

What Percentage of Patients with Duchene Muscular Dystrophy are Potentially Treatable with Gene Therapies?

Sir,

Antisense oligonucleotide (ASO)-mediated exon skipping is potentially useful in children with Duchene muscular dystrophy (DMD). The premise is that patients with in-frame mutations such as Becker's syndrome have milder disease. ASO's have the capability of masking a particular exon which is out of frame. This allows the cellular machinery to exclude that exon in the final mRNA, resulting in a smaller but still functional dystrophin protein.^[1] The most well-studied ASO for DMD is for exon-51 skipping. However, exon skipping of other deletions may also be possible. We analysed a small cohort of children with DMD from Central India and evaluated how many could theoretically benefit from exon-skipping technologies.

This was a retrospective analysis of all children with DMD, attending our pediatric neurology clinic in a teaching hospital in Central India, between 2014 and 2019 who had undergone genetic testing for mutations. Genetic testing included commercial PCR-based tests, multiplex ligation-dependant probe amplification (MLPA), or if required targeted exome sequencing using NextGen sequencing. Inclusion criteria were boys less than 18 years with progressive proximal muscle weakness, elevated creatine phosphokinase (CPK) levels, and documented genetic testing. Further, the type of mutations were analysed. The percentage of children who would benefit from exon skipping was analysed. Institutional ethics clearance was taken.

A total of 47 children with DMD were included in the study. The mean age was 7.7 years (range 4–15). Mutations were detected in 3/5 (60%) of patients tested using PCR technology

and 34/42 (80.9%) in those tested by MLPA. Exonic deletions were seen in 33 patients and duplications in four. The majority of exonic deletions i.e., 27/33 (81.8%) were seen in the distal hotspot spanning exons 45–55 and all the duplications were seen in the proximal hotspot involving exons 2–22 [Figure 1]. The commonest single exon deletion was in exon 45 and the commonest multi-exon deletion involved exons 48–50. Two children with negative results on PCR were lost to follow-up. Of the eight children with no mutations detected on MLPA, six underwent targeted exome sequencing. Two patients were detected to have mutations confirming limb-girdle muscular dystrophy (LGMD). The others had DMD; one had a 4 base pair deletion in exon 29 resulting in a frameshift mutation and premature truncation, one had a stop codon in exon 30, one had a single base pair duplication in exon 7, and one had an 11 base pair insertion in exon 55.

Of the 41 patients with mutations detected, 25/41 (60.9%) would have theoretically benefitted from exon-skipping technology. One child who had a nonsense mutation resulting in a stop codon in exon 30 would have theoretically benefitted from stop codon read-through drugs like ataluren. The breakup of the various mutations with potential benefit from exon skipping technologies are elaborated in Table 1.

DMD affects 15.9–19.5/100,000 newborns.^[2] It is caused by mutations in the dystrophin gene which comprises 79 exons and codes for the dystrophin protein. In literature, about 80% of the mutations are exonic deletions and duplications which result in loss of dystrophin. The remaining 20% cause small mutations including nonsense mutations, small insertions, or deletions resulting in a stop codon or frameshift mutations, and premature truncation of the reading frame.^[3]

