TANGO oligonucleotides for the treatment of Dravet Syndrome: Safety, biodistribution and pharmacology in the non-human primate

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1. Abstract

Rationale: Dravet syndrome 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 Dravet syndrome patients carry de novo mutations in the *SCN1A* gene leading to haploinsufficiency of the voltage-gated sodium channel α subunit $Na_V1.1$. Currently, there are no disease-modifying, targeted therapeutics available for these patients. Stoke's novel therapeutic approach utilizes antisense oligonucleotides (ASOs) to increase the endogenous expression of *SCN1A*. Based on encouraging efficacy data in a *Scn1a*+/- Dravet syndrome mouse model, we evaluated the safety and activity of lead ASOs in cynomolgus monkeys in a non-GLP study. The activity of an optimized ASO, STK-001, is described in detail.

Methods: Stoke's novel platform, Targeted Augmentation of Nuclear Gene Output (TANGO), exploits naturally-occurring non-productive splicing events to increase target protein expression via modulation of splicing. Two dose levels of an optimized ASO that was active in targeting a non-productive alternatively splicing event in *Scn1a* in rodent was evaluated in cynomolgus monkeys for safety, brain biodistribution, target engagement and pharmacodynamics. After a single intrathecal lumbar bolus injection (IT-L) of the ASO, animals were sacrificed and ASO, *SCN1A* mRNA and $Na_V1.1$ protein levels were measured on Day 3 (48 hours post dose) and on Day 29 (28 days post dose).

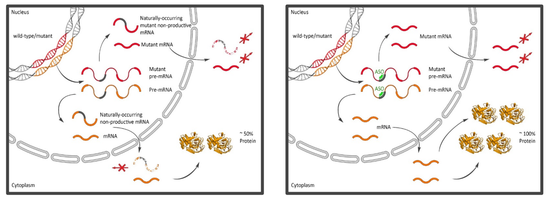
Results: The ASO was well tolerated at both dose levels with no changes on physical and neurological exams, no changes in food intake, body weight, hepatic function or platelet counts and no abnormal histopathology in all tissues examined. A broad ASO biodistribution was observed in the brain with the highest level found in different regions of the cerebral cortex. Endogenous levels of *SCN1A* gene expression varied between animals however there was a trend toward decreased non-productive and increased productive transcript. Importantly, an increase in $Na_V1.1$ protein was observed in the high dose group on Day 29 in regions of the cerebral cortex which are thought to be involved in disease pathology. The increase in $Na_V1.1$ protein varied in different regions of the cortex with as much as a two-fold increase observed in some regions.

Conclusions: We demonstrated that a single IT-L bolus injection of our lead TANGO ASO in NHP was safe and pharmacologically active in the animals tested. The favorable safety profile observed in animals that overexpressed $Na_V1.1$ protein above wild type levels is highly encouraging. These results support continued development of this oligonucleotide to provide a gene-specific, disease-modifying therapy for Dravet syndrome patients.

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

Experimental Design

Treatment	Dose	Day 3 Necropsy	Day 29 Necropsy
Vehicle (aCSF)	na	2 Male	2 Male
STK-001	Low	3 Male	3 Male
STK-001	High	3 Male	3 Male

2-3 year old naïve cynomolgus monkeys were administered a single, bolus intrathecal lumbar (IT-L) injection of artificial cerebrospinal fluid (aCSF) or one of two dose levels of STK-001on Study Day 1. After dosing, all animals underwent standard clinical and neurological observations and blood samples were collected. CSF and brains were collected at necropsy. Separate punches of various brain regions were collected for STK-001 concentration level, gene expression and protein expression measurement.

Methods

ASO Measurement: STK-001 levels were measured by Liquid Chromatography Mass Spectrometry (LCMS) in tissues and by Hybridization enzyme-linked immunosorbent assay (HELISA) in plasma and CSF.

SCN1A gene expression measurement: Productive *SCN1A* and non-productive (NMD-inducing) *SCN1A* mRNA transcripts were measured using Taqman qPCR. Data is shown as a percentage of productive transcript over total (productive + NMD-inducing).

NaV1.1 Protein measurement: $Na_V1.1$ protein was measured using a Mesoscale Discovery Electrochemiluminescence (MSD-ECL) assay which utilizes a recombinant fragment of $Na_V1.1$ as a standard.

4. Brain Tissue STK-001 Exposure

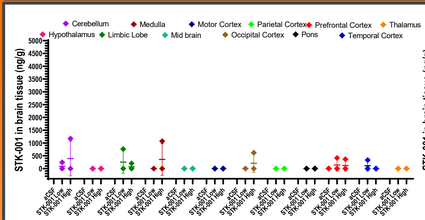


Figure 2. Levels of STK-001 in Cynomolgus Monkey Brain on Study Day 3
At the low dose, brain tissue STK-001 exposure was BLQ in most brain regions. At the high dose, STK-001 was detectable, primarily in cortex and cerebellum.

Note: Measured levels of STK-001 in predose and aCSF-dosed samples were below the limit of quantification (BLQ).

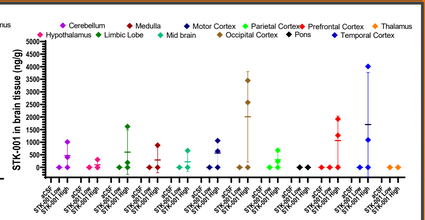


Figure 3. Levels of STK-001 in Cynomolgus Monkey Brain on Study Day 29
At the low dose, brain tissue exposure was BLQ in most brain regions. At the high dose level, exposure in cortical brain regions was generally higher than in deeper structures and, in general, was increased from Day 3.

