Genome editing

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Mutation corrections in spinal muscular atrophy

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Base editors can restore the expression of survival motor neuron protein to therapeutically beneficial levels in animal and cell models of spinal muscular atrophy.

Spinal muscular atrophy (SMA) is a progressive neuromuscular disorder that is caused by various homozygous loss-of-function mutations in the survival motor neuron 1 (*SMN1*) gene. It affects 1 in 10,000 infants worldwide, with the severity of disease presentation varying according to the copy numbers of the paralogous *SMN2* gene. *SMN2* and *SMN1* share more than 99.9% of their sequence identity, yet they differ by a synonymous C•G-to-T•A substitution at position 6 (C6T) of exon 7 (ref. 1). This mutation changes an exonic splicing enhancer into an exonic splicing silencer, causing alternative splicing of the SMN2 transcript, which results in exon-7 skipping and in the translation of a truncated protein that is rapidly degraded[2] (Fig. 1a, left). Consequently, because the translation of the *SMN2* gene does not ordinarily restore physiological levels of the SMN protein, patients with SMA experience motor neuron loss, paralysis and death at a young age[1].

Therapies for SMA approved by the United States Food and Drug Administration (the small molecule risdiplam, the antisense oligonucleotide nusinersen and the viral-vector-based gene therapy onasemnogene abeparvovec) all increase SMN-protein levels, improving motor function and lengthening the lifespan of patients with SMA[3–5]. Although they are potent, these therapies either use exogenous regulatory mechanisms, require repeated dosing, or do not address disease presentation across all affected tissues. Hence, new treatment methodologies are needed to fully address the complexities of the presentation of SMA. Now, reporting in *Nature Biomedical Engineering*, Christiano Alves, Benjamin Kleinstiver and colleagues describe an adenosine base-editing (ABE) strategy for the correction of the *SMN2* C6T substitution to restore physiological levels of the SMN protein[6]. Their findings confirm and complement a study, authored by David Liu and collaborators and recently reported in *Science*, that used a similar base-editing approach as well as Cas-mediated methods for the disruption of regulatory elements[7]. Together, the two studies highlight the promise of base editing for meaningfully expanding treatment options for patients with SMA.

A previously effective gene-editing strategy for treating SMA involves disrupting the splicing regulatory elements of the *SMN2* gene by using Cas9 nucleases and a single-guide RNA to specifically disrupt an intronic splicing silencer[8]. Although these nuclease-based methods can be effective in vitro[8] and in vivo[9], the double-stranded DNA breaks created by the nuclease can introduce unintended indels, which could have unknown consequences in patients, such as large chromosomal deletions and translocations[9]. Therefore, Alves, Liu and their respective colleagues separately developed base-editing strategies that function without the need for double-stranded breaks. These methods leverage base editors composed of Cas enzymes fused to deaminase domains that can edit specific DNA bases when directed by a targeting guide RNA. By means of rigorous optimization, both research teams designed combinations of base editors and guide RNAs that made use of Cas9 enzymes with relaxed protospacer-adjacent-motif requirements to specifically edit the *SMN2* C6T substitution with limited off-target and bystander effects (Fig. 1a, right). On the one hand, Alves and colleagues engineered the ABE8e base editor with a SpRY Cas9 variant and a guide RNA positioning the target adenosine at position 8 from the protospacer-adjacent motif. They achieved G-to-A editing efficiencies greater than 90% and a concomitant restoration of physiological SMN levels in three patient-derived fibroblast lines[6]. On the other hand, Liu and colleagues engineered a similarly efficient ABE8e-SpyMax base editor with an identically positioned guide RNA that led to an editing efficiency higher than 95%, as well as a larger than 40-fold increase in SMN protein levels (a notable 1.5-fold increase above that of nusinersen) in mouse embryonic stem cells with the truncated SMN protein[7]. The research teams also assessed off-target editing levels, with Alves and colleagues finding that 2 of 34 putative off-target sites were edited by ABE8e-SpRY in patient-derived fibroblasts[6], and Liu and colleagues observing in human embryonic kidney (HEK293T) cells that 9 of 23 putative off-target sites were edited by the ABE8e-SpyMax system[7]. These observations are in line with the expected dependence of off-target editing on cell type; in fact, Alves and co-authors measured 19 edits out of 24 potential off-target sites in HEK293T cells when using their ABE8e-SpRY construct[6].

