TH699

The Nav1.7-Targeting Small Interfering RNA ATL-301 Provides Months-Long Analgesia in Rodents

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Abstract

Background and Aims: The voltage-gated sodium ion channel Nav1.7 is a key gate for transmission of noxious sensory input from peripheral neurons into the spinal cord and CNS. Human and rodent genetics show that constitutive genetic loss of Nav1.7 results in the loss of pain without motor, cognitive, or autonomic effects (1, 2, 3), suggesting that Nav1.7 inhibition could be a safe and effective therapy for pain. Key challenges for systemic Nav1.7 therapeutics include distribution to cellular sites of action, achieving a sufficiently high level of inhibition or receptor knockdown, and selectivity for Nav1.7 versus the sodium channel paralogs that govern excitability of brain, skeletal muscle, and heart. Branched, or divalent, small interfering RNA (di-siRNA) is a novel technology that knocks down target gene expression with high specificity, has broad distribution throughout the CNS, and has a months-long duration of action following a single dose directly into the cerebrospinal fluid (4). Here we describe and characterize the effects of ATL-301, a novel di-siRNA targeting Nav1.7.

Methods: ATL-301 was designed to target a sequence identical among human, rodent, and primate SCN9A mRNAs and not shared with any other sodium channel or human gene. Reduction of transcript *in vitro* by ATL-301 was measured in HEK cells overexpressing SCN9A via reverse transcriptase quantitative polymerase chain reaction (RT-qPCR), and effects on functional Nav1.7 protein *in vitro* were determined using whole cell patch clamp electrophysiology. ATL-301 was formulated in saline and dosed in rats via intrathecal lumbar injection through implanted catheters. The evoked pain response was measured using the Hargreaves assay for sensitivity to painful thermal heat and with a pinprick assay for sensitivity to painful mechanical stimuli. Pain assessments were completed every two weeks following a single administration for a total duration of 12-18 weeks. Following takedown, transcript knockdown was assessed in sensory ganglia from the dorsal root by RT-qPCR and by RNAscope imaging, and protein knockdown was assessed via an in-house MesoScale Discovery ELISA.

Results: *In vitro*, ATL-301 reduced total cellular SCN9A mRNA expression by 60% and eliminated Nav1.7 currents, both with an IC50 of <10pM. There was no knockdown of SCN1A, SCN2A, SCN3A, SCN8A, SCN10A, or SCN11A *in vitro* at concentrations up to 10nM. ATL-301 administered to rats via intrathecal lumbar catheter resulted in dose-dependent analgesia, assayed as an increase in the time to paw withdrawal in the Hargreaves radiant heat test and by the number of withdrawals in the pinprick model of sharp mechanical pain. The analgesic effect paralleled effects reported upon complete genetic removal of Nav1.7 (2). In both pain models, analgesia from a single dose lasted approximately three months, and animals were bright, alert, and responsive throughout. ATL-301 reduced cytoplasmic but not nuclear SCN9A expression in L4-L6 ganglia and reduced protein expression in a dose-responsive manner. RNA-seq showed specific knockdown of SCN9A with no knockdown of other ion channels.

