Long-Lasting Analgesia in Rodents by siRNA-Mediated Silencing of Nav1.7

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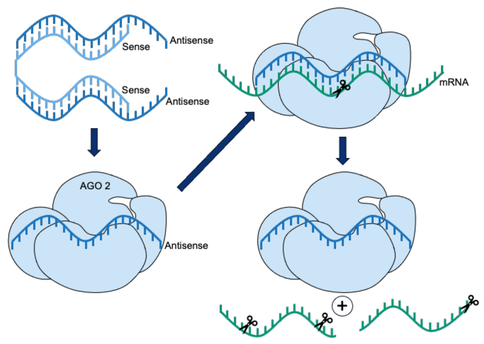
Abstract

Branched, or divalent, small interfering RNA (di-siRNA) is a novel version of siRNA developed for broad *in vivo* distribution throughout the CNS. Following direct administration into the cerebrospinal fluid, a single dose of di-siRNA knocks down target transcript and corresponding protein throughout the brain and spinal cord for at least six months. We have applied di-siRNA technology to target the voltage-gated sodium ion channel Nav1.7 encoded by the SCN9A gene, shown by human and rodent genetics to be a key gate for perception of pain. *In vitro*, a novel di-siRNA, ATL-301 reduced Nav1.7 transcripts by approximately 50% and eliminated voltage-gated fast tetrodotoxin-sensitive sodium channels, as recorded with patch-clamp electrophysiology. Dosed intrathecally in rats, ATL-301 gave a dose-dependent reduction of SCN9A transcript in sensory ganglia to a maximum of approximately 50%, consistent with in vitro results. Transcript knockdown was paralleled by dose-dependent analgesia, assayed as an increase in the time to paw withdrawal in the Hargreaves radiant heat test. All animals responded with at least 30% increase in paw withdrawal time, with many animals demonstrating the maximum increase. RNAscope imaging experiments on cultured cells and on neurons of peripheral sensory ganglia showed about half of SCN9A transcripts located in the cytoplasm and half in the nucleus, where transcripts are inaccessible to siRNA-mediated knockdown and do not contribute to protein translation. This suggests that knockdown of cytoplasmic transcript drove complete knockdown of Nav1.7 protein and produced analgesia. ATL-301 also gave strong analgesia in the pinprick model of mechanical pain. In both pain models, analgesia from a single dose lasted fourteen weeks or longer. ATL-301 was selective for SCN9A against all eight voltage-gated sodium channel paralogs, tested in live cells with RT-qPCR assays following active transfection. Despite decades of effort, small-molecule and large-molecule approaches to Nav1.7 have not produced clinically effective inhibitors due to limitations of tolerability, selectivity, and distribution; di-siRNA overcomes these challenges and is a promising modality for new therapies directed against severe chronic pain.

Background

- di-siRNAs are novel branched siRNAs that enable selective knockdown of individual transcripts via Argonaute-2 and the RNA-induced silencing complex (RISC). di-siRNAs distribute broadly throughout the CNS and have months-long duration of action.

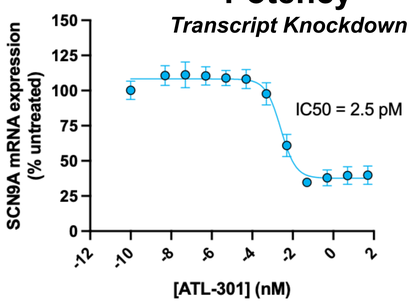


- **Study Goal:** Test whether di-siRNA mediated degradation of SCN9A (Nav1.7) produces lasting analgesia.

