Good news for the mdx mouse community: Improved dystrophin restoration after skipping mouse dystrophin exon 23

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Currently there are four exon-skipping phosphorodiamidate morpholino oligomer (PMO) drugs approved by the US Food and Drug Administration (FDA) for treating patients with Duchenne muscular dystrophy (DMD). Approval was based on dystrophin restoration (~1%), and the FDA label specifies that functional effects remain to be confirmed. While it is unclear whether these low dystrophin levels are sufficient to delay disease progression, it is clear there is room for improvement. In this issue, Gan et al. report on the use of an arginine-rich peptide conjugated to a PMO (pPMO), to achieve more efficient dystrophin restoration in mdx mice, not only in skeletal muscle but also in diaphragm and heart, where unconjugated PMO is very inefficient. However, the pPMO targets mouse dystrophin exon 23. While this Compound targets the majority of dystrophinopathy mice, it applies to exactly zero patients with DMD.

DMD is a severely progressive muscle-wasting disease that is caused by a lack of functional dystrophin. In patients with DMD, mutations cause a premature truncation of protein translation and non-functional proteins. Becker muscular dystrophy is a later-onset and less severely progressive disease that is also caused by mutations in the dystrophin gene. However, new mutations do not disrupt the reading frame and allow the production of internally deleted, but partially functional, dystrophins. The exon-skipping approach is based on the premise that most patients with DMD can produce a Becker-like dystrophin. This requires the skipping of one or more exons to restore the reading frame. Exon skipping can be achieved with oligonucleotides, chemically modified RNA analogs that bind specifically to a target exon and hide it from the splicing machinery.

There is a plethora of chemical modifications available for oligonucleotides, but the exon-skipping oligonucleotides approved for DMD all have the PMO chemistry. Despite very high doses of PMOs used for the clinical treatment of DMD (30–80 mg/kg) (Table 1), dystrophin restoration levels are relatively low. This is not surprising as PMOs have poor bioavailability and are cleared efficiently and rapidly by the kidney. Uptake by muscle is speculated to be dependent on muscle turnover and is inefficient. It has been known for years that arginine-rich peptides can improve uptake of PMOs in a non-tissue-specific manner. However, it is similarly known that arginine-rich peptides are toxic. Thus, the challenge is to find a peptide that has sufficient arginines to allow improved uptake without resulting in toxicity at the therapeutically effective dose. In the publication of Gan et al., the authors present results with a proprietary peptide that is one of the >350 peptides reported in the patent cited (US9161948B2). The peptide is conjugated to a PMO targeting mouse exon 23, which is mutated in the mdx mouse model. As this exon is in frame, skipping it will bypass the mutation and restore dystrophin production.

Compared with unconjugated PMO, the pPMO performed much better, with dystrophin levels of up to 68% in skeletal muscle and 41% in heart 30 days after a single intravenous dose of 80 mg/kg. A single dose of 40 and 80 mg/kg resulted in improvement in muscle function, strength, and muscle histology. The authors also studied the longevity of effects, revealing that exon-skipping levels reduced to (close to) 0% between 30 and 60 days. Dystrophin protein levels declined with time as well but were still detectable at 90 days for all muscles studied. Finally, the authors performed a study comparing 1, 2, or 3 monthly intravenous doses of 40 mg/kg. As expected, multiple doses resulted in accumulation of dystrophin levels up to 51% in skeletal muscle and 9% in heart. At the same dosing regimen, the unconjugated PMO resulted in <5% dystrophin in skeletal muscle and barely detectable dystrophin in diaphragm and heart.

It is clear that the pPMO is outperforming the PMO, with much higher exon skipping and dystrophin levels and notable dystrophin restoration in diaphragm and heart. However, the studies are done in mdx mice with a pPMO targeting a mouse dystrophin exon. This means that extrapolation has to occur at two levels when translating this to patients with DMD: from an antisense oligonucleotide (ASO) targeting mouse exon 23 to an ASO targeting human exon 51 and at a species level from mouse to human. It has become clear that human dystrophin exon 51 is one of the more difficult exons to skip. As such, using human-specific oligonucleotides in a humanized mouse model would be preferable. It is also known that the mdx mouse regenerates more efficiently than patients with DMD. Since the PMOs target dystrophin transcripts and these are produced by muscle tissue and not fibrotic and adipose tissues, the therapeutic effect in mdx is expected to be higher than in humans.