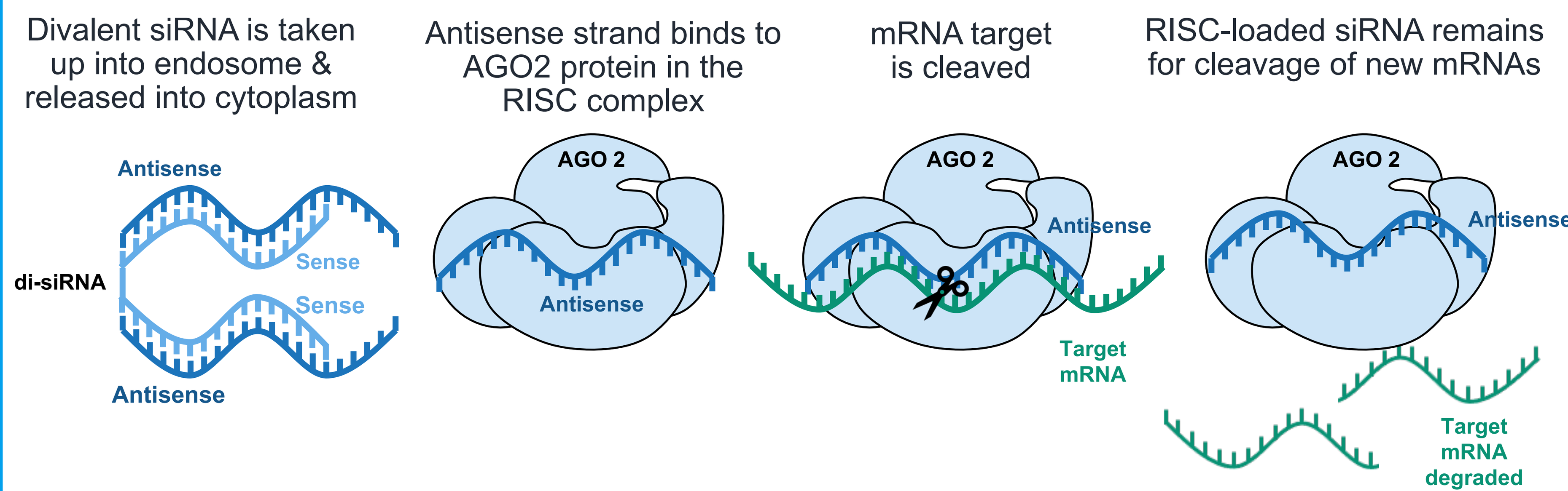
Conclusions: ATL-301 specifically knocked down SCN9A mRNA and Nav1.7 protein both *in vitro* and *in vivo.* A single intrathecal lumbar dose of ATL-301 resulted in sustained analgesia in rodents without apparent adverse effects. This effect was dose-dependent and durable for three months. These data suggest that ATL-301 may effectively target Nav1.7 and that di-siRNAs can address limitations to small and large molecule approaches such as CNS distribution, tolerability, and selectivity.

References:

1. Alsaloum et al. Nature Reviews Neurology 2020, 16:689-705
2. Shields et al. J Neuroscience 2018, 38(47):10180-10201
3. Goldberg et al. Clin Genet 2007, 71(4):311-319
4. Alterman et al. Nature Biotechnology 2019, 37:884-894

