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TANGO With SCN1A: Can This Molecular Dance Defeat Dravet Syndrome?

Antisense Oligonucleotides Increase Scn1a Expression and Reduce Seizures and SUDEP Incidence in a Mouse Model of Dravet Syndrome

Han Z, Chen C, Christiansen A, et al. *Sci Transl Med*. 2020;12(558):eaaz6100. doi:10.1126/ scitranslmed.aaz6100 PMID: 32848094.

Dravet syndrome (DS) is an intractable developmental and epileptic encephalopathy caused largely by de novo variants in the *SCN1A* gene, resulting in haploinsufficiency of the voltage-gated sodium channel α subunit $Na_V1.1$. Here, we used Targeted Augmentation of Nuclear Gene Output (TANGO) technology, which modulates naturally occurring, nonproductive splicing events to increase target gene and protein expression and ameliorate disease phenotype in a mouse model. We identified antisense oligonucleotides (ASOs) that specifically increase the expression of productive *Scn1a* transcript in human cell lines, as well as in mouse brain. We show that a single intracerebroventricular dose of a lead ASO at postnatal day 2 or 14 reduced the incidence of electrographic seizures and sudden unexpected death in epilepsy in the F1:129S-*Scn1a*$^{+/-}$ $\times$ C57BL/6J mouse model of DS. Increased expression of productive *Scn1a* transcript and $Na_V1.1$ protein was confirmed in brains of treated mice. Our results suggest that TANGO may provide a unique, gene-specific approach for the treatment of DS.

Commentary

Developmental and epileptic encephalopathies (DEEs) are severe disorders characterized by onset of intractable seizures in infancy or early childhood along with developmental and cognitive impairments. Mortality is increased in DEE, including elevated risk of sudden unexpected death in epilepsy. Dravet syndrome (DS) is a DEE caused by haploinsufficiency of the voltage-gated sodium channel gene *SCN1A*, which encodes the sodium channel alpha subunit $Na_v1.1$. Treatment options for DS are limited. Genetic approaches, including traditional gene therapy and CRISPR-aided approaches, have begun to be explored as more precise treatments of genetic disorders like DS. Han et al[1] report a new treatment strategy for DS that works via modulation of alternative splicing of the *SCN1A* gene.

Many genes are regulated to some extent by alternative splicing. The *SCN1A* genome sequence includes an alternative exon, exon 20N, that encodes a premature termination codon and initiates nonsense-mediated decay of the transcript.[2] Thus, transcripts that include exon 20N are nonproductive. These types of exons that result in nonproductive transcripts via alternative splicing are also called "poison exons." Normally, these nonproductive transcripts only account for a small percentage of *SCN1A* transcripts. In some cases, pathogenic variants that promote inclusion of exon 20N have been identified in individuals with DS,[3] demonstrating that transcripts containing exon 20N are nonproductive and lead to *SCN1A* haploinsufficiency. Poison exons are not a rare phenomenon. More than 1000 disease-associated genes with at least one nonproductive alternative splicing event have been identified.[4]

Because reduced *SCN1A* gene expression is a significant cause of DS, a logical potential treatment could be restoration of normal levels of *SCN1A* gene expression. One approach to elevating gene expression involves antisense oligonucleotides (ASOs) that target the *SCN1A* mRNA transcript. Antisense oligonucleotides are small, single-stranded nucleic acid molecules designed to bind directly to the mRNA transcript of the targeted gene. Antisense oligonucleotides are chemically modified to improve stability and cellular uptake and to lessen cytotoxicity. One type of ASO is called a gapmer, which has been only partially modified. When the gapmer binds its mRNA target, the unmodified section of the ASO creates a section of double-stranded RNA that catalyzes degradation of the transcript by RNase H. Gapmers are useful for downregulating RNA expression and have been used successfully in mouse models of epilepsy caused by gain-of-function mutations, such as *SCN8A* encephalopathy.[5] However, for DS, a different type of ASO that is fully modified can be employed. When the ASO binds to *SCN1A* mRNA, it will not catalyze degradation of the transcript. Instead, the ASO has been

designed to bind to the poison exon 20N to prevent its inclusion into the transcript. In this way, the proportion of productive *SCN1A* transcripts will be increased, presumably leading to more functional $Na_v1.1$ sodium channels.

