Durable Suppression of Spontaneous Seizures in a Mouse Model of KCNT1 Genetic Epilepsy with Divalent siRNA

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Abstract

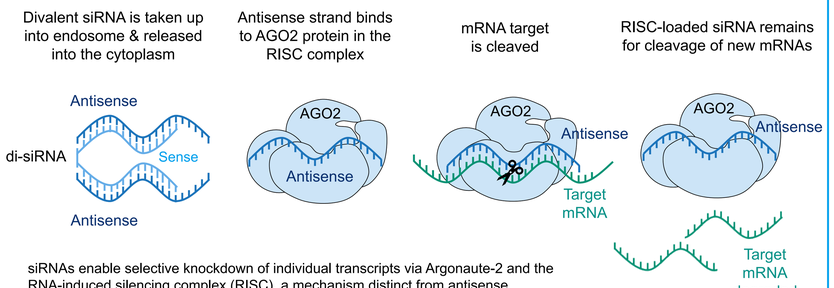
Background: Gain-of-function genetic variants in the *KCNT1* gene, which encodes a sodium-activated potassium ion channel, drive severe early-onset developmental epileptic encephalopathies including EIMFS (epilepsy of infancy with migrating focal seizures) and SHE (sleep-related hypermotor epilepsy). No therapy provides more than sporadic or incremental improvement. Here we report suppression of seizures in a genetic mouse model of KCNT1 epilepsy by targeting mouse *Kcnt1* with divalent small interfering siRNA (di-siRNA), an emerging variant of oligonucleotide technology developed for the CNS.

Methods: The ATL-201 molecule is two identical fully synthetic double-stranded siRNAs, covalently linked, with 100% base pair match to sequence identical in human *KCNT1* and mouse *Kcnt1*. Mechanistically, ATL-201 reduces potassium conductance by targeting both wildtype and mutant *KCNT1* alleles. ATL-201 was tested in mice homozygous for *Kcnt1-Y777H*, the mouse ortholog to the human pathogenic *KCNT1-Y796H* missense variant. Seizures were measured in freely-behaving mice with electrocorticogram from implanted leads combined with behavioral observations. The number and durations of seizures were measured in parallel groups dosed with ATL-201 or with PBS in a six-month durability study and in a two-month dose-efficacy study.

Results: ATL-201 reduced transcript from whole-cell lysate and eliminated potassium currents from cloned KCNT1 channels in heterologous expression. ATL-201 also eliminated sodium-activated potassium currents recorded from cultured mouse cortical neurons. In vivo, ATL-201 suppressed seizures in *Kcnt1-Y777H* homozygous mice in a dose-dependent manner with near-complete suppression from two weeks to at least four months. *Kcnt1-Y777H* mice had defects in nest-building behavior, whereas in ATL-201-treated mice nest-building was near equivalent to wildtype mice.

Conclusions: Patients with KCNT1-driven epilepsy experience up to hundreds of seizures a day and have severe impairment in cognitive, motor, and language development and high mortality. The dose-dependent efficacy and long durability of ATL-201 suggest that ATL-201 used clinically might suppress seizures and give improvements in overall function. ATL-201 targets sequence that does not encompass any known pathogenic variant in *KCNT1* and so in principle should be effective against any genotype of KCNT1 epilepsy. The efficacy and durability of the di-siRNA gives promise for a quarterly- or biannually-dosed disease-modifying treatment of KCNT1 epilepsy with a mechanism distinct from emerging antisense oligonucleotide and small molecule therapies.

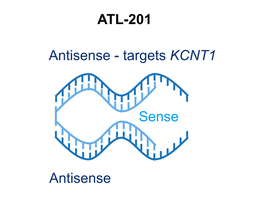
Background: Transcript Cleavage by siRNA



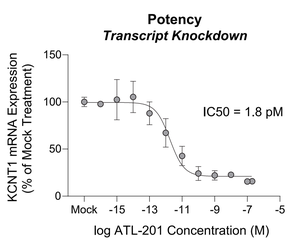
siRNAs enable selective knockdown of individual transcripts via Argonaute-2 and the RNA-induced silencing complex (RISC), a mechanism distinct from antisense oligonucleotides (ASOs)

Divalent siRNAs (di-siRNAs) distribute broadly through the CNS following dosing in saline into the cerebrospinal fluid, without lipid or other carriers

