TANGO – Targeted Augmentation of Nuclear Gene Output for the Treatment of Dravet Syndrome
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ABSTRACT

Results: Dravet syndrome is a severe pediatric epileptic encephalopathy characterized by high frequency, daily seizures and severe intellectual disability, and a high rate of sudden unexpected death in epilepsy (SUDEP). The majority of Dravet syndrome patients carry de novo mutations in SCN1A leading to haploinsufficiency. Currently, there are no disease-modifying, targeted therapies to treat Dravet syndrome. We hypothesized that restoration of Na1.1 physiological levels in patients would reduce or prevent seizures, decrease the risk of SUDEP and potentially improve cognitive development. Here, we tested a novel therapeutic approach utilizing antisense oligonucleotides (ASOs), an established and FDA-approved therapeutic modality, to increase the endogenous expression of Scn1a in a Scn1a Knockout (KO) Dravet syndrome mouse model. The model recapitulates many key features of the human disease, including severe seizures, developmental delay, severe cognitive impairment, and cardiotoxicity.

Methods: We employed Targeted Augmentation of Nuclear Gene Output (TANGO), which entails selectively-activating non-productive splicing events to drive expression of a target protein expression cassette. TANGO operates in a mutation-independent manner and does not alter protein coding splicing mechanisms to achieve its goal. We identified an alternatively spliced exon 20 cassette, mScn1a20, which we confirmed to produce sodium channel protein termination codons and the generation of a non-productive mRNA. We designed ASOs to target this non-proliferation antagonist splice variant and tested them in vivo and in vitro.

Results: Identified ASOs significantly increased the expression of Scn1a in different human neuronal progenitor cells and in disease-relevant mouse models, with a marked increase in expression of Scn1a from a single, bona fide ICV injection of the lead ASO in mice and a single dose administration of ASO to neonates of Scn1a KO mice, with a marked increase in protein expression of Scn1a as well as Na1.1 protein. Time course experiments in neonatal and adult wildtype mice showed a sustained increase in Scn1a expression from a single, bona fide ICV injection of the lead ASO maintained through 26 days. A single ICV injection of the lead ASO at postnatal day P12 or with injections at P0 and P12 in Scn1a KO mice on P12 (n=4) dramatically increased the expression of Na1.1 and Scn1a mRNAs in the brain and restored physiological levels of Na1.1 channel protein expression in neurons in vivo. ASOs selectively upregulated Scn1a mRNA but did not other sodium channel mRNAs.

Conclusions: These results indicate that TANGO technology, can be used to drive expression in a mouse model of Dravet Syndrome and may provide gene-specific, disease-modifying approach to reverse physiological Na1.1 levels to prevent seizure and SUDEP in patients.

RESULTS

A single dose of ASO administered at P2 rescues SUDEP in 99% of Scn1a Flm/KO Dravet syndrome mice

A single dose of ASO increases Scn1a mRNA and Nav1.1 protein in Dravet syndrome mice

F1 [129S-Scn1aFlm x C57Bl/6J] males and females

*ASO treatment significantly improved survival in Scn1a Flm/D mice (p<0.0001)

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SUMMARY & CONCLUSIONS

• TANGO exploits non-productive pre-mRNA splicing to address Dravet syndrome caused by SCN1A haploinsufficiency.
• We demonstrated the potential of this approach by testing this concept in human neuronal progenitor cells, wild-type C57Bl/6J mice, and Scn1a Flm/KO Dravet syndrome mice.
• We identified sequences that decrease inclusion of the nonsense-mediated mRNA decay (NMD) inducing exon and increase productive mRNA.
• The in vitro potency of the lead ASO was evaluated and calculated to have an EC50 of 167 nM (not shown).
• The selected ASO is active in vivo in restoring Scn1a mRNA and Nav1.1 protein levels in a mouse model of Dravet syndrome.
• A single dose of ASO administered at P2 rescues SUDEP in Dravet syndrome mice.

We have validated a unique approach that can potentially address Dravet syndrome caused by SCN1A haploinsufficiency. Restoration of expression via this approach could be regulated under the endogenous SCN1A promoter. This approach may translate to a better safety profile as only cells that already express SCN1A can increase its expression in response to ASO treatment. Furthermore, delivery of ASO to the CNS requires established technique and does not require complex delivery systems. Finally, unlike AAV-based gene therapy, the size of the mRNA is not a constraint via this approach.