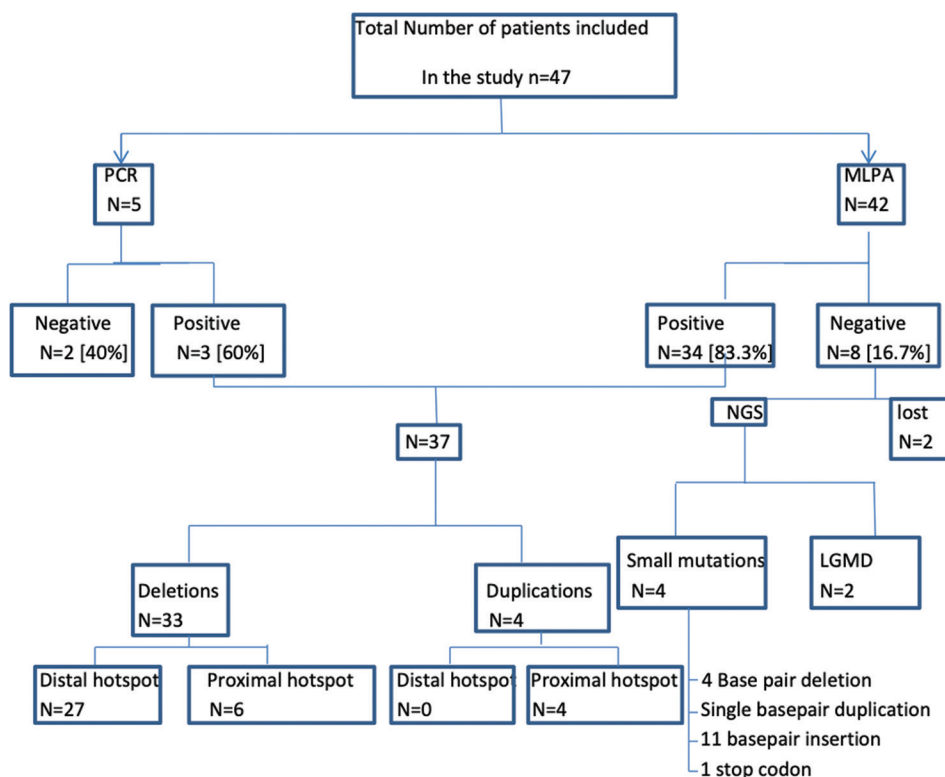


Figure 1: Breakup of mutations identified in children with DMD. (PCR = polymerase chain reaction, MLPA = multiplex ligand-associated probe amplification, NGS = NextGen sequencing)

Table 1: Mutations with potential benefit with exon skipping

Exon skipping ASO	No. who would potentially benefit	Percentage of all mutations	Exonic mutation (number of patients)
51	10	24.4%	48-50 (5), 49-50 (4), 45-50 (1)
53	6	14.6%	49-52 (2), 45-50 (1), 48-52 (1), 49-52 (1), 50-52 (1), 52 (1)
44	4	9.7%	45 (3), 45-54 (1)
45	2		46-47 (1), 46-53 (1)
7	2	4.8%	8-11 (2)
55	1	2.4%	50-54 (1)

Of the 41 patients in whom we identified a genetic mutation in the dystrophin gene, 37 (90.24%) had exonic deletions or duplications and four (9.76%) had small mutations. We also noticed more deletions in the distal hotspot between exons 45–54 and more duplications in the proximal hotspot (exons 2-22) previously documented in other studies from India and elsewhere.^[3,4]

Exon skipping is one of the potential therapies for DMD already in clinical trials. The principle is to mask the splicing signal of an out-of-frame exon so that there is a synthesis of a Becker Muscular Dystrophy (BMD)-like protein. The proof of concept was first documented in lymphoblastic cells

in 1996.^[5] Two ASO’s, namely, 2’OMePS (drisapersen) and PMO (etipirsen) were developed targeting exon 51.

After preclinical trials in animal models the first Phase I clinical trial using intramuscular (IM) injections of drisapersen into the tibias anterior was conducted in four non-ambulant patients with DMD in 2007 and showed dystrophin expression in the muscles.^[6] Since then there have been further trials that have shown some improvement in the 6-min walk test (6MWT) though it has not yet received approval from the Food and Drug Administration (FDA).^[7]

Studies using etipirsen have demonstrated a 40–60% increase in dystrophin in muscles of treated patients and improvements in the 6MWT.^[8] It has now received approval from the FDA under the brand name Exondys 51. The makers of Exondys 51, Sarepta also developed PMO ASOs to treat patients amenable to exon 45 or exon 53 skipping for which they have three clinical trials ongoing (NCT02500381, NCT02310906, and NCT02530905).^[1]

A study evaluating the theoretical possibility of exon skipping for various mutations found that the top ten mutations which would be amenable to skipping were exon 51 (13% of all mutations), 45 (8.1%), 53 (7.7%), 44 (6.2%), 46 (4.3%), 52 (4.1%), 50 (4%), 43 (3.8%), 6 and 7 (3%) similar to our findings.^[9]

Only 1/41 (2.4%) of children fulfilled the criteria for ataluren which is an oral drug approved by the European Union under

the trade name Translarna™ to treat patients with DMD due to stop codon mutations who are still ambulant and above 5 years. It works by interacting with the ribosome which reads through the stop codon and results in a functional protein. Real-world data from an ongoing multicentre registry indicate that ataluren with the standard of care, delays progression of muscle weakness in patients with DMD in routine clinical practise.^[10]

To summarise about 60% of a small cohort of children with DMD from Central India would theoretically be amenable to treatment with exon-skipping technologies.

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Conflicts of interest

There are no conflicts of interest.

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