Review

CellPress

The emperor's new dystrophin: finding sense in the noise

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Targeted dystrophin exon removal is a promising therapy for Duchenne muscular dystrophy (DMD); however, dystrophin expression in some reports is not supported by the associated data. As in the account of 'The Emperor's New Clothes', the validity of such claims must be questioned, with critical re-evaluation of available data. Is it appropriate to report clinical benefit and induction of dystrophin as dose dependent when the baseline is unclear? The inability to induce meaningful levels of dystrophin does not mean that dystrophin expression as an end point is irrelevant, nor that induced exon skipping as a strategy is flawed, but demands that drug safety and efficacy, and study parameters be addressed, rather than questioning the strategy or the validity of dystrophin as a biomarker.

Dystrophin, DMD, and exon skipping

Antisense oligonucleotide (AO)-induced splice intervention to treat DMD has progressed from a concept to clinical trials in less than two decades. Clinical trials have now been running for more than 3 years, some with encouraging results and others less so. In this review we briefly summarize the history and rationale behind exon skipping as a treatment for DMD, consider preclinical exon skipping studies, and discuss the oligomer chemistries under clinical evaluation. We reflect on potential challenges and discuss some limitations in the clinical evaluation and implementation of molecular therapies for an orphan disease such as DMD. We also address the added complication of treatments needing to be stratified because of different DMD mutations.

DMD and the rationale for exon skipping

DMD arises from protein-truncating mutations in the 2.4 Mb dystrophin (*DMD*) gene, comprising 79 exons, that result in an insufficiency of functional dystrophin, the 427-kDa skeletal muscle isoform that confers strength and stability on muscle fibers [1–3]. DMD patients are typically non-ambulant by 12 years of age and succumb to cardiac and/or respiratory complications in their mid-20s [4,5]. Recent advances in health care, particularly the use of steroids and assisted ventilation, have extended the patients' lifespan considerably [6–8]. Becker muscular dystrophy (BMD) also arises from *DMD* lesions, but these are predominantly in-frame deletions that allow expression of an internally deleted protein that retains some function [1]. BMD is clinically defined as the patient remaining ambulant until at least age 16 years or later, although severity may vary from severe (wheelchair bound by age 17 years) to asymptomatic [9,10]. The loss of exon 16 was serendipitously identified in three generations of males in one family, with no evidence of pathology and serum creatine kinase levels within the normal range [11]. Analysis of BMD patient cohorts with deletions that begin or end with exon 51 reported 'that all varieties of internally deleted dystrophin assessed in this study have the functional capability to provide a substantial clinical benefit to patients with Duchenne muscular dystrophy' [12]. Other detailed studies of BMD individuals similarly report milder phenotypes compared with DMD [13] and web-based utilities are now available to predict the functionality of the dystrophin isoforms [14,15].

The aim of AO-induced exon skipping is to alter dystrophin pre-mRNA processing to restore the reading frame of a DMD-causing gene transcript (Figure 1). Modulation of *DMD* pre-mRNA processing can allow the generation and subsequent translation of a BMD-like dystrophin mRNA. Chemically synthesized AOs induce no permanent modification of the recipient genome and sustained exon skipping is achieved only through periodic re-administration of the AOs. This issue has been addressed by expressing RNA antisense transcripts capable of inducing targeted exon skipping under the control of U7 promoters in an adeno-associated virus (AAV) construct. These vectors have shown long-term specific exon skipping and restoration of the reading frame in mouse [16,17] and dog [18–20] dystrophinopathy models.

AO-mediated exon skipping will not cure DMD but should slow the progression of the muscle wasting associated with the disease. The degree of amelioration will be influenced by the extent of pathology before commencement of the treatment, the efficiency of the induced exon skipping, the nature of the induced BMD-like dystrophin isoform, and, presumably, the genetic background of the patient. One would expect that commencing treatment before extensive muscle damage and fibrosis are established would offer the best outcome for the patient. However, even if 100% exon skipping and reframing of the dystrophin mRNA could be achieved, the induced dystrophin missing one or more exons remains a BMD-like

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Keywords: Duchenne muscular dystrophy; dystrophin; exon skipping; clinical evaluation.