ATL-201 Knocks Down *KCNT1* Transcript and Protein In Vitro

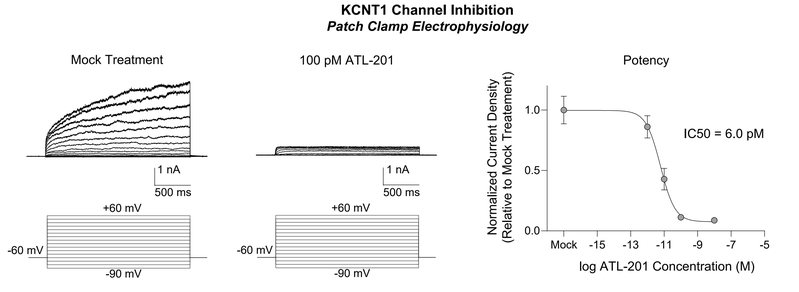


KCNT1-targeting sequence of ATL-201 is identical in mouse *Kcnt1* and human *KCNT1* and is not a 100% match to any other cDNA sequence in the human or mouse genome



Transcript knockdown by ATL-201 was tested using active transfection of multiple concentrations into HEK293T cells stably expressing human KCNT1

ATL-201 treatment produced potent, dose-dependent transcript knockdown that was nearly complete at high concentrations

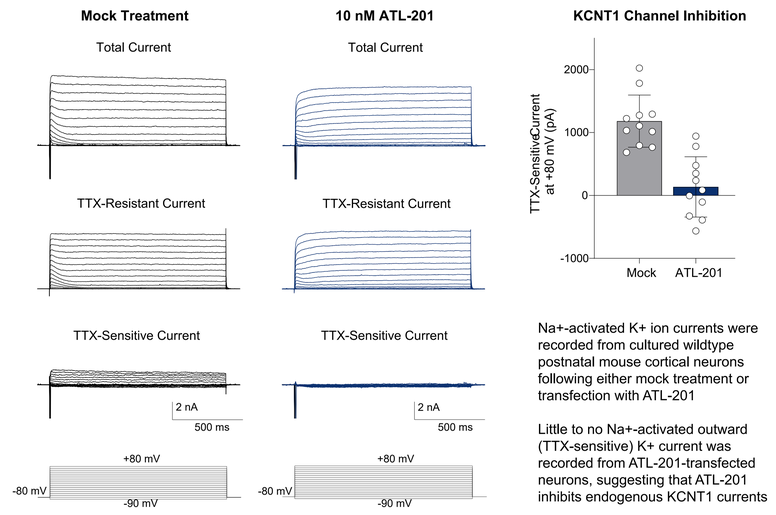


KCNT1 protein function was measured using whole-cell patch-clamp electrophysiology

Representative current traces recorded from mock-treated or ATL-201-treated KCNT1-expressing HEK293T cells with currents evoked by step depolarizations from -90 mV to +60 mV

Reduction in current was dose-dependent, with near-complete inhibition at a saturating concentration of 10 nM

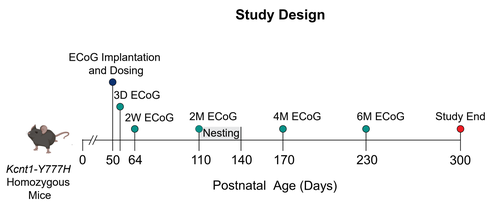
ATL-201 Inhibits Neuronal Sodium-Activated Potassium Currents

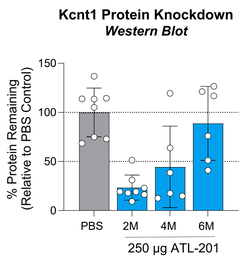


Na+-activated K+ ion currents were recorded from cultured wildtype postnatal mouse cortical neurons following either mock treatment or transfection with ATL-201

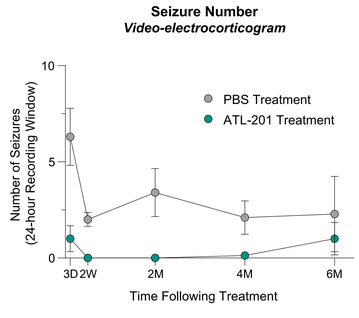
Little to no Na+-activated outward (TTX-sensitive) K+ current was recorded from ATL-201-transfected neurons, suggesting that ATL-201 inhibits endogenous KCNT1 currents

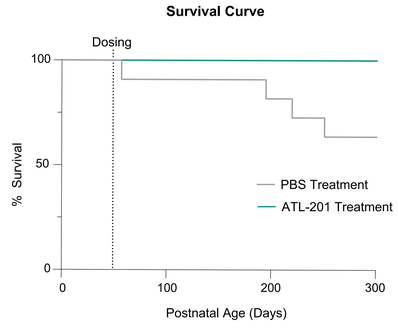
ATL-201 Durably Suppresses Seizures in the *Kcnt1-Y777H* Epilepsy Mouse Model

