area of the tibialis anterior muscle in the lower leg, but took only a small biopsy of the muscle 4 weeks after treatment. Kinali and co-authors targeted a 1 cm² area of the extensor digitorum brevis, a small muscle in the foot, and the muscle was completely removed for analysis; they also biopsied the saline-injected contralateral muscle. Kinali and co-authors showed that low-dose AVI-4658 did not induce expression of dystrophin that was greater than the background expression, whereas high-dose AVI-4658 induced a significant increase in dystrophin expression and the number of dystrophin-positive fibres seen with immunohistochemical staining.

The proportion of dystrophin-positive fibres and the amount of dystrophin detected vary between Duchenne muscular dystrophy and Exon skipping to reframe transcripts.

**Figure 1: Antisense-mediated exon skipping to reframe DMD transcripts**

Patients with Duchenne muscular dystrophy have mutations in DMD, the gene that encodes dystrophin. The mutations disrupt the open reading frame of dystrophin (in this example, exons 45–50 are deleted). Consequently, protein translation stops prematurely, resulting in a non-functional protein. By use of antisense oligonucleotides that target a specific exon in which there is a mutation that truncates the expression of dystrophin (exon 51 in this example), the reading frame can be restored. This enables the production of an internally deleted but partially functional dystrophin. AON=antisense oligonucleotide.
van Deutekom and co-authors

<table>
<thead>
<tr>
<th>Compound</th>
<th>Placebo</th>
<th>Dose</th>
<th>Number of patients</th>
<th>Target muscle</th>
<th>Proportion of positive fibres</th>
<th>Proportion of dystrophin expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRO051</td>
<td>No</td>
<td>0.8 mg</td>
<td>4</td>
<td>Tibialis anterior</td>
<td>64–97%†</td>
<td>17–35%‡</td>
</tr>
<tr>
<td>AVI-4658</td>
<td>Yes</td>
<td>0.9 mg</td>
<td>5†</td>
<td>Extensor digitorum brevis</td>
<td>44–79%§</td>
<td>22–32%§</td>
</tr>
</tbody>
</table>

*The two antisense oligonucleotides target the same region in exon 51. AVI-4658 contains the sequence of PRO051 (underlined). The PMO is a DNA analogue, whereas 2´OMePS is an RNA analogue, which explains why the antisense oligonucleotides contain the nucleotides thymine and uracil. †Two other patients received a lower dose (0.09 mg), which was ineffective. ‡Not corrected for positive fibres in saline injected contralateral muscle. §Corrected for number of positive fibres in saline injected contralateral muscle (0.3–5.0% positive fibres). ¶Corrected for background staining in saline injected contralateral muscle (11–21%). §§Not corrected for background staining in saline injected contralateral muscle. ¶¶Up to 42% when selecting only positive fibres; this figure is not available for the other study.

Table 1: Comparison of studies of intramuscular antisense oligonucleotides in patients with Duchenne muscular dystrophy.

Finally, the sequence-specific approach has implications for future personalised therapy. Although skipping of exon 51 is applicable for 13% of patients with Duchenne muscular dystrophy, it will not benefit the other 87%, whereas skipping of 10 exons might be beneficial for more than 70% of patients with a deletion mutation in DMD, or 40% of all patients. Other approaches are being developed that could raise the number of patients who can be treated: for example, antisense oligonucleotides to duplications and point mutations through double-exon11–12 and multi-exon skipping.13,14 Although these approaches look promising on paper, not all are successful in practice, and we might need to learn much more about the complex splicing of this large gene (2.5 Mbp and 79 exons) to optimise its therapeutic correction.15 In all cases, this process will require close coordination with regulatory agencies,5,10 because there are too few patients with the rarer mutations to set up independent clinical trials.

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AA-R and G-JBvO are employees of Leiden University Medical Center and coinventors on patent applications for antisense sequences and exon-skipping technology. Leiden University Medical Center has licensed the rights to the patents on PRO051 exclusively to Prosensa. The inventors specified on the patents, who include AA-R and G-JBvO, are jointly entitled to a share of any future royalties paid to Leiden University Medical Center, should the therapy eventually be brought to the market. G-JBvO is an unpaid member of the external scientific advisory board of Prosensa.