Lori Isom's group at University of Michigan teamed up with Stoke Therapeutics to test this ASO approach utilizing a system called TANGO (Targeted Augmentation of Nuclear Gene Output). Candidate ASOs were screened in cell culture for their ability to elevate expression of *SCN1A*. The most active ASO elevated productive *SCN1A* transcript by greater than 15-fold. This ASO did not affect expression of the highly homologous sodium channel genes *SCN2A*, *SCN3A*, *SCN8A*, or *SCN9A* and was selected for further study in mice. Wild-type mice were given various doses of ASO via single intracerebroventricular injection at postnatal day 2. Five days after treatment, productive *SCN1A* transcripts increased in a dose-dependent manner, up to a maximum of 6-fold above baseline levels. Importantly, $Na_v1.1$ protein levels also increased. Elevated *Scn1a* expression was observed for at least 30 days posttreatment, with up to 25-fold increase in productive *Scn1a* transcript and approximately 30% increase in $Na_v1.1$ protein levels compared to untreated adult mouse brain. The ASO injection was then tested in the $Scn1a^{+/-}$ mouse model of DS. Untreated $Scn1a^{+/-}$ mice exhibited approximately 70% mortality by 30 days of age. However, the $Scn1a^{+/-}$ mice treated with ASO at P2 were almost completely protected, with 97% of mice surviving up to 90 days of age. $Scn1a^{+/-}$ mice treated at P14, a time point closer to seizure onset in this model, exhibited 85% survival up to 90 days of age compared to 64% survival of untreated $Scn1a^{+/-}$ mice. Thus, the ASO treatment significantly prolonged survival in a mouse model of Dravet syndrome. Importantly, the study also quantified the effect of ASO on seizure frequency. $Scn1a^{+/-}$ mice given ASO or PBS (phosphate-buffered saline) at P2 were monitored by electroencephalography from postnatal day 22 to 46. Antisense oligonucleotide–treated mice had significantly fewer seizures and a longer latency to first seizure than their PBS-treated littermates. This study provides compelling preclinical evidence that precision therapy targeting haploinsufficiency of *SCN1A* could be a promising treatment for DS.

Several genetic approaches to modification of *Scn1a* expression are currently being studies for use in treatment of DS. For example, a viral-mediated strategy that delivers nuclease-deficient Cas9 along with guide RNAs targeting the *SCN1A* promoter was able to ameliorate febrile seizures in a mouse model of DS.[6] Features of ASO treatment that may be advantageous compared to viral-based strategies are (1) ASO therapy is not dependent on gene size and (2) ASO administration would not be predicted to provoke an immune response. Delivery of ASOs can be achieved through lumbar puncture, a strategy which has proven successful in trials for spinal muscular atrophy (SMA)[7] and Huntington disease.[8]

However, outstanding questions remain about timing and long-term consequences of ASO treatment. In their study, Han et al treated mice close to onset (P14) but did not treat mice after onset of seizures. They also did not follow mice past 90 days of age. When would treatment need to be administered to have a meaningful effect? Is there a therapeutic window beyond which elevating *SCN1A* expression would no longer be able to quiet the epileptic circuitry in the brain? Would multiple or lifelong treatment be necessary to maintain the therapeutic effect? What are the long-term adverse effects of ASO treatment? In addition, Han et al do not report whether cognitive or behavioral comorbidities were improved in ASO-treated $Scn1a^{+/-}$ mice. Despite these issues, the efficacy of ASOs against seizures in mouse models of DEE and the successful delivery and modulation of target gene expression in clinical trials for SMA and Huntington disease provide hope for precision treatment of severe genetic epilepsies.

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