


## RESEARCH ARTICLE

# Ataluren for drug-resistant epilepsy in nonsense variant-mediated Dravet syndrome and CDKL5 deficiency disorder

Orrin Devinsky , LaToya King, Judith Bluvstein & Daniel Friedman

Department of Neurology, NYU Langone Comprehensive Epilepsy Center, NYU Grossman School of Medicine, New York, New York

## Correspondence

Orrin Devinsky, NYU Langone Medical Center, 223 East 34th Street, New York, NY 10016, USA. Tel: 646-558-0802; E-mail: od4@nyu.edu

## Funding information

PTC Therapeutics, (Grant / Award Number: 'NCT02758626') Epilepsy Foundation, (Grant / Award Number: "NCT02758626") Dravet Syndrome Foundation, (Grant / Award Number: 'NCT02758626') Finding A Cure for Epilepsy and Seizures, (Grant / Award Number: 'NCT02758626').

Received: 8 December 2020; Revised: 4 January 2021; Accepted: 5 January 2021

*Annals of Clinical and Translational Neurology* 2021; 8(3): 639–644

doi: 10.1002/acn3.51306

## Abstract

**Objective:** Ataluren is a compound that reads through premature stop codons and increases protein expression by increasing translation without modifying transcription or mRNA stability. We investigated the safety and efficacy of ataluren in children with nonsense variants causing Dravet Syndrome (DS) and CDKL5 Deficiency Syndrome (CDD). **Methods:** This single-center double-blind, placebo-controlled crossover trial randomized subjects to receive ataluren or placebo for 12 weeks (period 1), a 4-week washout, then another 12-week treatment (period 2). The primary outcome was ataluren's safety profile. The secondary outcome measures were (1) changes in convulsive and/or drop seizure frequency and (2) changes in minor seizure types during ataluren treatment compared to placebo. Exploratory objectives assessed changes in cognitive, motor, and behavioral function as well as quality of life during ataluren therapy. **Results:** We enrolled seven subjects with DS and eight subjects with CDD. Three treatment-related adverse events (AE) occurred during the blinded phases. Two subjects withdrew due to AE. Ataluren was not effective in reducing seizure frequency or improving cognitive, motor, or behavioral function or quality of life in subjects with either DS or CDD due to nonsense variants. Limitations included a small sample size and 12-week treatment phase, possibly too short to identify a disease-modifying effect. **Significance:** There was no difference between ataluren and placebo; ataluren is not an effective therapy for seizures or other disorders in children with DS or CDD due to nonsense variants. There were no drug-related serious AE during the double-blind period, consistent with ataluren's favorable safety profile in larger studies. (Funded by Epilepsy Foundation, Dravet Syndrome Foundation, Finding A Cure for Seizures and Epilepsy and PTC Therapeutics, Inc.; ClinicalTrials.gov number, NCT02758626).

## Key points

- Ataluren was used to treat children with CDKL5 Deficiency Disorder (CDD) and Dravet Syndrome due to premature stop codons.
- This was a single site double-blind placebo-controlled randomized study.
- Ataluren was not associated with a reduction in seizures in positive impact on nonseizure outcomes.

## Introduction

Patients with epileptic encephalopathies such as Dravet syndrome (DS) and CDKL5 deficiency disorder (CDD) suffer from global developmental delays and treatment-resistant epilepsy, leading to severe morbidity and elevated mortality. CDKL5 is an X-linked genetic epileptic encephalopathy most frequent in females.<sup>1</sup> CDD patients exhibit signs of poor developmental skills (e.g., poor

sucking, poor eye contact) in the first months of life. Later, impaired fine and gross motor skills, lack of speech, and reduced eye contact and social interactions are evident. Many patients never walk independently.<sup>1</sup> DS often presents in the first year of life in an otherwise normal infant with fever-related generalized tonic-clonic seizures and/or alternating unilateral hemiconvulsions.<sup>2</sup> Later, myoclonic, atypical absence, and partial seizures often develop. Developmental delays develop in the second year of life and intellectual disability affects nearly all patients.<sup>3</sup> Seizures are usually drug-resistant, but epilepsy severity often diminishes by adolescence. Ataxic gait disorder, dysarthria, and intention tremor often develop in later childhood.<sup>4</sup> The mortality rate due to seizures and status is high; 15% of patients die by adolescence and 20% die by early adulthood.<sup>5</sup>

