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TANGO – Targeted Augmentation of Nuclear Gene Output for the Treatment of Genetic Diseases

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Introduction

Most human genetic diseases are due to loss or reduction of function of a single gene. Stoke is developing antisense oligonucleotide medicines that target RNA splicing to increase gene expression for the treatment of severe genetic diseases. TANGO exploits naturally occurring non-productive splicing events to restore target protein to near normal levels. Using proprietary bioinformatics, we have identified a large number of potentially druggable disease-associated genes via TANGO.

Dravet syndrome (DS) is a devastating childhood genetic disease characterized by severe seizures, cognitive & motor impairments and death. The primary cause of DS is decreased expression of the sodium voltage-gated channel type 1 alpha subunit (Nav1.1). Here, we show that antisense oligonucleotides (ASO) therapy can be used to specifically increase productive *Scn1a* mRNA and consequently restore levels of Nav1.1 protein. We are currently testing the impact of these ASO in a mouse model of DS that faithfully replicates a number of the disease manifestations in humans.

Bioinformatic Selection Strategy

Non-productive alternative splicing events identified via transcriptome-wide analysis of various RNAseq datasets

n = 127,774

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Build a disease database from multiple curated sources

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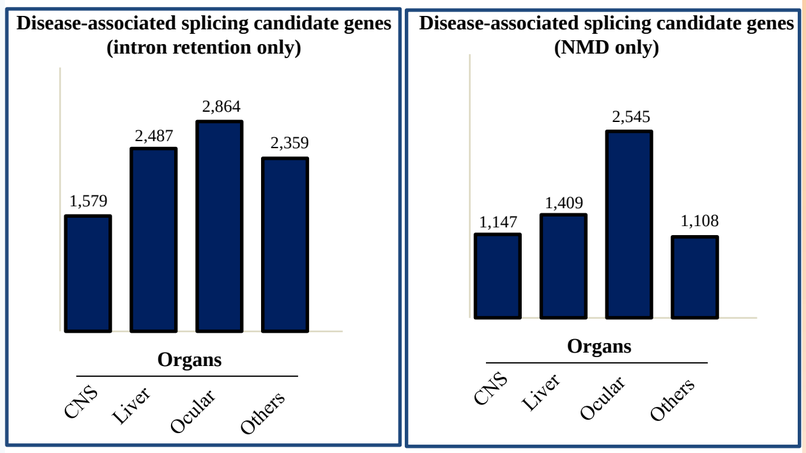

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Disease-Associated Splicing Candidates Targetable by anti-sense oligonucleotides (n = 3,634 unique genes)

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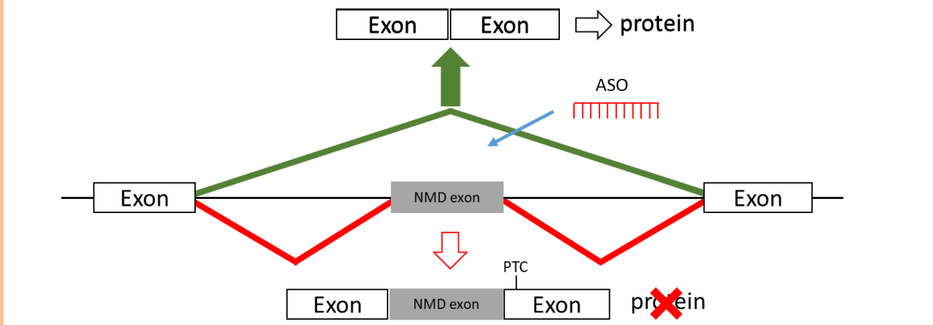
Stoke Evaluation & Prioritization

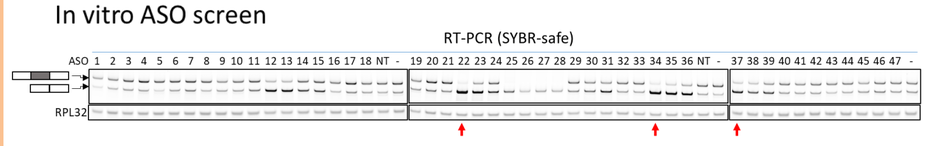
- Inheritance status (AD/AR)
- Haploinsuffiency
- Sequence conservation
- In vivo/ex vivo model
- Clinical development path
- Disease prevalence & unmet need
- Competitive landscape

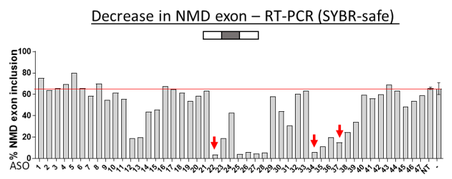
RESULTS

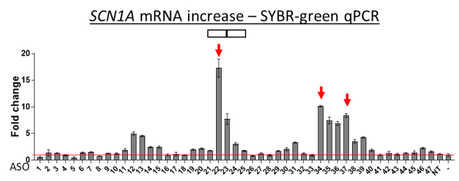
Converting non-productive mRNA into productive mRNA for SCN1A via antisense oligonucleotides