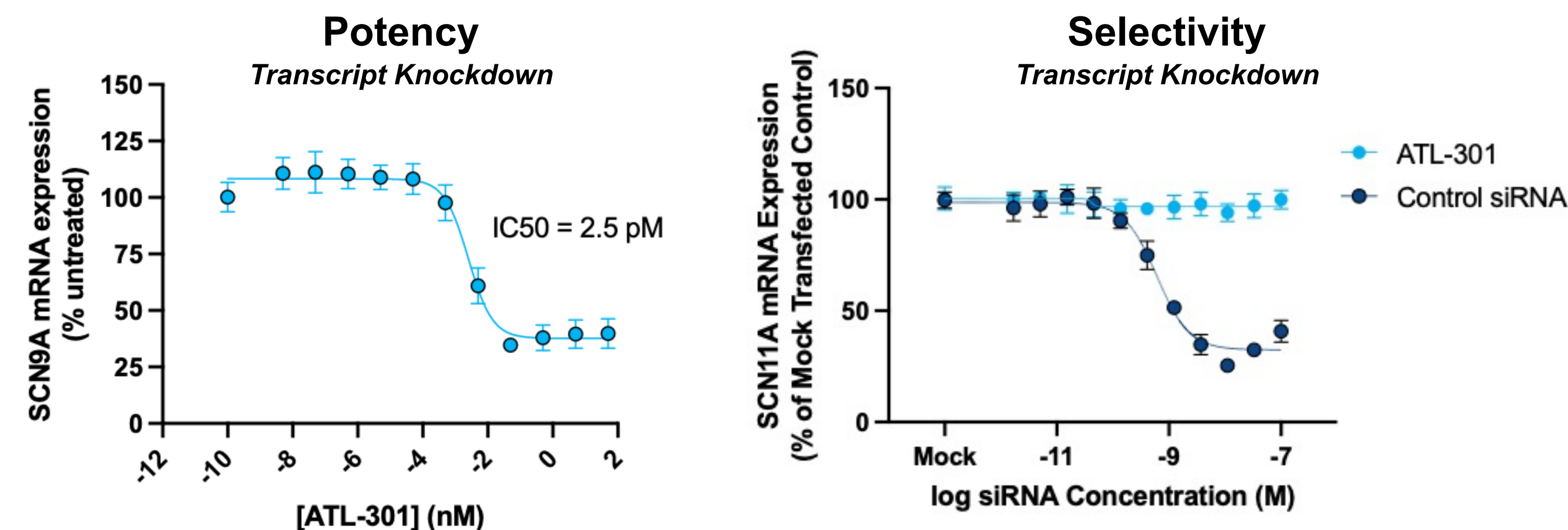
Relevance for Patient Care: Despite decades of effort, small molecule and large molecule approaches to Nav1.7 have not produced clinically effective inhibitors. The tolerability, selectivity, distribution, and slow onset of the oligonucleotide di-siRNA ATL-301 make it a promising candidate for a non-opioid therapy with the potential for large, durable effects on pain.

