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Commentary

Base editing in humanized dystrophic mice

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In vivo gene editing and, more recently, base editing have shown great promise in correcting the dystrophin mutations in animal models and patient cells. A recent study published in *Molecular Therapy Nucleic Acids* demonstrates efficient correction of the nonsense human dystrophin mutations in humanized mouse models of Duchenne muscular dystrophy (DMD) by adenine base editing (ABE).[1]

DMD is characterized by the absence of functional dystrophin protein resulting from mutations in the dystrophin gene, leading to progressive muscle wasting, cardiac complications, and premature mortality. Large deletion mutations account for approximately 70% of all DMD cases. The major therapeutic efforts for DMD are centered on restoring functional dystrophin expression. One strategy is to deliver a miniaturized version of dystrophin cDNA, known as micro-dystrophin, through adeno-associated virus (AAV). In 2023, the US Food and Drug Administration (FDA) granted accelerated approval for Sarepta's Elevidys (AAVrh.74 carrying micro-dystrophin cDNA) for patients with DMD who are 4–5 years of age.[2] This represents an important milestone in DMD gene therapy development. Another strategy utilizes antisense oligonucleotides to induce targeted exon skipping and restore the reading frame of the dystrophin gene. Exon skipping converts DMD-associated frame-disrupting mutations into Becker muscular dystrophy-like deletions, which can produce more functional, truncated dystrophin protein. The first exon-skipping drug for *DMD* exon 51 skipping, eteplirsen, received FDA approval in 2016, followed by VyonDys-53 and viltolarsen for exon 53 and AmonDys-45 for exon 45 skipping. More recently, the CRISPR-Cas9 gene editing system has shown great promise to permanently restore the reading frame of the dystrophin gene, as demonstrated by numerous preclinical animal studies and cell culture studies.[3]

While large deletions are the most common cause of DMD, nonsense mutations are estimated to account for approximately 10%–15% cases. Correcting such nonsense mutations could take different strategies, with the potential to restore full-length dystrophin expression. One strategy involves modulating translation termination efficiency through the utilization of pharmacological agents or engineered suppressor tRNAs.[4,5] Ataluren, an oxadiazole compound facilitating ribosomal readthrough of premature stop codons, was approved to treat patients with DMD with nonsense mutations in European and several other countries.

Another highly promising approach leverages the base-editing technology, in particular ABE, to reverse premature stop mutations, offering a potential solution to permanently restore wild-type, full-length dystrophin expression in patients with DMD with nonsense point mutations. This has previously been demonstrated in mouse models of DMD.[6,7] As base editing is highly sequence-context dependent, Jin et al. further explored this therapeutic strategy in humanized mouse models carrying patient-derived nonsense mutations.[1] The authors identified 12 nonsense point mutations in a cohort of 27 patients with DMD. By screening a panel of single guide RNAs (sgRNAs) targeting these mutations using a fluorescence reporter assay in HEK293T cells, they investigated the feasibility of correcting these 12 mutations using SpG-ABE. The efficiency of SpG-ABE-mediated correction varied across different nonsense mutations, with an efficiency reaching up to 80% for some and being marginal for others. SpG-ABE-mediated correction with ∼60% efficiency was confirmed in a human induced pluripotent stem cell (iPSC) model that was generated from a DMD patient with the c.4174C>T mutation in exon 30. After differentiating single corrected iPSC colonies into cardiomyocytes, dystrophin expression was restored to a level comparable to normal cardiomyocytes derived from H9 cells using immunofluorescence staining. Deep sequencing analysis revealed minimal bystander and off-target editing events, demonstrating that the selected sgRNA combined with SpG-ABE can be highly specific to correct human nonsense mutations.

To assess the *in vivo* performance of SpG-ABE/sgRNA, the authors generated two humanized DMD mouse models, each harboring a specific patient-derived nonsense mutation in the dystrophin gene. The split intein version of SpG-ABE was packaged into AAV9 vectors, a commonly used capsid for delivering genes to skeletal and cardiac muscles. Both ubiquitous and muscle-specific promoters were tested to drive ABE expression, with the former exhibiting superior efficiency in undifferentiated patient iPSCs and the latter performing better in animals, suggesting that the selection of promoters can affect the editing outcomes. Intraperitoneal delivery of AAV9 vectors carrying SpG-ABE components resulted in widespread rescue of dystrophin expression in the treated animals. This treatment also reduced serum creatine kinase levels and improved muscle function in DMD mice, as evidenced by improved performance on rotarod and treadmill running tests. Muscle fibrosis was also reduced by SpG-ABE treatment, as revealed by histological analysis.

In comparison to ataluren and exon skipping, which act at the transcript level, *in vivo* base editing directly corrects the

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disease-causing mutations at the chromosomal DNA level, potentially leading to more robust and long-lasting therapeutic benefits. In addition, base editing could also be used to induce permanent exon skipping by targeting the splicing sites or enhancers.[8,9] Besides DMD, base editing has been widely pursued to target many genetic or even non-genetic diseases, including hearing loss, blindness, immunodeficiency, cardiovascular diseases, blood disorders, heart failure, liver diseases, neurodegenerative diseases, and viral infection, among others.[10] Excitingly, the first *in vivo* base-editing clinical trial involving nonviral delivery of ABE targeting *PCSK9* has shown promising initial results for the treatment of heterozygous familial hypercholesterolemia.[11]

Overall, the findings presented by Jin et al.[1] together with previous preclinical and ongoing clinical studies offer compelling evidence for the therapeutic potential of ABE in the treatment of DMD and many other diseases. However, further investigations are warranted to address the challenges associated with *in vivo* base editing, such as AAV delivery efficiency/specificity, off-target effects, host immune responses, and long-term safety. Nevertheless, the rapid advancement in *in vivo* base-editing therapy instills hope among patients and families affected by many currently incurable diseases.

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DECLARATION OF INTERESTS

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