5. $Na_V1.1$ Expression Levels in Brain Tissue

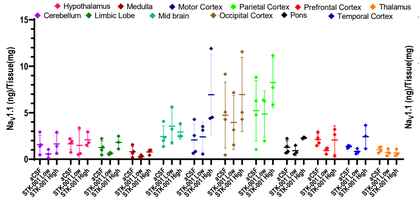


Figure 4. Levels of $Na_V1.1$ Protein in Cynomolgus Monkey Brain Regions on Day 3
All brain regions assessed had measurable levels of $Na_V1.1$, with midbrain, motor cortex, occipital cortex and motor cortex being the highest. No or marginal change in $Na_V1.1$ levels in brain tissues were observed at the low or high dose levels of STK-001.

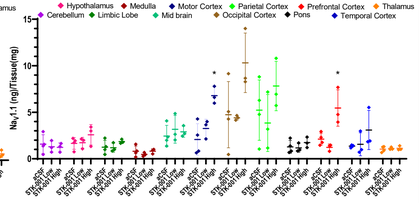


Figure 5. Levels of $Na_V1.1$ Protein in Cynomolgus Monkey Brain Regions on Day 29
No or marginal change in $Na_V1.1$ levels in brain tissues were observed at the low dose of STK-001. At the high dose of STK-001, $Na_V1.1$ protein levels increased by 1.2- to 3-fold in motor cortex, occipital cortex, parietal cortex, and prefrontal cortex compared to aCSF treated animals. .* = p<0.05

6. *SCN1A* Expression Levels in Brain Tissue

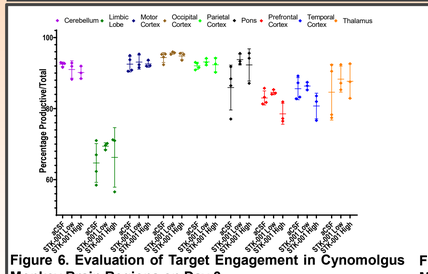


Figure 6. Evaluation of Target Engagement in Cynomolgus Monkey Brain Regions on Day 3
Target engagement was evaluated by comparing the level of productive *SCN1A* gene expression/total *SCN1A* gene expression No target engagement was observed in any of the 9 brain regions on Day 3 at either dose level.

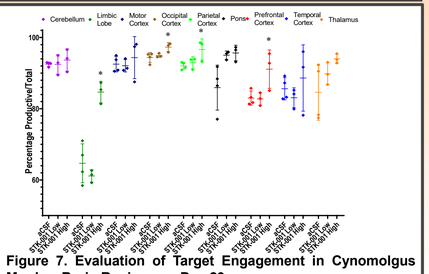


Figure 7. Evaluation of Target Engagement in Cynomolgus Monkey Brain Regions on Day 29
Significant target engagement as determined by measurement of productive *SCN1A* gene expression/total *SCN1A* gene expression was observed in prefrontal cortex, parietal cortex, occipital cortex and limbic lobe on Day 29 at the high dose of STK-001. * = p<0.05

7. Plasma and CSF STK-001 Exposure

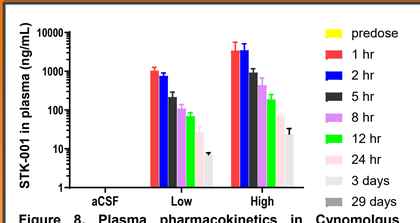


Figure 8. Plasma pharmacokinetics in Cynomolgus Monkey after Intrathecal Administration of STK-001
STK-001 reached mean peak plasma levels ~1 hr (first time of collection) for the low dose and 2 hr for the high dose. The concentrations declined in a biphasic manner. Peak and total exposures of each compound increased with increasing dose.

Note: Measured levels of STK-001 in predose and aCSF-dosed samples were below the limit of quantification of each assay.

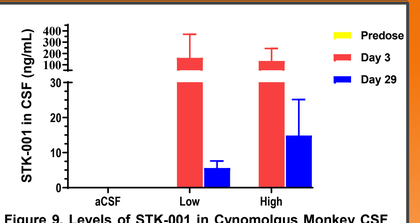


Figure 9. Levels of STK-001 in Cynomolgus Monkey CSF on Day 3 and 29
At Day 3, the observed CSF exposure in the low and high dose groups were similar. CSF STK-001 levels decreased markedly from Day 3 to Day 29 for both doses suggesting a transition from distribution to clearance phase during this period. Mean exposure levels were variable, but slightly higher in the high compared to the low dose group at Day 29.

8. Safety and Tolerability Assessment

Key safety measures in non-human primates

No complement system activation	✓
No decrease in platelet counts	✓
No change in hepatic function	✓
No clinical signs or symptoms over 28 day period after administration	✓
Normal histopathology in brain, liver and kidney	✓

9. Conclusions and Next Steps

Conclusions

- STK-001 distributes broadly in the non-human primate brain at the higher dose level.
- 48hr post injection, STK-001 is still in the distribution phase.
- STK-001 elicits the predicted effects on *SCN1A* gene expression indicating target engagement throughout brain cortex of the non-human primate after a single IT injection.
- STK-001 elicits the desired pharmacological response by increasing $Na_V1.1$ protein expression throughout brain cortex of non-human primate after a single IT injection.
- CNS and systemic exposure resulting from a single IT dose of STK-001 is well-tolerated in non-human primate at both dose levels tested.

Next Steps

The current study is part of the IND-enabling data package to support initiation of a clinical trial for Dravet syndrome in 2020.