Both sets of authors investigated the translational potential of their base-editing strategies in an SMA mouse model that expresses the truncated SMN protein and exhibits disease phenotypes at a young age. They used intein-mediated dual adeno-associated virus 9 (AAV9) vectors with the optimized base editors split between the two vectors and delivered through intracerebroventricular (ICV) injection (Fig. 1b). Alves and colleagues performed ICV injections in neonatal mice at the pre-symptomatic stage, and observed A-to-G editing efficiencies 12 days later of approximately 6% and 4% in the animals' brain and spinal cord, respectively; when injecting 13-day-old heterozygous mice, these numbers increased respectively to about 10% and 8% at 12 weeks post-injection. Interestingly, they also investigated the administration of their base-editing systems via simultaneous ICV and intravenous (IV) injection (Fig. 1b). This strategy led to levels of editing in the brain and spinal cord that were comparable to those of ICV injection alone; however, simultaneous ICV and IV injection resulted in substantially higher editing in the liver and the heart. In fact, this injection strategy led to detectable levels of SMN protein in the liver, to an overall improvement in motor function, and to a modest increase in survival when compared to untreated mice (Fig. 1b)[6]. In the study by Liu and co-authors, 25 weeks after ICV injection in newborn mice the authors measured an approximately 37% editing efficiency in cortical nuclei, as well as improved motor function and an