1471-4914/
 http://dx.doi.org/10.1016/j.molmed.2015.04.006

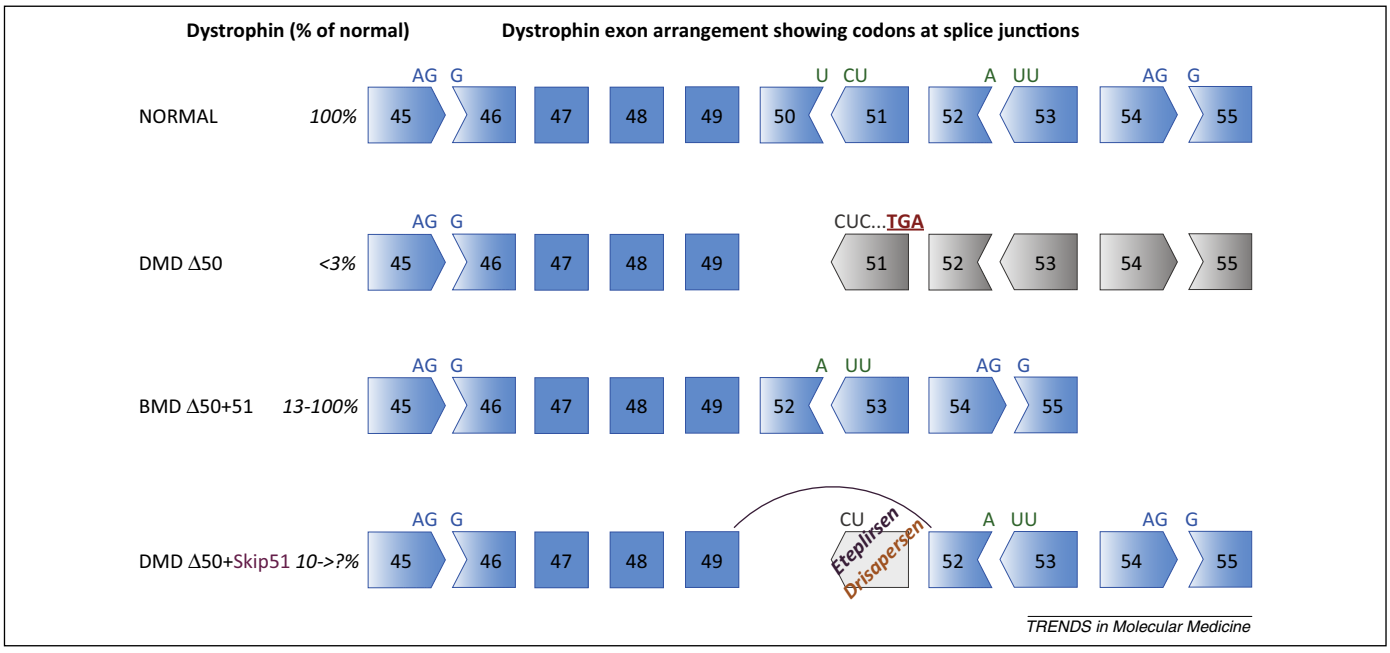

Figure 1. Arrangement of *DMD* exons 45–55 indicating codons interrupted by splice junctions. In-frame exons are represented by rectangles whereas those that carry partial codons at the exon boundary are shown with angled sides. Codons disrupted by exon junctions are shown in upper case, above. Deletion of exon 50 disrupts the reading frame (grey shading) through the loss of a single U in the 'UCU' serine codon, leading to premature termination of translation of nine codons downstream of the splice junction (red text). Deletion of exons 50 and 51 in BMD does not disrupt the reading frame and allows a shorter isoform to be synthesized. Skipping of exon 51 induced by an antisense oligomer such as eteplirsen [56] or drisapersen [47] restores the reading frame.

isoform. Typically, BMD individuals with a confirmed deletion of, for example, exons 48–51, assessed at the mRNA level, have mild–asymptomatic features [12]. However, it is unreasonable to expect 're-framed' dystrophin in AO-treated DMD patients to exhibit the same phenotype as a BMD individual who has been making this dystrophin isoform since birth.

Eighty-three percent of all DMD patients could potentially benefit from targeted exon skipping [21] and while many whole-exon deletions and intraexonic mutations will require single exon skipping, other *DMD* lesions will require skipping of multiple exons to restore the reading frame. Genomic deletion of exon 6 will require the removal of both exon 7 and 8 to reframe the mRNA, whereas intraexonic mutations in any of these dystrophin exons (6–8) will demand the removal of all three to generate a BMD-like dystrophin isoform. *DMD* exon 2 is the most commonly duplicated exon, either individually or in a multi-exon block, and, unless skipping of a single copy of the duplicated exon can be achieved, will require five exons (3–7) to be excised in addition to the duplicated exon 2 to restore the reading frame [22].

Most DMD-associated deletions are clustered in the major mutation hotspot encoded by exons 45–55 in the rod domain and are therefore likely to benefit from dystrophin re-framing. Patients with deletion of exons 45–55 are reported to have a mild clinical presentation and multi-exon skipping to emulate this dystrophin isoform has been proposed as a one-size-fits-all treatment for patients with any mutation in this ten-exon block [23]. Not all DMD mutations will be amenable to exon skipping. Loss of 36 or more exons, or lesions involving those exons encoding crucial functional domains, is unlikely to be addressed by exon skipping [24]. Interrogation of DMD patient databases shows that in-frame deletions downstream of exon 55 leading to BMD are rare. The paucity of BMD patients with lesions in this region may be due to the complex exon structure and/or the stability of the latter third of the gene and there is little indication of what to expect from induced exon skipping in this region.

Over two decades ago, low levels of dystrophin were reported in approximately two out of three DMD individuals; rare, brightly staining dystrophin-positive 'revertant' fibers [25,26] and more common very weakly staining fibers [27,28]. These researchers insightfully postulated 'the very weak labeling on a high proportion of fibers might be due to a compensatory event at RNA transcript level. In either case, exon skipping or other forms of abnormal splicing may be involved. Any mechanism by which, for example, an exon 44 deletion (out-of-frame) could be enlarged to an exon 44–45 deletion (in-frame) would have the required effect' [29]. Although no suggestions were provided at the time regarding how to reproduce the mechanism responsible for this phenomenon, the seeds of exon skipping as a therapeutic strategy had been planted.

Published a month before the manuscript by Nicholson and colleagues was accepted in 1993, the use of AOs to correct aberrant β-globin gene pre-mRNA processing was described [30]. Mutations in β-globin gene introns 1 and 2 activated cryptic splice sites that were used in preference to the normal splice sites and AOs targeting the abnormal splice sites forced the splicing machinery to default to the normal pattern [30]. Nevertheless, a few years passed before the pieces came together and exon skipping was applied to dystrophin pre-mRNA processing.

The development of exon skipping therapy

The development of AO-induced dystrophin exon skipping arose independently and around the same time in laboratories in Japan, the UK, Australia, and The