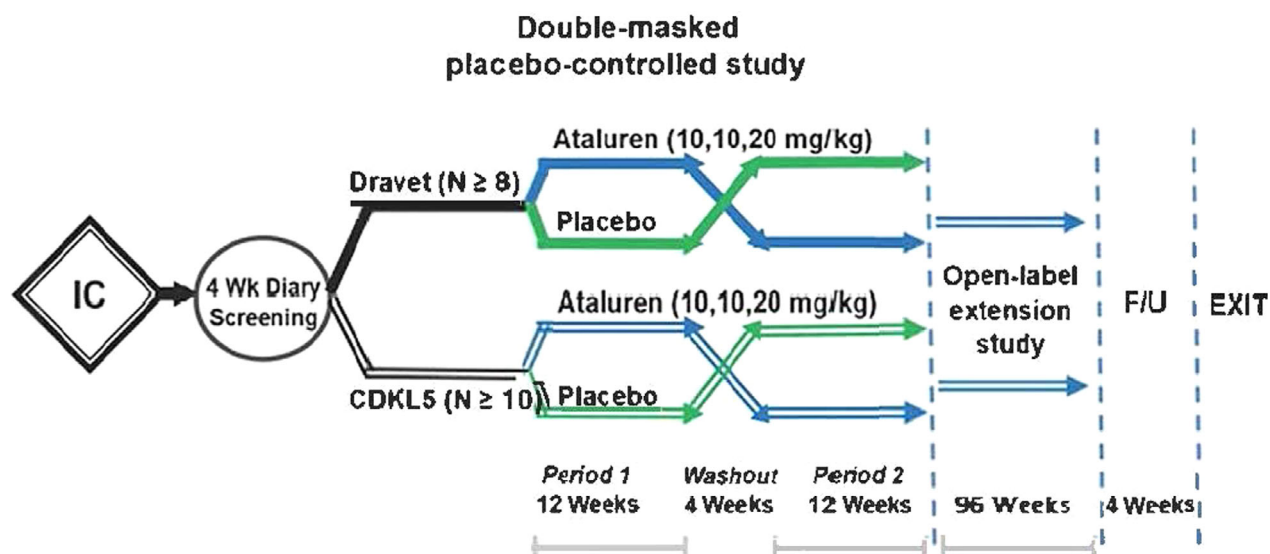
Ataluren is a novel 1,2,4-oxadiazole linked to two ring structures: fluorobenzene and benzoic acid (C<sub>15</sub>H<sub>9</sub>FN<sub>2</sub>O<sub>3</sub>) that promotes read-through of premature stop codons and increases protein expression in animal and human studies. In cellular assays and animal models of genetic disease, ataluren selectively enables read-through of mRNA containing a premature stop codon, producing full-length functionally active proteins localized to the appropriate cellular location.<sup>6,7</sup> Ataluren does not read-through normal stop codons.<sup>8</sup> Ataluren is selective for translation and does not alter levels of mRNA with premature stop codons or wild-type mRNA; that is, it does not modify transcription or mRNA stability. Ataluren has a favorable safety profile and is approved to treat nonsense-mediated Duchenne's muscular dystrophy in more than 30 countries. In this randomized blinded cross-over pilot study, we examined the effect

of ataluren on seizure control and cognitive function in patients with DS and CDD due to nonsense variants which account for ~15–20% of cases.<sup>1,9,10</sup>

## Methods

We screened children with DS and CDD due to a nonsense variant in one allele documented by a CLIA-certified laboratory report at the NYU Langone Comprehensive Epilepsy Center. Additional inclusion criteria included: (1) Age  $\geq 2$  years old and  $\leq 12$  years old, (2) male or female, (3) failure to control seizures despite appropriate trial of 2 or more antiseizure drug (ASDs) at therapeutic doses, (4) current regimen of 1–3 baseline ASDs at stable doses for a minimum of 4 weeks before enrollment (i.e., Screening Visit). For children with a vagus nerve stimulator or on the ketogenic or modified Atkins diets, these therapies were not considered an ASD but had to be unchanged for 3 months before enrollment, (5)  $\geq 6$  convulsive or drop seizures with duration  $\geq 3$  sec over the 4 weeks of diary screening before randomization and  $\geq 6$  convulsive or drop seizures with duration  $> 3$  sec during the 4 weeks from Screening to Baseline.