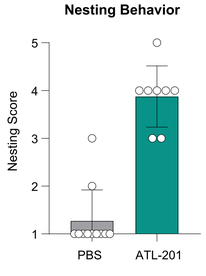




Kcnt1-Y777H mice (orthologous to the human disease-causing Y796H variant) underwent electrocorticogram electrode implantation and were dosed with PBS or 250 µg ATL-201 in a durability study





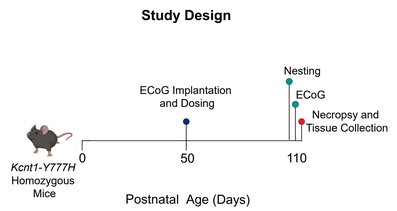


Near-complete suppression of seizures was observed from two weeks to at least four months in ATL-201-treated animals

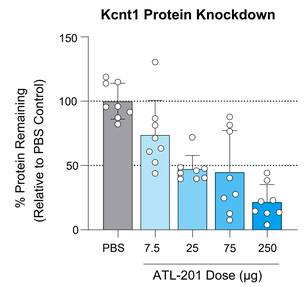
ATL-201 promoted survival of *Kcnt1-Y777H* mice, with no deaths observed in the treament group over the 300 day study period

ATL-201 treatment in *Kcnt1-Y777H* mice promoted nest building, a behavioral measure with social, thermoregulatory, and motor components that is sensitive to genetic and to pharmacological perturbation

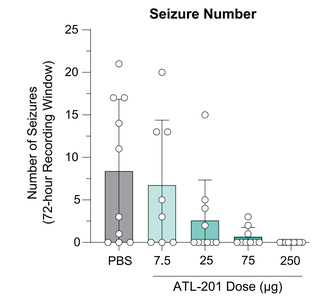
ATL-201 Efficacy is Dose-Dependent

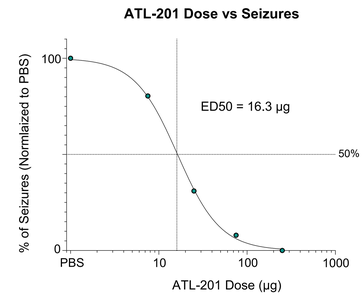


Kcnt1-Y777H mice were dosed with PBS or with a dose of ATL-201 ranging at half log units from 7.5 µg to 250 µg in a two-month dose-efficacy study



Kcnt1 protein knockdown was dose-dependent, with knockdown apparent at 7.5 µg and peaking at 78% ± 14% (mean ± SD) at 250 µg

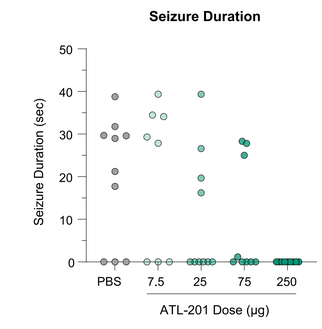




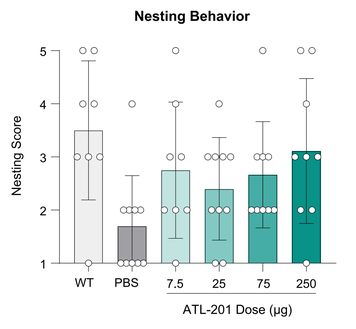
ATL-201 produced dose-dependent suppression of seizures, again with complete reduction at 250 µg

A nonlinear regression fit of the data showed that a 28% reduction in Kcnt1 protein correlated to a 50% reduction in seizures at an anticipated dose of 16 µg

Data suggest that full knockdown of KCNT1 protein is not required for significant reduction in seizures



Seizure duration was unaffected by ATL-201



Kcnt1-Y777H mice lost nest-building ability, which then was restored to near-wildtype levels by ATL-201, although not in a clearly dose-dependent manner

Summary

- Divalent siRNA, an emerging small interfering RNA technology distinct from antisense oligonucleotide technology, has potency, distribution, and durability appropriate for neurological disease
- ATL-201, a novel di-siRNA targeting the human *KCNT1* gene, knocks down *KCNT1* transcript with consequent near-complete knockdown of KCNT1 protein
- A single dose of ATL-201 into cerebrospinal fluid gives durable, dose-dependent reduction in seizures and improvement in nesting behavior in a relevant preclinical model of human disease
- ATL-201 shows promise for disease-modifying treatment of a severe early-onset developmental epileptic encephalopathy that has no effective treatments