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

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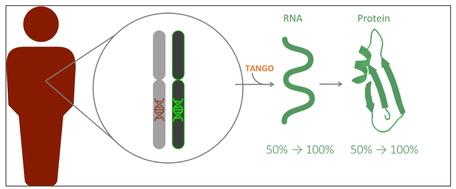
ABSTRACT

Rationale: Dravet syndrome is a severe pediatric epileptic encephalopathy characterized by high seizure frequency and severity, intellectual disability, and a high risk of sudden unexpected death in epilepsy (SUDEP). The majority of Dravet syndrome patients carry *de novo* mutations in *SCN1A* leading to haploinsufficiency of the voltage-gated sodium channel α subunit Nav1.1. Currently, there are no disease-modifying, targeted therapeutics to treat Dravet syndrome. We hypothesized that restoration of Nav1.1 physiological levels in patients should reduce or prevent seizures, decrease the risk of SUDEP, and potentially improve cognitive development. Here, we test a novel therapeutic approach using antisense oligonucleotides (ASOs), an established and FDA-approved therapeutic modality, to increase the endogenous expression of *Scn1a* in a *Scn1a*$^{+/-}$ Dravet syndrome mouse model. The model recapitulates many patient phenotypes, including severe seizures, developmental delay, ataxia, sleep disorders, and SUDEP.

Methods: We employed Targeted Augmentation of Nuclear Gene Output (TANGO), which exploits naturally-occurring non-productive splicing events to increase target protein expression via modulation of splicing. TANGO operates in a mutation-independent manner and does not alter protein coding splicing isoforms to achieve its goal. We identified an alternatively spliced exon conserved in human and mouse *SCN1A* that leads to the incorporation of a premature termination codon and the generation of a non-productive mRNA. We designed ASOs to target this non-productive alternatively spliced exon and tested them *in vitro* and *in vivo*.

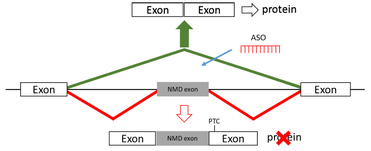
Results: Identified ASOs significantly increased the expression of *SCN1A* in cultured human neural-progenitor cells and in differentiated neurons with no effect on the expression of other voltage-gated sodium channel genes. This increase in *SCN1A* expression resulted from a gene-specific reduction in non-productive mRNA and an increase in productive mRNA. Intracerebroventricular (ICV) injection of the lead ASO in neonate and adult C57BL/6J wildtype mice yielded a substantial, dose-dependent increase in *Scn1a* mRNA as well as Nav1.1 protein. Time course experiments in neonate and adult wildtype mice showed a sustained increase in *Scn1a* expression from a single, bolus ICV injection of the lead ASO monitored through 80 days. A single ICV injection of the lead ASO at postnatal day (P) 2 in F1:129S x C57BL/6J *Scn1a*$^{+/-}$ Dravet syndrome mice prevented generalized seizures and SUDEP in 99% (79 of 80) of *Scn1a*$^{+/-}$ mice tested, with a subset of mice monitored through ~90 days of development. In contrast, approximately 50% of littermate *Scn1a*$^{+/-}$ mice treated with a control ASO seized and died. There were no deleterious effects on 101 ASO-treated littermate *Scn1a*$^{+/+}$ mice tested, with a subset of mice monitored through ~90 days of development. A smaller cohort of Dravet syndrome mice (5 *Scn1a*$^{+/-}$ and 1 *Scn1a*$^{+/+}$) received a single ICV injection of the lead ASO at P14. We have monitored these animals through ~45 days and found that generalized seizures and lethality were rescued in 100% of the *Scn1a*$^{+/-}$ mice with no deleterious effects on the *Scn1a*$^{+/+}$ mouse. A larger cohort of mice injected at P14 is being monitored.