Background



- siRNAs enable selective knockdown of individual transcripts via Argonaute-2 and the RNA-induced silencing complex (RISC)
- Divalent siRNAs (di-siRNAs) distribute broadly through the CNS following dosing into the cerebrospinal fluid

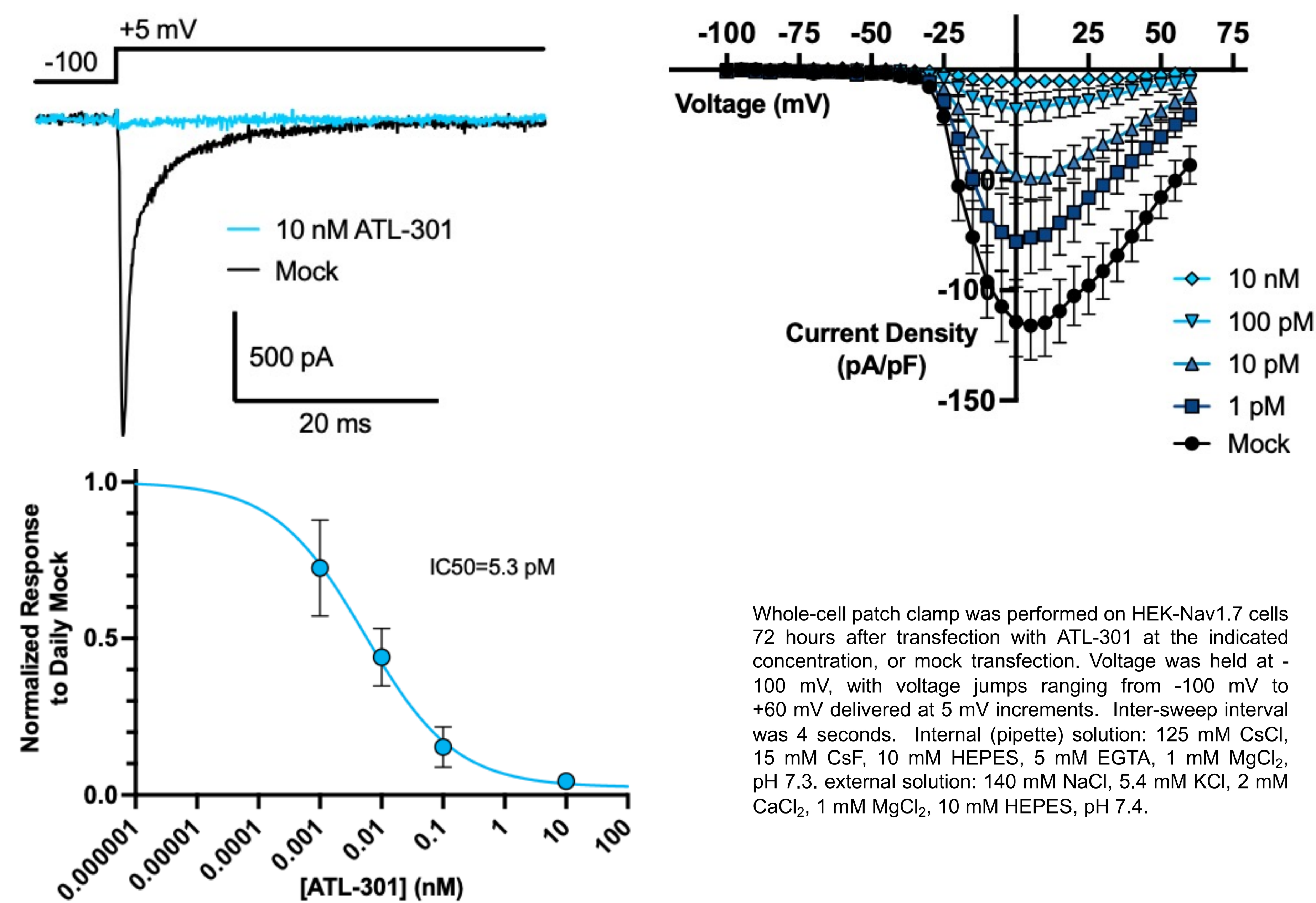
di-siRNA ATL-301 Selectively Inhibits SCN9A *In Vitro*



