Exon skipping in Duchenne muscular dystrophy

Although the publication by Judith van Deutekom et al. [1] is not the first exon skipping trial reported to treat Duchenne Muscular Dystrophy (DMD), it is without doubt the most convincing demonstration that splice intervention has real potential. In 2006, Takeshima et al. [2] reported an ambitious single patient study administering an oligodeoxyribonucleotide designed to excise exon 19 and restore the reading frame in a DMD patient missing exon 20. Dystrophin expression was equivocal, and many factors could have accounted for this, including the nature of the oligomer chemistry and dosage regimen.

In contrast, the Leiden trial [1] was designed to first assess safety and adverse events, with local restoration of dystrophin around the injection sites providing proof-of-concept that exon skipping can be induced in human dystrophic muscle. Exon 51 was chosen as the first target, as PRO051, an oligomer with 2’-O-methyl modified bases on a phosphorothioate backbone, could be of benefit to an estimated 25% of DMD deletion patients.

Pre-screening patient cell cultures confirmed that PRO051 was effective at by-passing the primary gene lesion, as there was a remote but real possibility that dystrophin gene variations at the oligomer annealing site could compromise efficiency, or, that a second protein truncating mutation could lie elsewhere in the gene. Each of the four reported cases carried a different deletion (of exon 50, 52, 48–50 or 49–50), that would be addressed by skipping exon 51 and the in vitro studies indicated its effectiveness (up to 90% in one case).

The participants then each received four injections (200 μg of the oligomer in saline), along a line and 5 mm apart, into the tibialis anterior muscle. The site was biopsied 28 days later. Each patient reported one or more adverse events, with mild local pain at the injection site in one case, while other events could be attributed more to the muscle biopsy process.

Both RNA and protein studies clearly demonstrated exon skipping and induction of some dystrophin synthesis in all participants. Relatively low levels of the skipped gene transcripts were detected by “high sensitivity PCR conditions”, employed due to the small amount of section material available. Details of these high sensitivity conditions were not clear but they were, apparently, different from those used for the in vitro studies.

It is possible that exon skipping may have been more pronounced at earlier time-points, but immunostaining and Western blotting unequivocally demonstrated dystrophin in all four participants, with amounts of the induced protein ranging between 3% and 12% of the levels found in normal healthy muscle, although these figures may be underestimates considering the nature of the dystrophic tissue.

Despite these very positive outcomes and confirmed proof-of-concept, there must be some concerns regarding the amounts of dystrophin transcript detected and levels of protein laid down relative to the amount of oligomer administered. While any extrapolation from in vitro studies can only be made with caution, it is worth noting the disparity between in vitro and in vivo oligomer concentrations evaluated. Substantial exon skipping was induced after in vitro transfection with 100 nM oligomer, complexed with PEI for enhanced delivery. In vivo, a total of 800 μg of PRO051 was injected at a concentration of 1 mg/ml (about 150 μM), over three orders of magnitude higher than that used in vitro. Overall, it was most encouraging that no local inflammatory or toxic responses were observed in the muscle biopsies, although it may be of concern that more dystrophin was not produced considering the amounts of oligomer injected, albeit only once. More substantial dystrophin expression might be expected after repeated administration.

While no adverse events were observed in rats and monkeys receiving repeated intramuscular or intravenous administration of PRO051 at dosages up to 50 mg/kg, there was no mention of any assessment of exon skipping in these animals. Despite possible mismatches with the rat dystrophin sequence that might compromise oligomer action, it would seem that an obvious extension of this study would be to attempt to detect dystrophin RNA transcripts missing the targeted exon. It could be of great relevance to future human trials to ascertain whether exon skipping is induced in the treated animals.

Many technical challenges and questions have yet to be addressed, including enhanced bioavailability and uptake, developing appropriate delivery regimens, the consequences of long term oligomer exposure and application to other responsive DMD mutations. It is possible that regulatory issues may prove as great a challenge as some technical considerations, but nevertheless a benchmark has now been set for future clinical trials, including the one currently under way in the United Kingdom. The crucial take-home
message from Judith van Deutekom and her colleagues is that splice intervention and targeted exon skipping can be induced in dystrophic muscle, and is definitely worth pursuing as a potential future therapy for DMD.

References


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Myostatin blockade – therapy or body building?

Since the original description of a mutation in the myostatin gene that is responsible for the “double-muscled” phenotype in cattle [1], a number of strategies have been developed to stimulate muscle growth by interfering with the function of the encoded protein, myostatin [2]. In their recent report, Qiao et al. [3] blocked myostatin activity in mice by systemically overexpressing the myostatin propeptide using an adeno-associated viral (AAV8) vector and a cytomegalovirus (CMV) promoter. One may argue that their findings simply add to the growing list of promising results showing the effect of myostatin blockade on skeletal muscle since no novel strategy was used in this study [4], the results mainly confirm previous reports [5], and the form of gene expression (relaying on the CMV promoter) is not applicable for therapeutic strategies in humans. Nonetheless, the results of the functional evaluation of treated muscle require attention. Qiao et al. demonstrated that myostatin blockade in wild-type mice did not change the force generation despite an excessive muscle growth. In contrast to wild-type mice, treatment of mdx mice (the mouse model for Duchenne muscular dystrophy) resulted in increased muscle size and increased tetanic force. However, Qiao et al. additionally investigated the exercise behaviour after myostatin blockade and found that treated mdx mice had a diminished endurance when running on the treadmill, which may be explained by the fact that lack of myostatin results in a loss of oxidative fibres and a fibre type conversion towards fast glycolytic fibres [6]. What do we learn from these experiments? Firstly, myostatin blockade makes normal muscle bigger but not stronger. Secondly, myostatin blockade makes mdx muscle bigger and stronger, but mice cannot run as far. Therefore, from a functional perspective, the question remains unanswered: can myostatin blockade provide a possible new therapeutic strategy, ultimately delivering functional benefits for patients, or will it, instead, constitute a new approach to body building?

References


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