di-siRNA ATL-301 Inhibits Nav1.7 *In Vitro*

Potency

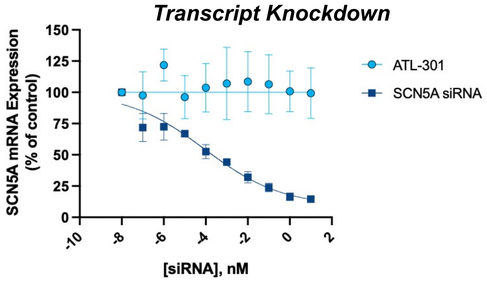
Transcript Knockdown



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

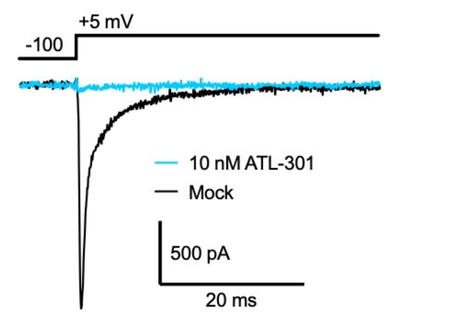
Selectivity

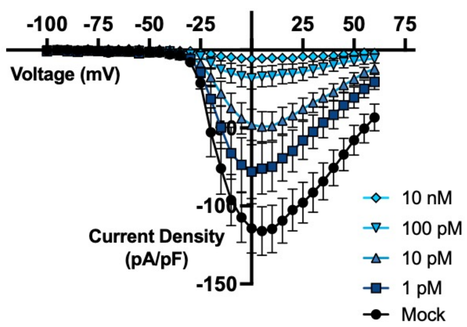
Transcript Knockdown

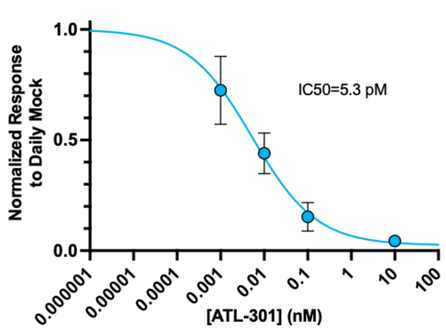


Human iPSC-cardiomyocytes (FujiFilm) were actively transfected with ATL-301 or an SCN5A control siRNA (Horizon Discovery) using RNAi max. Total RNA was isolated after 48 hours and mRNA expression was assessed using RT-qPCR, normalized to GAPDH. ATL-301 did not show appreciable knockdown of any other sodium channel genes, tested in SKN-BE2-M17, NCCIT, MM127, and HEK-Nav1.9 cells with PCR primers verified to be isoform-specific.

- ATL-301 gave complete knockdown of Nav1.7 current *in vitro*



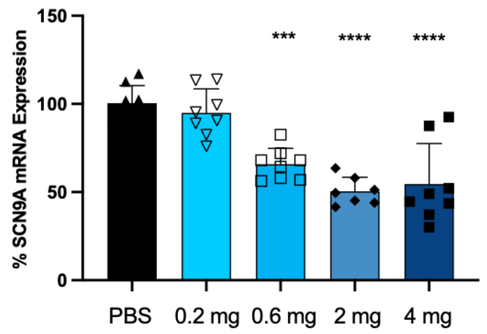




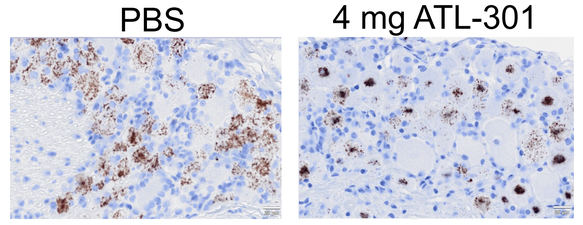
Whole-cell patch clamp was performed on HEK-Nav1.7 cells 72 hours after transfection with ATL-301 at the indicated concentration, or mock transfection. Voltage was held at -100 mV, with voltage jumps ranging from -100 mV to +60 mV delivered at 5 mV increments. Inter-sweep interval was 4 seconds. Internal (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 Knocks Down Nav1.7 *In Vivo*

- ATL-301 produced dose-dependent reduction of cytoplasmic Nav1.7 transcripts in rat dorsal root ganglia



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 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 following IT-L ATL-301 treatment

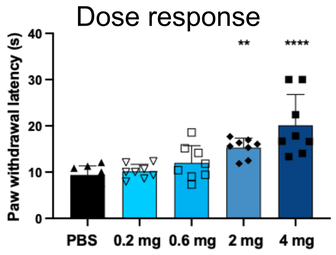
RNAscope imaging of lumbar DRGs 8 weeks after IT-L treatment with vehicle or ATL-301. Each brown speckle represents one molecule of SCN9A mRNA detected via stack hybridization assay.

