Targeted Augmentation of Nuclear Gene Output (TANGO) of *Scn1a* Prevents SUDEP in a mouse model of Dravet Syndrome

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1. Summary

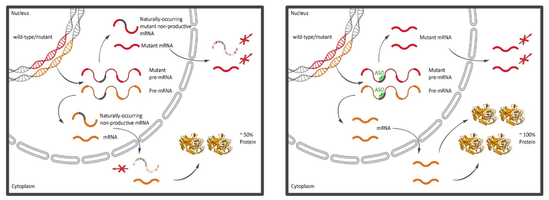
- Dravet syndrome (DS) is a severe developmental and epileptic encephalopathy characterized by high seizure frequency and severity, intellectual disability, and a high risk of sudden unexpected death in epilepsy (SUDEP). The majority of DS patients carry de novo mutations in *SCN1A* leading to haploinsufficiency of the voltage-gated sodium channel α subunit $Na_V1.1$.
- We have developed a novel therapeutic approach to treat DS using STK-001, an antisense oligonucleotide (ASO), to increase the endogenous expression of *SCN1A* mRNA and $Na_V1.1$ protein by inhibiting generation of a splice variant transcript that contains a premature termination codon leading to degradation by nonsense mediated mRNA decay (NMD).
- The current studies test this approach using the *Scn1a*^{tm1Kea}, F1:129S-*Scn1a*+/- x C57BL/6J DS mouse model (DS mouse) that has been shown previously to recapitulate many phenotypes of DS including severe seizures and SUDEP (Miller et al, 2014). Efficacy of STK-001 in this DS mouse was evaluated by quantification of spontaneous seizure by electroencephalography (EEG) and survival monitoring.
- These results provide evidence that TANGO technology can be used to rescue both the seizure and survival phenotypes in a mouse model of *Scn1a*-linked DS and has the potential to provide a gene-specific, disease-modifying treatment to restore $Na_V1.1$ to physiological levels to provide therapeutic benefits for DS patients.

Miller AR1, Hawkins NA, McCollom CE, Kearney JA. Genes Brain Behav. 2014 Feb;13(2):163-72.

2. TANGO (Targeted Augmentation of Nuclear Gene Output)

TANGO uses ASOs to specifically increase protein expression by targeting naturally-occurring non-productive alternative splicing events. TANGO reduces non-productive messenger RNAs (mRNA), which are normally targeted for degradation by nonsense-mediated mRNA decay (NMD) as shown in Figure 1. In turn, TANGO increases productive mRNA and protein. TANGO specifically increases expression of canonical target mRNA and full-length protein, only in tissues with endogenous gene expression. As these events are naturally-occurring, TANGO can upregulate the wild-type alleles in the context of autosomal dominant haploinsufficiency, thus providing a potentially unique opportunity to treat these diseases.

Figure 1. TANGO Mechanism



3. Experimental Design and Methods

STK-001 Administration

Mice were administered a single intracerebroventricular (ICV) injection of STK-001 or vehicle (PBS) at postnatal day (PND)2 or PND14.

Efficacy endpoints

Survival: Mice were monitored for survival out to PND90.

Seizure: Mice are implanted with an 8201-EEG Headmount (Pinnacle Technology, Inc., Lawrence, KS) and seizure activity was continuously monitored from PND22-PND46.

Analytical Methods

STK-001 Measurement: STK-001 levels were measured by Liquid Chromatography Mass Spectrometry (LCMS).

Scn1a gene expression measurement: Productive *Scn1a* mRNA transcripts were measured using Taqman qPCR assays.

$Na_V1.1$ Protein measurement: $Na_V1.1$ protein was measured using a Mesoscale Discovery electrochemiluminescence (MSD-ECL) assay which utilizes $Na_V1.1$ expression in wild-type mouse brain tissue lysates as a standard.

4. STK-001 administration at PND2 prevents SUDEP in the DS mouse model

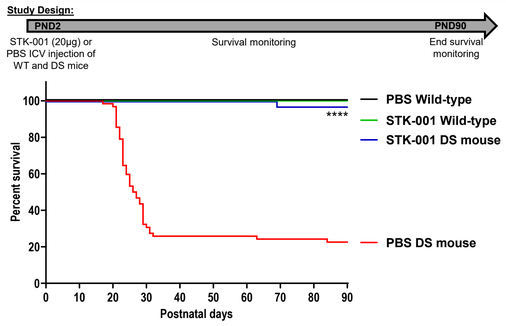


Figure 2. Percent survival of DS and WT mice after a single ICV injection of STK-001 or PBS on PND2
Kaplan-Meier curve showing DS and WT littermate mice monitored to PND90 for survival. Administration of STK-001 significantly ($p<0.0001$) improved survival in the Dravet mouse model 33/34 survived up to PND90, compared with 14/62 animals in PBS-treated group. **** = $p<0.0001$

5. STK-001 administration at PND2 significantly reduces spontaneous seizures in the DS mouse model

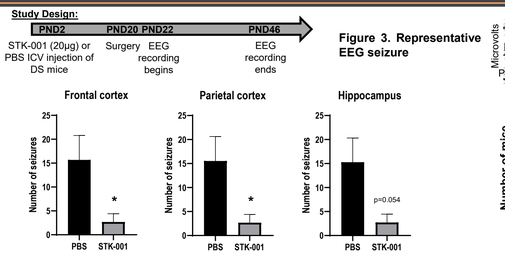


Figure 4. Seizures recorded between PND22-46 after a single ICV injection of STK-001 or PBS
STK-001 significantly reduced the number of spontaneous seizures recorded in DS mice between PND22 and PND46. In all mice, seizures generalized with a few exceptions. * = $p<0.05$

Figure 3. Representative EEG seizure

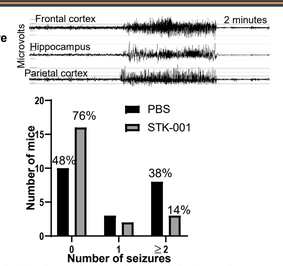


Figure 5. Number of mice that had 0, 1, or 2 or more seizures
The number of seizure-free mice increased after STK-001 administration at PND2, while the number of mice having 2 or more seizures decreased compared to control. Wild-type mice had no seizures regardless of treatment (data not shown).

