1. Abstract

Rationale: Dravet syndrome is a severe developmental and epileptic encephalopathy characterized by high seizure frequency and severity, co-segregating with SCN1A haploinsufficiency, and a high burden of non-sustained awake-state brain damage (CSD). The majority of Dravet syndrome patients do not have a ready candidate for gene-directed therapeutics, necessitating targeted therapies available for these patients. Stroke is a therapeutic approach suitable for an intravenous oligonucleotide (ASO) to increase the endogenous expression of SCN1A. Based on encouraging efficacy data in a Dravet Syndrome mouse model, we evaluated the safety and activity of lead ASOs to a cynomolgus monkey in a non-clinical PD study. The activity of an optimized ASO, STK-001, is described in detail.

Methods: Stroke was administered in a single non-human primate (NHP) Toxicology (TANGO) study as a recombinant fragment of NaV1.1, to increase target protein expression via modulation of splicing. Two dose levels of an optimized ASO that was active in targeting a non-autosomal dominant haploinsufficiency, thus providing a potentially unique opportunity to treat these diseases. However there was a trend toward decreased non-productive (NMD)-occurring, TANGO can upregulate the wild-antisense (aCSF) in cynomolgus monkeys and mice. 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 does not use AOS to specifically increase protein expression by targeting naturally occurring non-productive alternative splicing events. TANGO uses mimetic non-prospective messenger RNAs (mRNAs), which are non-mammally 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 carnosine target mRNAs and full-length protein, only in tissues with exogenous NaV1.1 protein levels in brain tissues were below the limit of quantification (BLQ).

3. Experimental Design and Methods

Supervised Design

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time</th>
<th>Sample Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>Day 0</td>
<td>Pre-dose</td>
</tr>
<tr>
<td>STK-001</td>
<td>Day 0</td>
<td>Pre-dose</td>
</tr>
<tr>
<td>STK-001</td>
<td>Day 29</td>
<td>Day 29</td>
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</tbody>
</table>

3.2 vehicle- and non-vehicle- treated monkeys were administered a single, brain-throughout beta (ST) injection of artificial cerebrospinal fluid (aCSF) or vehicle AOS in non-human primates. Treatment using the vehicle AOS was administered i.m. (200 μg/kg) and followed by i.t. (100 μg/kg) injections on Days 0 and 29, with the first dose being 72 hours before the second dose. Animals were sacrificed at Day 1000, aCSF (100 μg/kg) or vehicle (100 μg/kg) were measured on Day 100 (4 h before post dose and on Day 29 24 h post dose, respectively).

Notes: The ASO was well tolerated at both dose levels with no changes on physical and neurological exam. No changes in liver, kidney, or any other tissue with no changes in general health. No history of seizures or spasticity.

4. Brain Tissue STK-001 Exposure

**Figure 2. Levels of STK-001 in Cynomolgus Monkey Brain on Study Day 3**

All the low-dose, brain tissue STK-001 exposure was BLQ in all brain regions at the low dose of STK-001. At the high dose of STK-001, brain tissue STK-001 exposure was measured in all brain regions at the high dose of STK-001.

**Figure 3. Levels of STK-001 in Cynomolgus Monkey Brain on Study Day 29**

At the low dose, brain tissue STK-001 exposure was BLQ in all brain regions at the low dose of STK-001. At the high dose of STK-001, brain tissue STK-001 exposure was measured in all brain regions at the high dose of STK-001.

5. NaV1.1 Expression Levels in Brain Tissue

**Figure 4. Levels of NaV1.1 Protein in Cynomolgus Monkey Brain Regions on Day 3**

All the low-dose, brain tissue NaV1.1 protein levels were below the limit of quantification (BLQ) in all brain regions at the low dose of STK-001. At the high dose of STK-001, brain tissue NaV1.1 protein levels were measured in all brain regions at the high dose of STK-001.

**Figure 5. Levels of NaV1.1 Protein in Cynomolgus Monkey Brain Regions on Day 29**

At the low dose, brain tissue NaV1.1 protein levels were below the limit of quantification (BLQ) in all brain regions at the low dose of STK-001. At the high dose of STK-001, brain tissue NaV1.1 protein levels were measured in all brain regions at the high dose of STK-001.

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 levels of SCN1A gene expression in treated and control animals. 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 was determined by measurement of increased SCN1A gene expression in treated monkey brain compared to aCSF treated animals. * = p<0.05.

7. Plasma and CSF STK-001 Exposure

**Figure 8. Plasma pharmacokinetics in Cynomolgus Monkey after intrathecal administration of STK-001**

Figure 9. Levels of STK-001 in Cynomolgus Monkey CSF on Day 3

**Figure 9. Plasma pharmacokinetics in Cynomolgus Monkey after intrathecal administration of STK-001**

At Day 3, the observed CSF exposure in the low and high dose groups were similar. CSF STK-001 levels decreased from Day 0 to Day 30 before both doses suggesting a transition from clearance to distribution 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.
- 48 hr 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 NaV1.1 protein expression throughout brain cortex of non-human primates after a single IT injection.
- CNS and systemic exposure resulting from a single IT dose of STK-001 is well-tolerated in non-human primates 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.