This was a single-center randomized, double-blind, placebo-controlled crossover trial. Subjects received either placebo or ataluren, 10 mg/kg in morning and midday and 20 mg/kg in the evening, for 12 weeks in period one and then after a 4-week washout, received the other treatment for 12 weeks (Fig. 1). A dose increase was allowed for subjects who increased  $\geq 10\%$  of body weight. Ataluren is a powder for oral suspension. There was no titration. Partway through the study, the Sponsor (PTC



**Figure 1.** Study trial design. EOS, end of study; F/U, follow-up; IC, informed consent; Wk, week.

Pharmaceuticals) changed dosage based on weight for subjects who were  $14 \pm 2$  kg. They originally took 125 mg/375 mg/375 mg daily and were changed to 125 mg/250 mg/250 mg. This affected three subjects.

The main outcome was percent change in motor seizure frequency. Other outcomes included safety and tolerability of ataluren; changes in cognitive, motor, and behavioral function; and quality of life. These outcomes were assessed by the following neuropsychological scales: Behavior Assessment System for Children (BASC), Vineland Adaptive Behavior Scales (VABS), and Quality of Life in Childhood Epilepsy (QOLCE).

## Results

We enrolled seven children with DS and eight children with CDD (Table 1). The Dravet subjects included five girls and two boys; mean age 6.4 years (median 7; range, 2–11). The Dravet subjects had a mean weight of 24 kg (median 23.6; range 13.6–48.1) and mean body mass index of 16.8 (range, 13.6–23.8). The eight CDD subjects were all girls; mean age 4.0 years (median 3.5; range, 2–11). The CDD subjects had a mean weight of 14.5 kg (range 10.0–19.1) and body mass index of 14.6 (range 12.5–18.1).

All subjects with DS completed the double-blind phase. One subject in the ataluren/placebo group completed the open-label extension phase. Four subjects, two each in the ataluren/placebo and the placebo/ataluren group were still in the open-label extension phase at the time of the data cut; and two subjects, one each in the ataluren/placebo (due to AE) and placebo/ataluren group (consent) discontinued during open-label extension phase. Of the eight CDD subjects, six completed the double-blind phase and two discontinued due to an adverse event. Two subjects, one each on ataluren/placebo and placebo/ataluren group, completed the open-label extension phase; two subjects on ataluren/placebo were still ongoing at the time of data cut; three subjects on placebo/ataluren discontinued (1 AE, 1 Consent and 1 lost of follow-up) during open-label extension phase.

## Safety during the blinded phase

### Dravet Syndrome

Six of seven subjects had adverse events (AEs) while on ataluren. Among them, three subjects had serious adverse

events (SAEs) considered unlikely related to the study medicine. One subject had two mild SAEs (decreased valproic acid level and increased seizure frequency) and two subjects had a moderate SAE (each with influenza). Three of seven subjects had AEs while on placebo. There were no study withdrawals due to an AE.

### CDKL5 deficiency disorder

Seven of eight subjects had AEs while on ataluren. One subject had a mild SAE (decreased valproic acid level; considered unrelated to study medicine). Two subjects had AEs leading to study withdrawal. One had GI symptoms (burping, flatulence, vomiting) of moderate severity, possibly related to study medicine. The other had increased seizures of mild severity, unlikely related to study medicine. Two of seven subjects had AEs while on placebo; one had a mild SAE (Croup; unrelated to study medicine). There were no study withdrawals on placebo.

## Safety during the open-label period

### Dravet syndrome

Six of seven subjects had AEs during the open-label period. One subject had two mild SAEs (ear infection and tooth infection) that were unrelated to study medicine. One subject withdrew from the study due to an AE (increased seizure frequency).

### CDKL5 deficiency disorder

Six of seven subjects had AEs during the open-label period. Two subjects had SAEs: one subject had elevated liver function enzymes twice (mild and severe), both possibly related to study medicine and both resolved. The other subject had *Mycoplasma pneumoniae*, moderate severity, unrelated to study medicine. One subject withdrew from the study due to an AE (elevated hepatic enzymes).

