Exon skipping with morpholino oligomers: new treatment option for cardiomyopathy in Duchenne muscular dystrophy?

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This editorial refers to ‘Long-term improvement in mdx cardiomyopathy after therapy with peptide-conjugated morpholino oligomers’ by N. Jearawiriyapaisarn et al., pp. 444–453, this issue.

The dystrophin gene encodes an essential component of the trans-membrane dystrophin–glycoprotein complex (DGC), which plays a critical role in maintaining membrane stability in cardiac and skeletal muscles.1 Defects in dystrophin destabilize the entire complex, which results in an abnormal susceptibility to sarcolemmal injury in response to contractile stress.2 Mutations that cause slight sequence alterations or loss of internal exons but conserve the reading frame result in Becker muscular dystrophy with a mild phenotype. Complete absence of dystrophin causes Duchenne muscular dystrophy (DMD), a X-chromosomal, fatal, and inherited muscle disease. Affected boys show progressive muscle weakness, leading to early immobility, respiratory failure, and markedly reduced life expectancy. Cardiomyopathy is an almost invariable complication of this inherited disease has translated into crucial to reduce mortality. However, there now seems to be hope since the identification of new therapeutic principles to causally treat dystrophin deficiency has steadily improved. In older patients, however, there is an increasing proportion of patients experiencing premature death due to ventricular dysfunction. Therefore, appropriate treatment strategies to target the cardiomyopathy become more and more crucial to reduce mortality.

Thus far, there is no established curative approach to DMD. However, there now seems to be hope since the identification of the molecular basis of this inherited disease has translated into recent therapeutic principles to causally treat dystrophin deficiency: cell replacement (myoblasts or stem-cells), pharmacological approaches to induce ribosomal readthrough of premature termination codons, viral gene transfer with micro-mini-dystrophin CDNA, and antisense oligonucleotide-mediated exon skipping are the most promising treatment strategies.4–10 Although these therapies have been shown to restore dystrophin expression locally in dystrophic skeletal muscles, they may leave the cardiomyopathy essentially untreated.

Currently, the approach of exon skipping is especially considered to have the potential for efficient treatment of boys with DMD. Antisense oligonucleotide (AON)-mediated exon skipping functions to restore the open-reading frame by removing specific exons from the altered dystrophin transcript, creating shortened, but functional, proteins that should be able to convert the Duchenne into a Becker phenotype. Recently, different two types of AONs have successfully been tested in a small number of patients with DMD: AONs of a 2′-O-methyl phosphorothioate RNA chemistry or phosphorodiamidate morpholino oligomers (PMOs).9,10 In these first clinical proof-of-concept studies, local intramuscular injections of AONs appeared to be safe and to have induced local expression of a significant amount of dystrophin protein in defined ‘isolated’ muscles.9,10 However, an improvement of morbidity or mortality probably requires treatment of comprehensive muscle groups with higher doses of the respective agent. Thus far, systemic applications of AONs have only been tested in animal models of DMD.7,8 Weekly intravenous injections of PMOs into dystrophin-deficient mdx mice, a murine model of DMD with a nonsense-mutation in exon 23 of the dystrophin gene, resulted in body-wide expression of functional levels of dystrophin in skeletal muscles.9 However, these effects were disappointingly absent in cardiac muscle of these mice. This might be a crucial limitation of the treatment with AONs, especially if one bears in mind that an increase in physical activity by targeted repair of skeletal muscles in mdx mice worsens cardiac injury and dilated cardiomyopathy.11

Jearawiriyapaisarn et al.12 report on a new strategy for using PMOs. On the basis of the recent observation that conjugation of arginine-rich, cell-penetrating peptides to PMOs allows an efficient cardiac transfer,13–15 the authors investigated the effect of a peptide-conjugated PMO (PPMO AVI-5225) on morphological alterations and contractile function in mdx mouse hearts. Skipping of exon 23 induced by PPMO AVI-5225 achieved cardiac expression of a shortened but functional dystrophin protein sufficient to ameliorate sarcolemmal damage, hypertrophy, and diastolic dysfunction in the hearts of mdx mice.12 The authors highlight that the combination of positively charged peptides with neutral oligomers that sequence-specifically target pre-mRNA is responsible for the effective cardiac expression

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of a functional dystrophin protein. It is remarkable that a restoration of just 30% of cardiac dystrophin leads to an attenuation of cardiac hypertrophy. In addition, transient expression of dystrophin before the onset of cardiac pathology seems to be sufficient to persistently slow down the progression of the cardiomyopathy in mdx mice when analysed after 7 months, a time point at which exon skipping and dystrophin expression no longer occur.

Jearawiriyapaisarn et al. have presented a proof-of-concept study for this new curative approach with the use of an established mouse model for DMD. The present study thus raises hope for future clinical studies. A word of caution seems appropriate, however. In general, mouse models with muscular dystrophy-associated cardiomyopathy, and especially mdx mice, do not entirely replicate the human clinical phenotype. The mdx mouse has a relatively mild cardiomyopathy and a normal life expectancy compared with the severe phenotype with early onset of ventricular dysfunction in DMD patients. However, patients present with diastolic dysfunction at an early stage of development of the cardiac disease, similar to the case with mdx mice. In contrast to the uniformity of animal models, the genetic background is more heterogeneous in patients with DMD, and the sequence-specific exon-skipping approach is not applicable to all DMD patients. The systemic application of substances harbours the risk of side effects, and higher doses may be needed in patients to achieve comparable effects. Thus, treatment effects in mice cannot be easily extrapolated to patients.

Overall, when we consider future clinical trials with AONs in patients, a number of questions have to be answered first: which patients do we want to treat and when shall we start treatment? Which dose is the most effective? Which side effects will we face, and how many negative effects of AONs do we have to accept? How long will such a therapy be effective, and how many cycles of the treatment will be required? Is an attenuation of the cardiac disease adequate, or do we want to prevent the development of cardiomyopathy completely?

Jearawiriyapaisarn et al. have cleared the way for clinical studies that will have to answer these questions. Further preclinical studies will show whether AON-mediated exon skipping might also become a therapeutic option for familial cardiomyopathies.

References