HEK-293T cells that express human Nav1.7 using a p-EEV vector (HEK-Nav1.7) were actively transfected with ATL-301 using RNAimax. Total RNA was isolated after 48 hours and mRNA expression was assessed using RT-qPCR, normalized to ATP5B housekeeping gene.

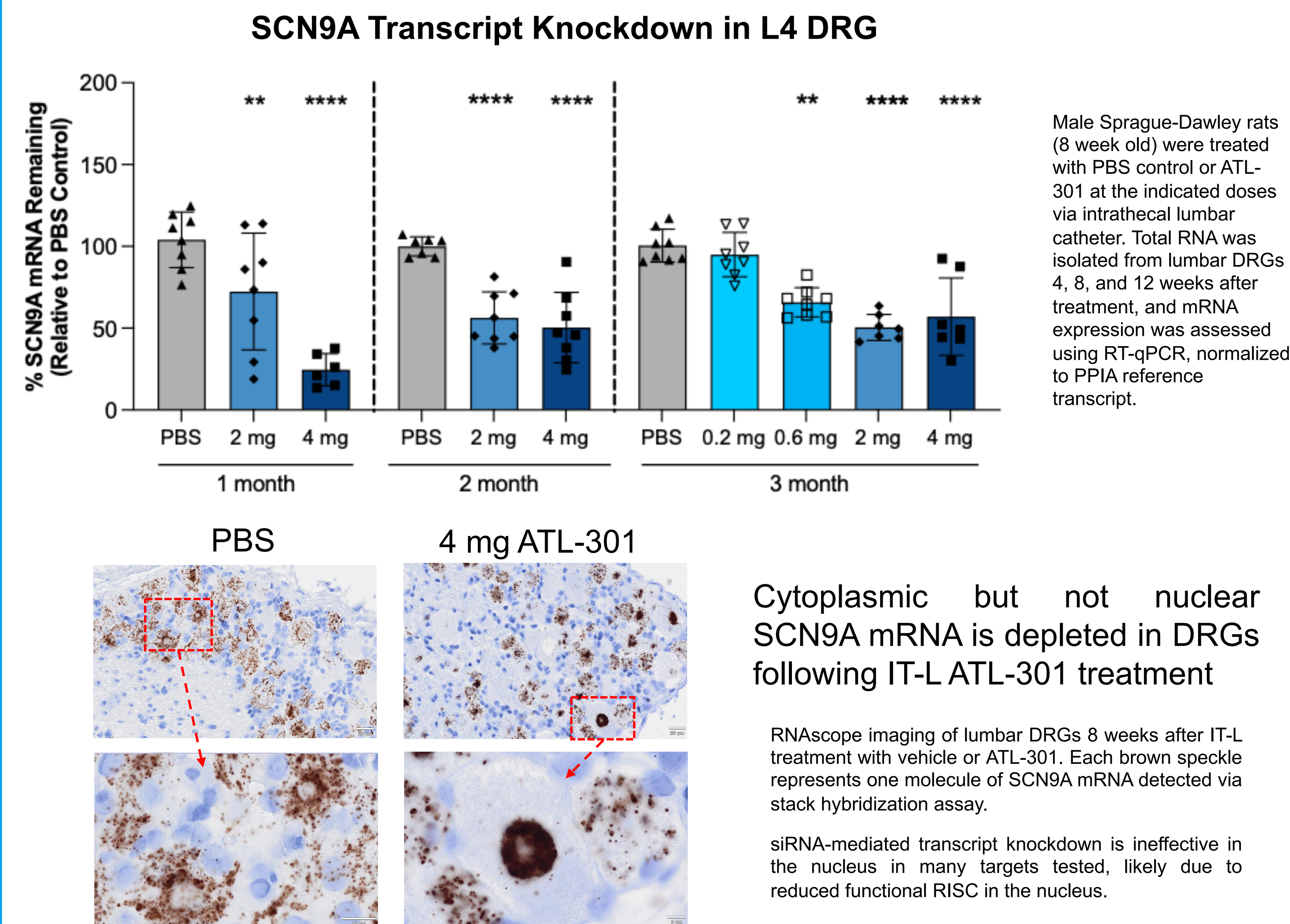
Human MM127 cells expressing SCN11A were actively transfected with ATL-301 or an SCN11A control siRNA (Horizon Discovery) using RNAimax. Total RNA was isolated after 48 hours and mRNA expression was assessed using RT-qPCR, normalized to TBP. ATL-301 did not show appreciable knockdown of SCN1A, SCN2A, SCN3A, SCN8A, SCN10A, or SCN11A, tested in SKN-BE2-M17, NCCIT, MM127, and HEK-Nav1.9 cells with PCR primers verified to be isoform-specific.

ATL-301 Inhibits Nav1.7 Current *In Vitro*



Whole-cell patch clamp was performed on HEK-Nav1.7 cells 72 hours after transfection with ATL-301 at the indicated concentration, or mock transfection. Voltage was held at -100 mV, with voltage jumps ranging from -100 mV to +60 mV delivered at 5 mV increments. Inter-sweep interval was 4 seconds. Internal (pipette) solution: 125 mM CsCl, 15 mM CsF, 10 mM HEPES, 5 mM EGTA, 1 mM $MgCl_2$, pH 7.3. external solution: 140 mM NaCl, 5.4 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, pH 7.4.