6. STK-001 administration at PND2 results in lasting drug exposure and increases in *Scn1a* and $Na_V1.1$ protein expression in a DS mouse model

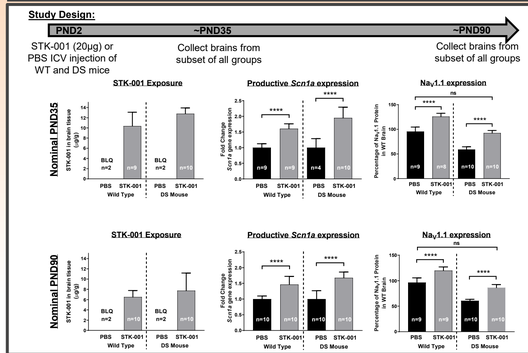


Figure 7. Exposure, *Scn1a* expression, and $Na_V1.1$ expression in brain tissues at ~PND35 and ~PND90 after a single ICV injection of STK-001 or PBS on PND2
Brain tissues had robust and long lasting exposure to STK-001 (~13 µg/g, ~PND35 and ~8 µg/g, ~PND90).

Expression levels of *Scn1a* productive transcript were increased after STK-001 administration ~1.5-2 fold in both WT and DS mice at ~PND35 and ~PND90.

$Na_V1.1$ protein increased in STK-001 treated DS mouse brains to levels indistinguishable from PBS-treated WT brains at ~PND35 and ~PND90. $Na_V1.1$ protein in the brains of STK-001 injected WT animals also increased over PBS-treated WT mice with no obvious adverse effects.

**** = $p<0.0001$, ns = non-significant

7. STK-001 administration at PND14 significantly reduces SUDEP in the DS mouse model

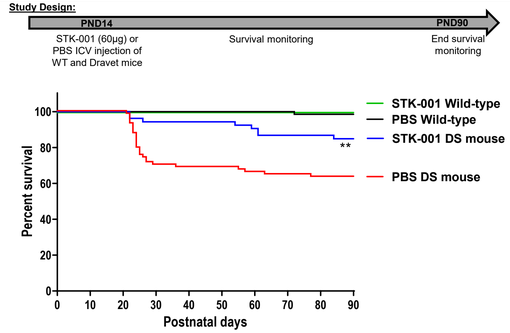


Figure 8. Percent survival of DS and WT mice after a single ICV injection of STK-001 or PBS on PND14
Kaplan-Meier curve showing survival to PND90 of DS and WT littermate mice following dosing at PND14 (around time of disease onset in the model). Administration of STK-001 significantly ($p < 0.005$) improved survival in the DS mouse model 45/53 survived up to PND90, compared with 47/74 PBS-treated group. ** = $p<0.005$

8. STK-001 administration at PND14 results in lasting drug exposure and increases in *Scn1a* and $Na_V1.1$ protein expression in a DS mouse model

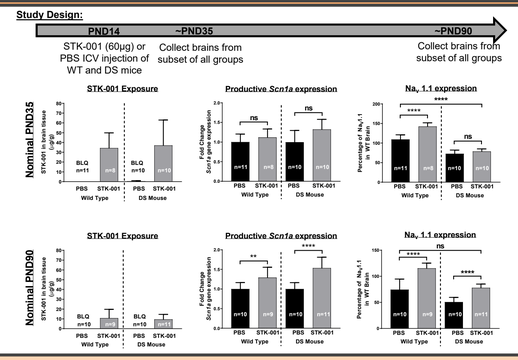


Figure 9. Exposure, *Scn1a* expression, and $Na_V1.1$ expression in brain tissues at ~PND35 and ~PND90 after a single ICV injection of STK-001 or PBS on PND14
Brain tissues had robust and long lasting exposure to STK-001 (~36 µg/g, ~PND35 and ~10 µg/g, ~PND90).

Expression levels of *Scn1a* productive transcript were increased after STK-001 administration ~1.5 fold in both WT and DS mice at ~PND90.

$Na_V1.1$ protein increased in STK-001 treated DS mouse brains to levels indistinguishable from PBS-treated WT brains at ~PND90. $Na_V1.1$ protein in the brains of STK-001 injected WT animals also increased over PBS-treated WT mice with no obvious adverse effects.

**** = $p<0.0001$, *** = $p<0.001$, ns = non-significant

9. Conclusions and Next Steps

Conclusions

- STK-001 treatment at PND2 significantly reduces spontaneous electroencephalographic seizures and SUDEP in the DS mouse model.
- STK-001 treatment around the time of disease phenotype onset (PND14) significantly reduces SUDEP in the DS mouse model.
- Collectively, the current studies demonstrate efficacy using the TANGO approach to selectively increase *Scn1a* gene expression and restore $Na_V1.1$ protein expression back to wild type levels in a DS mouse model.
- STK-001 has the potential to provide a gene-specific, disease-modifying treatment to restore $Na_V1.1$ to physiological levels and to provide therapeutic benefits for DS patients.

Next Steps

The current preclinical pharmacology studies strongly support continued development of STK-001 for treatment of Dravet Syndrome patients.