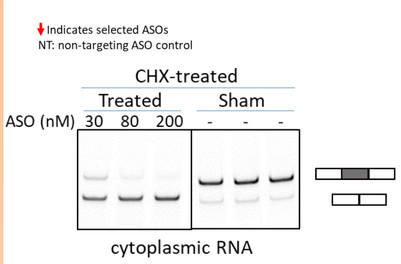
In vitro ASO screen

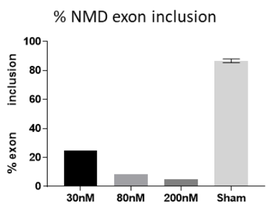


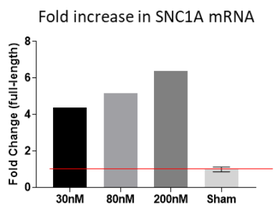




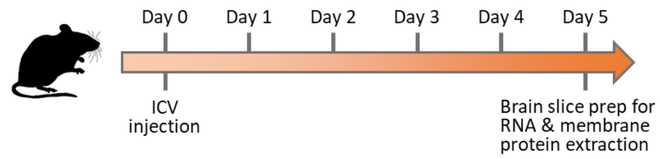
Indicates selected ASOs
NT: non-targeting ASO control

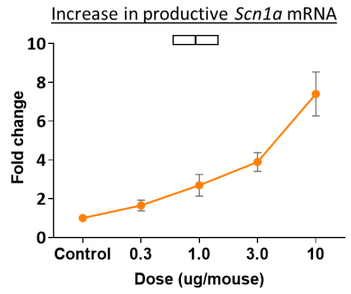


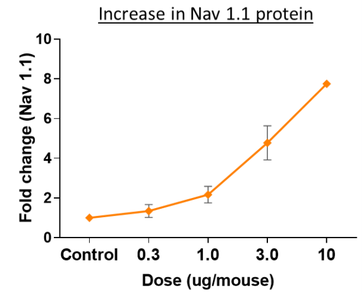




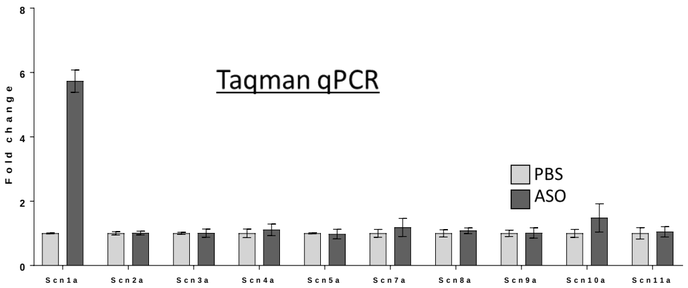
ASO increases *Scn1a* mRNA and protein *in vivo*







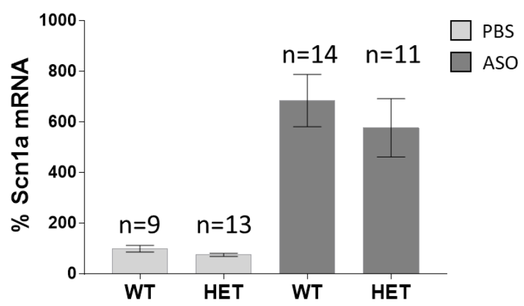
ASO selectively upregulates *Scn1a* but not closely related ion channel mRNA



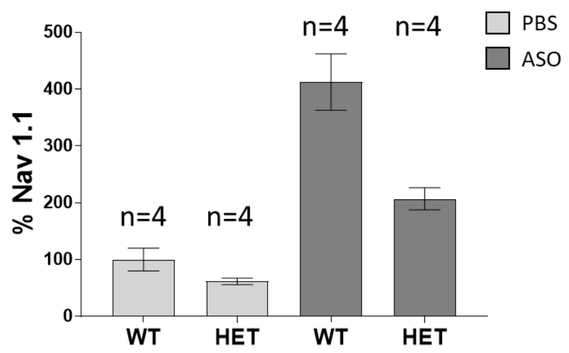
ASO increases *Scn1a* mRNA and Na_V 1.1 protein in Dravet mice

- F1 from 129S-scn1a^{tm1Kea} x C57BL/6 cross (postnatal day 2)
- N=9-14/group
- Single Dose: 20ug, administered ICV
- Total RNA & total protein extracted & analyzed

Scn1a mRNA increase – Taqman qPCR



Na_V 1.1 protein increase – western blot



Summary & Conclusion

- TANGO exploits non-productive mRNA splicing to address loss/reduced function diseases
- A proprietary bioinformatic approach is used to identify non-productive alternative splicing events in disease-associated genes
- We demonstrated the potential of this approach by testing this concept in Dravet Syndrome
- We identified sequences that can decrease inclusion of the non-sense mediated decay (NMD)-inducing exon and increase productive mRNA
- The selected ASO is active in vivo in restoring Nav1.1 protein levels and functional tests are ongoing in a mouse model of DS

In conclusion, we have validated a unique approach that can potentially address a number of devastating human genetic diseases caused by haploinsufficiency or hypomorphs. Restoration of expression via this approach permits regulation under the endogenous promoter. This can potentially translate to a better safety profile as only cells that already express the target gene can increase its expression in response to ASO treatment. Furthermore, delivery of ASO to the CNS, eye, liver and kidney is established 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. We are now building a portfolio of therapies via TANGO.