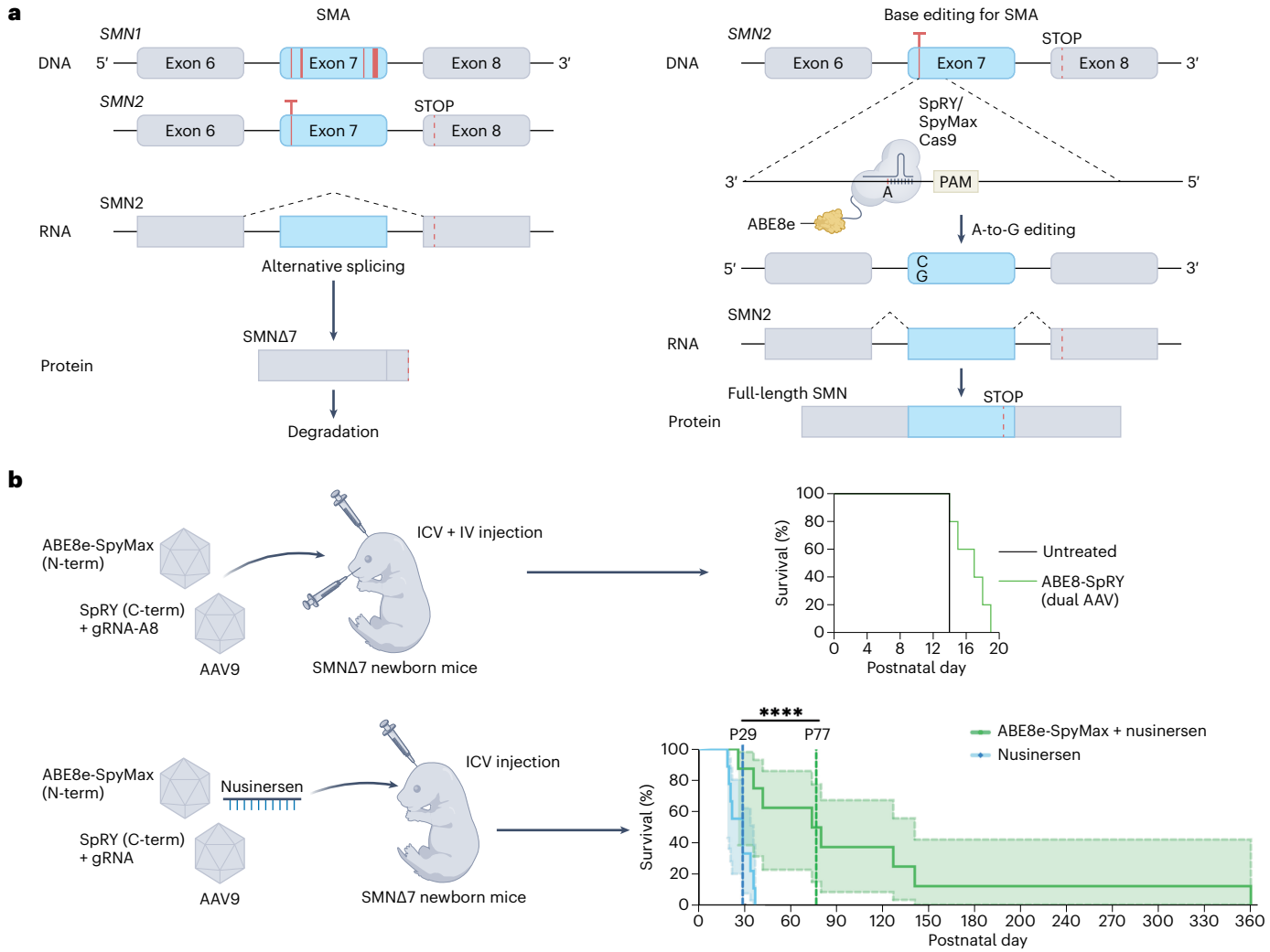

Fig. 1 | Base editors for the treatment of SMA. a, Left: the genotype of SMA. *SMN1* carries mutations that render the protein translated from it non-functional. The nearly identical *SMN2* gene carries a substitution (at position 6 of exon 7) that leads to alternative splicing of the pre-mRNA molecule and to the translation of the RNA into a truncated SMN protein (SMNΔ7) that is rapidly degraded. Right: ABE can be used to edit the exon-7 substitution in *SMN2* via a SpRY or SpyMax Cas9 fused to the ABE8e deaminase domain and paired with a single-guide RNA that places the target adenosine (A) at position 8 from the protospacer-adjacent motif (PAM). A-to-G editing led to wild-type splicing of the pre-mRNA molecule and to the production of functional full-length SMN. **b**, In vivo strategies used by Alves and co-authors[7] (top) and Liu and co-authors[8] (bottom). Both teams used a split-intein dual AAV9 vector as a delivery system to administer their optimized base editors (ABE8e-SpRY for Alves and colleagues, and ABE8e-SpyMax for Liu and colleagues) and guide RNAs to newborn mice expressing the truncated SMN protein. Alves and co-authors performed simultaneous ICV and IV injections, and observed a modest survival benefit[6]. Liu and co-authors used IV injection in combination with the regulatorily approved therapy nusinersen and observed a substantial survival benefit (green line) over that of nusinersen alone[8] (blue line). The shadings represent the 95% confidence interval, and P29 and P77 denote post-natal days 29 and 77, respectively. ****$P < 0.001$. The survival plot in the top right is adapted from ref. 6, Springer Nature Ltd and the survival plot in the bottom right is adapted with permission from ref. 7, AAAS.

overall survival benefit in these mice. These authors also evaluated the timing of the slower-acting AAV-based approaches both alone and in combination with the faster-acting splice-switching drug nusinersen. This combinatorial treatment outperformed nusinersen alone, both in terms of motor function and survival benefit (Fig. 1b), which brings much-needed attention to the importance of therapeutic timing for patients with SMA, as the timing of symptomatic onset differs across patients. With the promise of new genome-engineering tools that preserve native transcript levels and that use endogenous regulatory elements comes the necessity of thoughtfully designed clinical trials that assess the efficacies of these tools when administered on their own and in combination with existing therapeutics.

The two studies highlight the utility of performing meticulous optimizations in physiologically relevant cell types in order to refine a system for maximal therapeutic benefit in vivo. Although the investigators highlight minimal off-target editing and inconsequential bystander edits in their systems partly because of their optimizations, additional long-term studies will be essential to attain an optimal

balance between editing specificity and editing efficiency. Lessons from more advanced gene-therapy applications of base editing, such as those for Hutchinson–Gilford progeria syndrome[8] and neurodegenerative ataxias[9], will be critical for the successful translation of base editing for the treatment of SMA.

Furthermore, as genome-engineering tools continue to generate improved editing efficiencies with limited off-target consequences, the need for equally efficient delivery vehicles continues to grow. Liu and colleagues report up to 43% transduction efficiency in spinal motor neurons following ICV injection of AAV9, with substantial editing also present in the liver, heart and other peripheral organs[7]. As restoration of SMN levels in these peripheral tissues is essential[10], additional capsid engineering and transgene-expression efforts, alternative or combinatorial routes of administration such as those used by Alves and colleagues, and careful titration of the administered doses could all prove essential to achieving maximal therapeutic benefit[11]. Recent evidence has also highlighted the importance of species-specific and cell-type-specific epigenetic interactions with recombinant AAV genomes and the role that various vector modifications have on the longevity of protein expression, and thus on the therapeutic benefit for the patient[12]. With this in mind, translational studies should not be limited to AAV-mediated delivery, especially in the case of base editing for SMA, where a split-intein dual-AAV strategy is required. The delivery strategy can have profound implications for specificity and efficiency, and we anticipate that viral vectors (AAVs, adenoviruses and retroviruses) and non-viral vectors (gold nanoparticles, viral-like particles, lipid nanoparticles and polymers) will be evaluated in due course[13].

With developments in base editing continuing to expand the genomic toolkit for treating genetic disorders, it is exciting to think about the various therapeutic possibilities. Although editing the C6T substitution was the primary in vivo strategy in the studies by Alves, Liu and their respective colleagues, both teams also explored promising in vitro strategies targeted at other splicing regulatory elements. Alternative approaches, such as artificial splicing factors[14] and RNA editing via adenosine deaminases acting on RNA[15], could be effective for the modulation of splicing in SMA and beyond. As knowledge of SMA phenotypes, their molecular underpinnings, and the complexities of genome-engineering tools continue to expand, the treatment options for patients with SMA will only broaden.

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Competing interests

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