ATL-301 Inhibits SCN9A mRNA Expression *In Vivo*



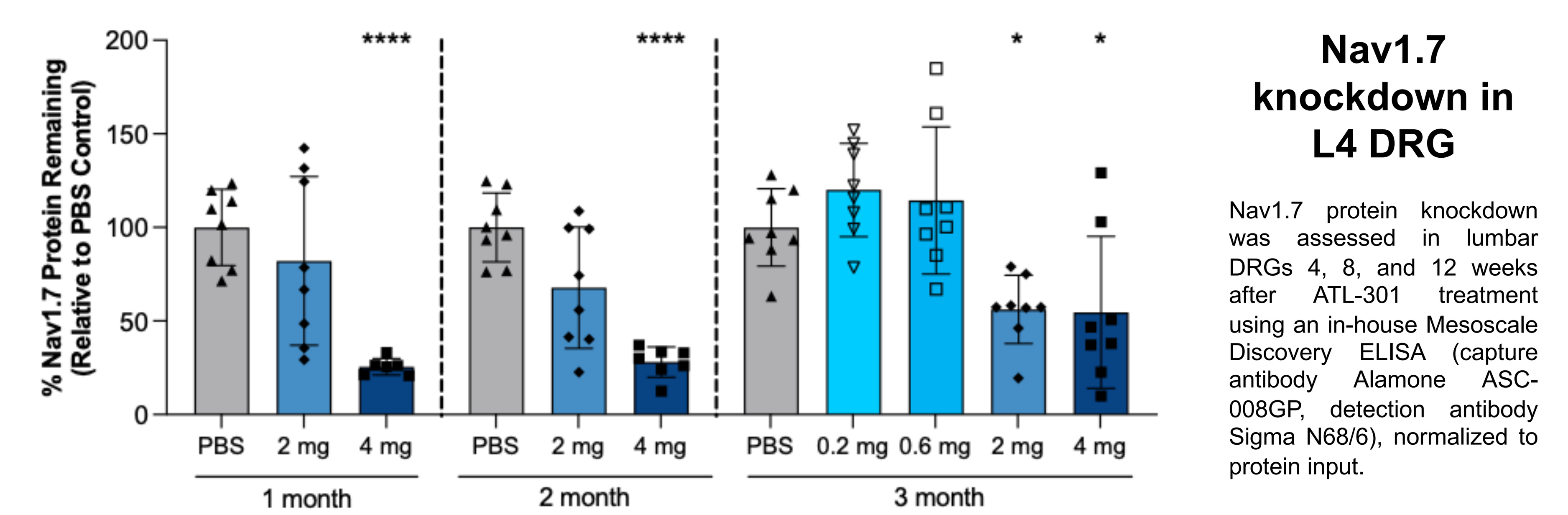
Male Sprague-Dawley rats (8 week old) were treated with PBS control or ATL-301 at the indicated doses via intrathecal lumbar catheter. Total RNA was isolated from lumbar DRGs 4, 8, and 12 weeks after treatment, and mRNA expression was assessed using RT-qPCR, normalized to PPIA reference transcript.

Cytoplasmic but not nuclear SCN9A mRNA is depleted in DRGs following IT-L ATL-301 treatment

RNAscope imaging of lumbar DRGs 8 weeks after IT-L treatment with vehicle or ATL-301. Each brown speckle represents one molecule of SCN9A mRNA detected via stack hybridization assay.

siRNA-mediated transcript knockdown is ineffective in the nucleus in many targets tested, likely due to reduced functional RISC in the nucleus.

