Targeted Augmentation of Nuclear Gene Output (TANGO) of *SCN1A* reduces seizures and rescues parvalbumin positive interneuron firing frequency in a mouse model of Dravet syndrome

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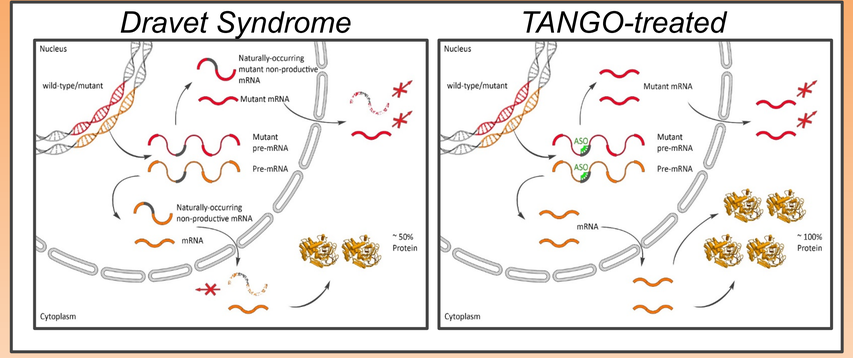
Summary

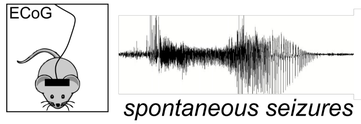
- Dravet syndrome (DS) is severe and progressive genetic epilepsy characterized by frequent, prolonged, and refractory seizures, beginning the first year of life. Cognitive regression, ataxia, speech impairment, sleep disturbances and an increased risk of sudden unexpected death in epilepsy are other aspects of the disease. Approximately 85% 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) (Zhou et al, 2020).
- The current studies test this approach using the *Scn1a*^{tm1Kea}, F2:129S-*Scn1a*+/- x C57BL/6J DS mouse model (DS mouse) that has been shown previously to recapitulate many phenotypes of DS (Miller et al, 2014). We evaluated the effects of STK-001 in this model by quantification of spontaneous seizure by electroencephalography (EEG) pre (P13-19) and post (P20-40) weaning. In addition, this model was crossed with mice hemizygous for a parvalbumin (PV)-tdTomato fluorescent reporter to produce WT and DS mice expressing the tdTomato specifically in PV expressing interneurons. Electrophysiological recordings were taken from tdTomato-expressing cells in the somatosensory cortex between P17-23.
- These results provide evidence that TANGO technology can be used to rescue the seizure phenotypes in a mouse model of *Scn1a*-linked DS. Taken together, these data provide further evidence that STK-001 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.

TANGO (Targeted Augmentation of Nuclear Gene Output)

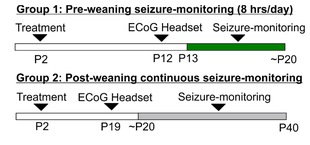


Approach

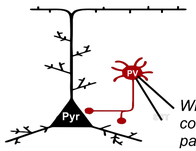
1. *Seizure monitoring*

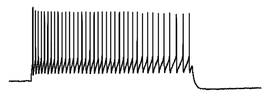


spontaneous seizures

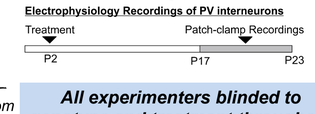


2. *Parvalbumin (PV) Interneuron Neuronal Excitability*



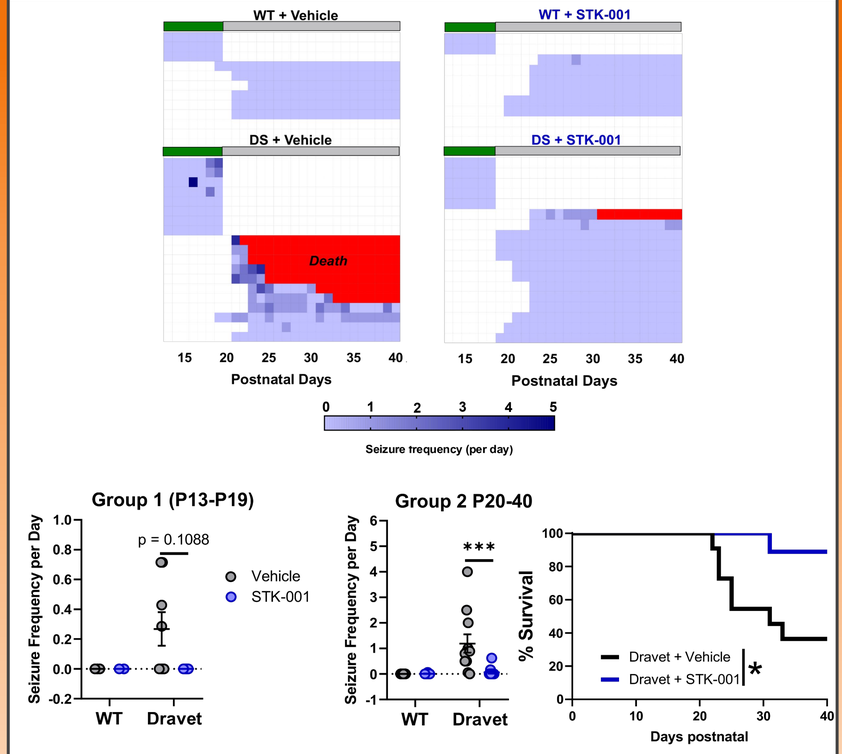


Whole-cell current-clamp recordings from cortical layer V tdTomato-positive parvalbumin-positive interneurons