Conclusions: These results indicate that TANGO technology can be used to rescue a mouse model of *Scn1a*-linked Dravet syndrome and may provide a gene-specific, disease-modifying approach to restore physiological Nav1.1 levels to prevent seizures and SUDEP in patients.



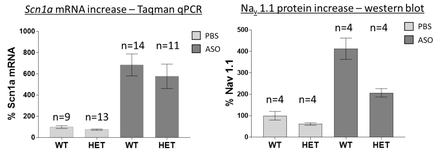
RESULTS

Antisense oligonucleotides prevent non-productive splicing and increase productive *Scn1a* mRNA and Nav1.1 protein



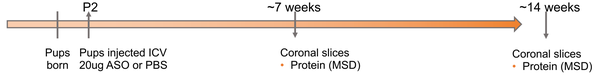
A single dose of ASO increases *Scn1a* mRNA and Nav1.1 protein in Dravet syndrome mice

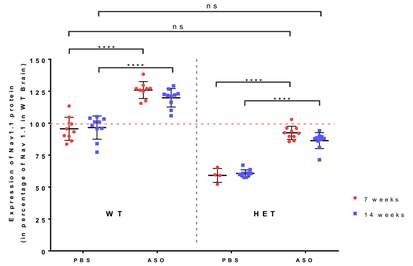
- F1[129S-*Scn1a*^{tm1Kea} x C57BL/6J], P2
- N=9-14 per group
- Single dose, 20 μg, ICV
- Total RNA and protein extracted and analyzed



A single dose of ASO restores Nav1.1 to WT levels in *Scn1a*$^{+/-}$ Dravet syndrome mice

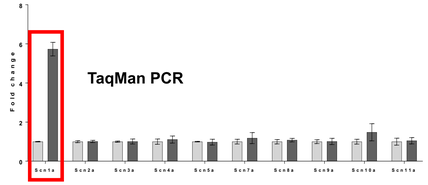
Study Design:





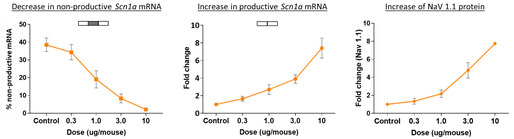
F1 [129S-*Scn1a*^{tm1Kea} x C57BL/6J] males and females
ASO treatment increased Na$_V$1.1 protein levels in +/+ and +/- brains vs. PBS at both timepoints (p<0.0001).

ASO selectively upregulates *Scn1a* mRNA but not other sodium channel mRNAs



ASO increases *Scn1a* mRNA and Nav1.1 protein *in vivo*

- C57BL/6J mice, postnatal day (P)1
- N=3-6/group (mRNA), N=2/group (protein)
- Single ASO dose: 0.3 μg, 1.0 μg, 3.0 μg, or 10 μg
- Total RNA extracted from coronal brain slices
- Membrane protein prepared from cortex



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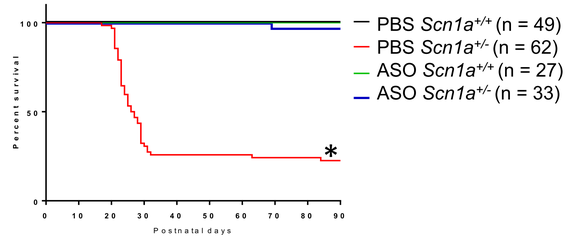
- Total RNA extracted from coronal brain slices
- Membrane protein prepared from cortex

A single dose of ASO administered at P2 rescues SUDEP in 99% of *Scn1a*$^{+/-}$ Dravet syndrome mice



F1 [129S-*Scn1a*^{tm1Kea} x C57BL/6] males and females
***ASO treatment significantly improved survival in *Scn1a*$^{+/-}$ DS mice (p<0.0001)**

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 neural progenitor cells, wild-type C57BL/6J mice, and *Scn1a*$^{+/-}$ 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 retains regulation 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 is an 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.