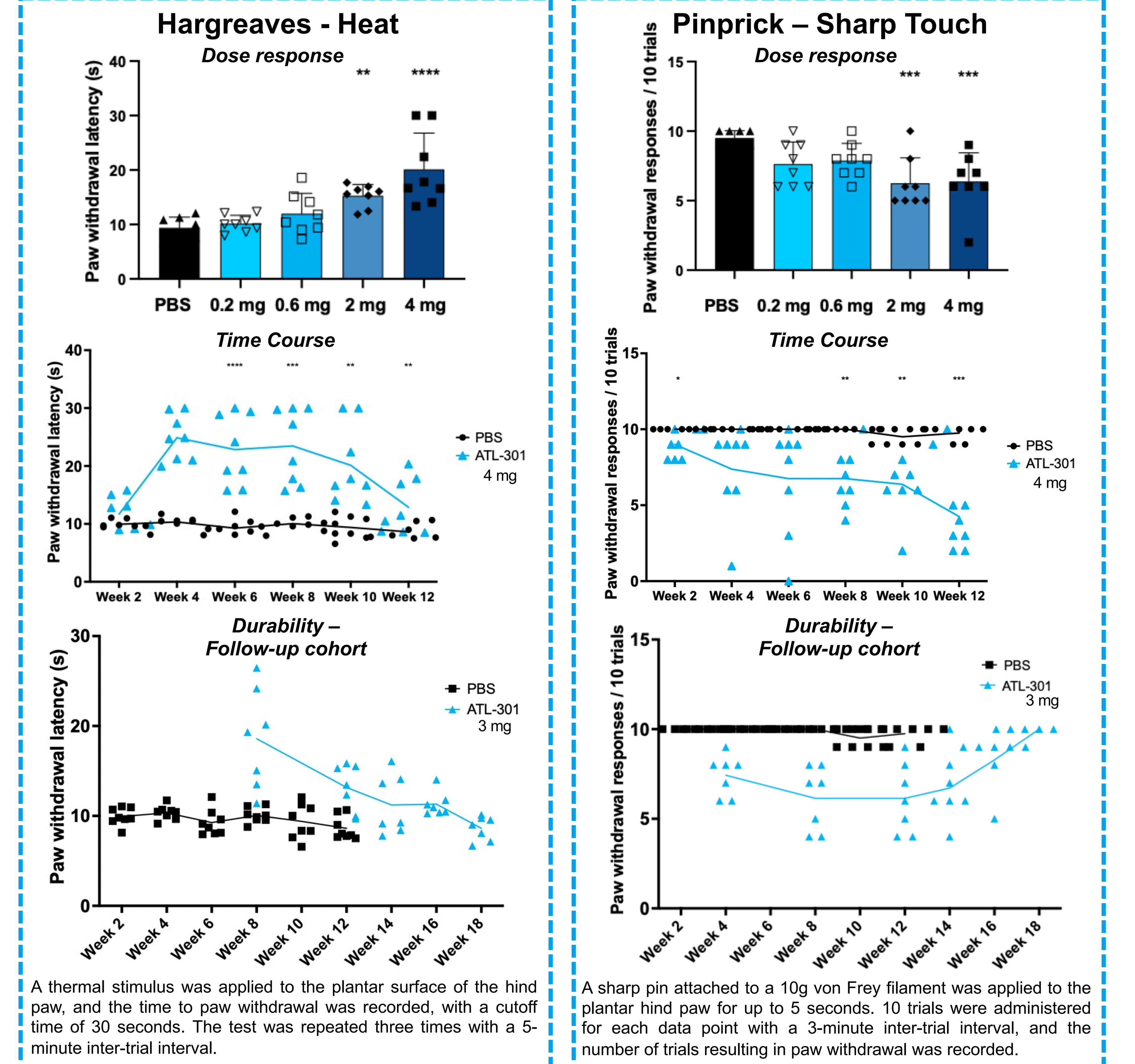
ATL-301 Inhibits Nav1.7 Protein Expression *In Vivo*



Nav1.7 knockdown in L4 DRG

Nav1.7 protein knockdown was assessed in lumbar DRGs 4, 8, and 12 weeks after ATL-301 treatment using an in-house Mesoscale Discovery ELISA (capture antibody Alamone ASC-008GP, detection antibody Sigma N68/6), normalized to protein input.

ATL-301 Produces Dose-Dependent Analgesia



A thermal stimulus was applied to the plantar surface of the hind paw, and the time to paw withdrawal was recorded, with a cutoff time of 30 seconds. The test was repeated three times with a 5-minute inter-trial interval.

A sharp pin attached to a 10g von Frey filament was applied to the plantar hind paw for up to 5 seconds. 10 trials were administered for each data point with a 3-minute inter-trial interval, and the number of trials resulting in paw withdrawal was recorded.

Summary

- ATL-301, a novel divalent siRNA, inhibits SCN9A expression and Nav1.7 current *in vitro*
- A single dose of ATL-301 di-siRNA delivered intrathecally in rats produced durable dose-dependent mRNA and protein knockdown
- ATL-301 treatment resulted in sustained analgesia, > 3 months in duration