Two clinical programs are currently ongoing with pPMOs targeting exon 51: SRP-5051 and PGN-EDO51. It is clear that pPMOs are more efficient than the PMOs in patients with DMD and healthy volunteers. However,
Table 1. Overview of doses of PMO and pPMOs used in clinical applications and clinical trials for Duchenne muscular dystrophy

| Compound      | Type | Target exon | Dose used (mg/kg) | Regimen                  | Dystrophin levels                           |
|---------------|------|-------------|-------------------|--------------------------|---------------------------------------------|
| Eteplirsen PMO| 51   | 30          | weekly intravenous | 0.4% increase           |
| Eteplirsen pPMO| 51 up to 200 | weekly intravenous | pending clinical trial |
| Golodirsen PMO| 53   | 30          | weekly intravenous | ~1% increase             |
| Viltolarsen PMO| 53 up to 80 | weekly intravenous | ~5% increase             |
| Casimersen PMO| 45   | 30          | weekly intravenous | ~1% increase             |
| SRP-5051 pPMO| 51   | 30          | monthly intravenous | 6.5% increase             |
| PGN-EDO51 pPMO| 51 up to 20 | single-dose intravenous | unknown (2% exon skipping in healthy volunteers) |
| RC-1001 pPMO  | mouse exon 23 | up to 80 | single-dose intravenous | 68% in muscle, 41% in heart |
| RC-1001 pPMO  | mouse exon 23 | 40 | monthly intravenous | 51% in muscle, 8% in heart |

Compared with the results reported in the mouse model, efficiency is more limited (Table 1). This could be due to the lower doses used (30 mg/kg for SRP-5051 and 20 mg/kg for PGN-EDO51). Indeed, in mdx mice, the most notable results were only achieved with 40 and 80 mg/kg doses. However, these higher doses are probably not tolerated by humans. At the currently used doses, hypomagnesemia has been reported for both compounds. This has resulted in a temporary hold by the FDA for the SRP-5051 trial in June 2022, which was lifted in September. The hypomagnesemia is likely a consequence of proximal tubular toxicity, which has an impact on the energetically demanding reabsorption of magnesium ions. The ongoing clinical trials will have to reveal whether longer-term treatment with arginine-rich pPMO is tolerable by patients with DMD or not.

For SRP-5051, dystrophin levels of up to 6.5% were reported after 3 treatments over a period of 12 weeks in patients with DMD. This is more than the ~0.4% of dystrophin increase after a year of treatment with the PMO, but obviously it all boils down to a key question: how much dystrophin is needed to have a therapeutic effect? This is a multifaceted question for which we refer the reader to a more focused publication. However, it is clear that for exon skipping, we are currently in the dynamic range, where more dystrophin is very likely to result in more therapeutic effects.

DECLARATION OF INTERESTS
A.A.-R. discloses being employed by LUMC, which has patents on exon-skipping technology, some of which has been licensed to BioMarin and subsequently sublicensed to Sarepta. As co-inventor of some of these patents, A.A.-R. is entitled to a share of royalties. A.A.-R. further discloses being an ad hoc consultant for PTC Therapeutics, Sarepta Therapeutics, Regenxbio, Alpha Anomeric, BioMarin Pharmaceuticals, Inc., Eisai, entrada, Takeda, Splicense, Galapagos, and Astra Zeneca. Past ad hoc consulting has occurred for CRISPR Therapeutics, Summit PLC, Audentes Santhera, Bridge Bio, Global Guidepoint and GLG Consultancy, Grunenthal, Wave, and BioClinica. A.A.-R. also reports having been a member of the Duchenne Network Steering Committee (BioMarin) and being a member of the scientific advisory boards of Eisai, Hybridize Therapeutics, Silence Therapeutics, and Sarepta Therapeutics. Past SAB memberships include ProQR and Philae Pharmaceuticals. Remuneration for these activities is paid to LUMC. LUMC also received speaker honoraria from PTC Therapeutics and BioMarin Pharmaceuticals and funding for contract research from Italfarmaco, Sarepta, Eisai, Galapagos, Synnaffix, and Alpha Anomeric. Project funding is received from Sarepta Therapeutics through an unrestricted grant.

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