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

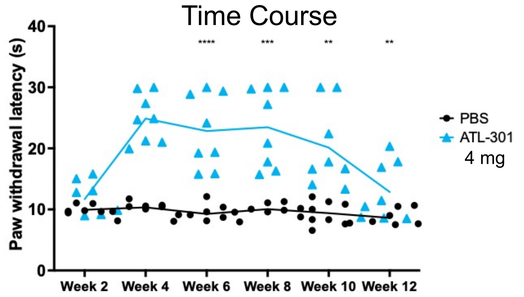
ATL-301 Produced Dose-Dependent Analgesia in Both the Hargreaves Radiant Heat and Pinprick Tests

Hargreaves - Heat

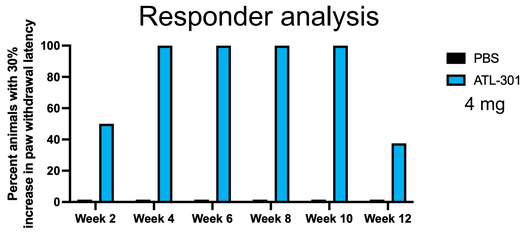
Dose response



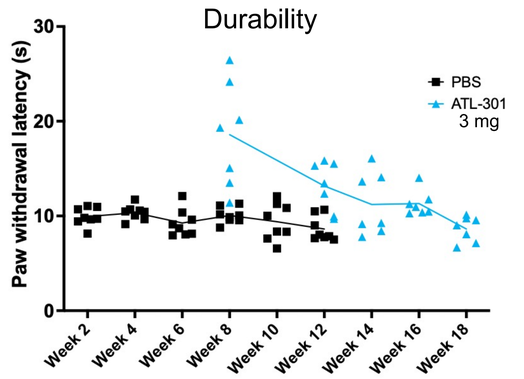
Time Course



Responder analysis



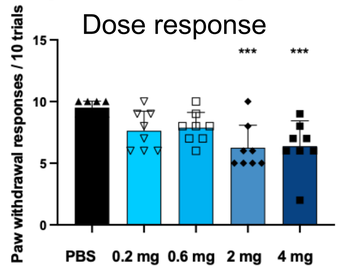
Durability



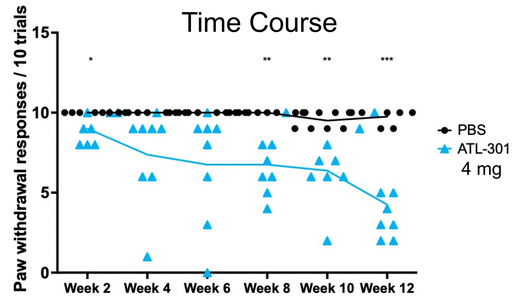
The Hargreaves test was administered using an apparatus with a heated glass base (IITC Life Science). All rats were well-acclimated to the apparatus before any testing and given a 20-min acclimation period before the first trial on each test day. The thermal stimulus was applied to the plantar surface 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.

Pinprick – Sharp Touch

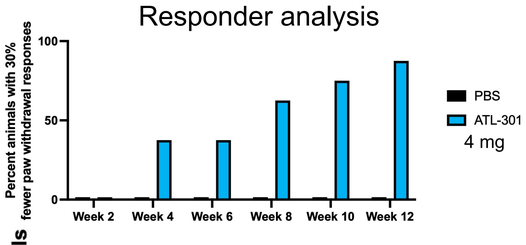
Dose response



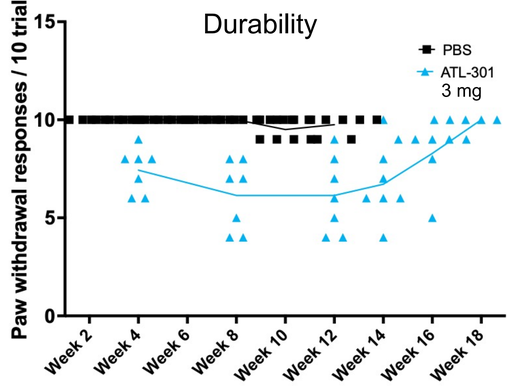
Time Course



Responder analysis



Durability



The pinprick test was performed in an apparatus with a wire mesh floor. All rats were well-acclimated to the apparatus before any testing and given a 20-min acclimation period before the first trial on each test day. 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.