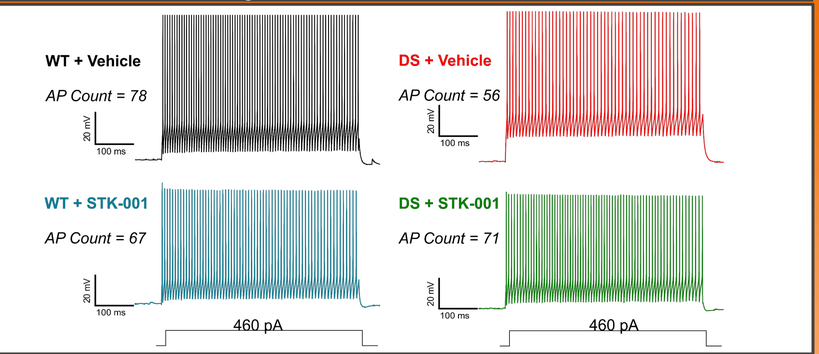


All experimenters blinded to genotype and treatment throughout data collection and analysis

STK-001 administration reduces seizure frequency and improves survival in the DS mice



Representative example traces of WT and DS PV interneuron excitability treated with vehicle or STK-001

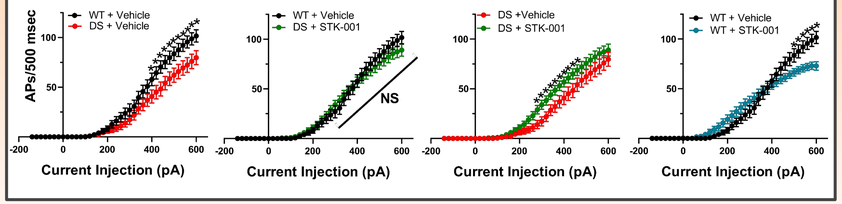


Membrane and action potential properties of WT and DS PV interneurons treated with vehicle or STK-001

	Resting Membrane Potential (mV)	Input resistance (MΩ)	Action Potential Threshold (mV)	Rheobase (pA)	Action Potential Amplitude (mV)	Upstroke Velocity (mV/ms)	Downstroke Velocity (mV/ms)	APD50 (ms)
WT Vehicle (n=22)	-68.9 ±1.4	110±6	-34±1	239±24	56±2	355±14#&^	-225±13#	0.37±0.03 #
WT STK-001 (n=22)	-68.3±1.0	139±13&^	-36±1	168±19&	62±2&	290±13*	-158±10*^	0.55±0.02* ^
DS Vehicle (n=27)	-69.9±1.0	102±7#	-35±1	290±22#^	55±2#	299±11*	-197±10	0.46±0.05
DS STK-001 (n=33)	-67.2±0.9	103±5#	-37±1	214±21&	57±2	301±11*	-197±9#	0.41±0.02 #

*indicates significance p≤0.05 compared to WT Vehicle
# indicates significance p≤0.05 compared to WT STK-001
& indicates significance p≤0.05 compared to Dravet Vehicle
^ indicates significance p≤0.05 compared to Dravet STK-001

STK-001 administration restores the firing frequency of DS PV interneurons to control PV interneurons



References:
Miller AR, Hawkins NA, McCollom CE, Kearney JA. Genes Brain Behav. 2014 Feb;13(2):163-72.
Tai C, Abe Y, Westenbroek R, Scheuer T, Catterall W PNAS 2014 July; 11(30) E3139-E3148.
Han Z, Chen C, Christiansen A, Ji S, Lin Q, Anumonwo C, Liu C, Leiser SC, Aznarez I, Liau G, Isom LL. Science Translational Medicine. 2020 Aug 26;12(558):1-14.

Conclusions and Future Directions

Conclusions

- TANGO ASO (STK-001) decreases seizure frequency and extends survival in a mouse model of *SCN1A*-linked DS.
- STK-001 restores high-frequency action potential firing in PV interneurons from DS mice.
- The current data support the hypothesis that the improvement in DS phenotype is, in part, due to restoration of excitability of cortical PV-expressing interneurons.

Ongoing/Future Directions

- Directly assess voltage-gated sodium channel function in isolated PV interneurons in mice treated with ASO.
- First patient dosed with STK-001 Aug 5, 2020 in open-label study of children and adolescents ages 2-18 with Dravet syndrome