## Protocol variances

Of 15 randomized subjects, 12 subjects missed an average of three doses, usually midday and less often evening due to school, therapy, or sleep. Some parents reported that their child spit out some of the

**Table 1.** Subject demographics.

	DRAVET syndrome			CDKL5 deficiency disorder		
Sex	5 male	2 female		8 female		
Age	Mean 6.4 years	Median 7 years	Range 2–11 years	Mean 4.0 years	Median 3.5 years	Range 2–11 years
Weight	Mean 24 kg	Median 23.6 kg	Range 13.6–48.1 kg	Mean 14.5 kg	Median 14.1	Range 10.0–19.1 kg

medication and others reported that they had to split the dose to have their child take it all. Ten parents did not return all medication sachets at all study visits, which limited our ability to assess adherence. The following inconsistencies with diary data included: (1) forgetting to document: time, duration or severity or seizure type, (2), caregiver recall is uncertain of exact data, and provided an average or “guesstimate,” or (3) forgetting to return diary. Questionnaire-related inconsistencies included (1) missed questions and (2) excess “Not applicable” choices that limited scoring.

## Efficacy

There was no change in motor seizure frequency or cognitive, motor, or behavioral functions or quality of life in the ataluren group compared to placebo among these patients with nonsense variant CDKL5 or Dravet Syndrome.

For DS, the median percent change from baseline in motor seizure, total seizure, and myoclonic seizure frequency is summarized in Table 2.

For the primary endpoint of percent change from baseline in motor seizures, the median difference between the two treatment groups along with 95% Hodges–Lehmann confidence interval is 54.9% (6.95, 1648.8).

For CDD, the median percent change from baseline in motor seizure, total seizure, and myoclonic seizure frequency is summarized in Table 3.

For the primary endpoint of percent change from baseline in motor seizures, the median difference between the two treatment groups along with 95% Hodges–Lehmann confidence interval is 11.2 (–32.7, 40.5).

**Table 2.** Median percent change from baseline in different types of seizures in DS by treatment.

	Ataluren ( <i>n</i> , median [IQR])	Placebo ( <i>n</i> , median [IQR])
Motor seizure	<i>n</i> = 7, 28.6 (163)%	<i>n</i> = 7, –22.7 (54.2)%
Total seizure	<i>n</i> = 7, 12.5 (42.1)%	<i>n</i> = 7, –19.0 (82.2)%
Myoclonic seizure	<i>n</i> = 4, –46.7 (78.3)%	<i>n</i> = 4, 28.0 (299)%

**Table 3.** Median percent change from baseline in different types of seizures in CDD by treatment.

	Ataluren ( <i>n</i> , median [IQR])	Placebo ( <i>n</i> , median [IQR])
Motor seizure	<i>n</i> = 7, 16.0%	<i>n</i> = 7, –9.10 (59.6)%
Total seizure	<i>n</i> = 7, 42.9 (51.9)%	<i>n</i> = 7, 18.6 (105.4)%
Myoclonic seizure	<i>n</i> = 2, 1251 (2098)%	<i>n</i> = 2, 630 (1021)%

## Discussion

In this pilot randomized placebo-controlled cross-over trial, ataluren did not reduce seizure frequency or improving cognitive, motor, or behavioral function or quality of life in subjects with DS and CDD due to nonsense variants. Our trial was limited by small sample size and a treatment-phase of 12 weeks, which may be too short to identify a disease-modifying effect. Ataluren had a good safety profile in these children with severe developmental delays and treatment-resistant epilepsy. The reduced levels of valproic acid seen in two patients likely reflect the known variability in serum valproic acid levels.<sup>11</sup> Ataluren is metabolized primarily by glucuronidation by UGT1A9,<sup>12</sup> which is one of eight UGT enzymes that collectively metabolize ~50% of valproic acid (40% by beta-oxidation in mitochondria, 10% by hepatic cytochromes).<sup>13</sup> However, any interaction at UGT1A9 is predicted to insignificantly raise valproic acid levels.<sup>14</sup>

This is the first assessment of a read-through compound in patients with primary brain disorders. Most prior human studies with ataluren were performed on patients with Duchenne’s muscular dystrophy (DMD). The efficacy of ataluren in DMD led to approval by the European Medical Agency and multiple other countries.<sup>15,16</sup>

There are many potential reasons why ataluren may not have reduced seizures or functional measures in these subjects. First, ataluren may not be effective in treating these disorders. However, the numerous preclinical models showing efficacy in other disorders<sup>17,18</sup> make this unlikely if the drug reached affected neurons at therapeutic levels. The drug may not be effective in central nervous system disorders due to limited or erratic crossing of the blood–brain barrier. Evidence that ataluren crosses the blood–brain barrier comes from studies in single oral dose administration of radiolabeled ataluren to Sprague–Dawley and Long Evans rats as well as the Hurler mouse model.<sup>19</sup> However, it is possible that for some drugs, such as ataluren, there may be large species or inter-individual differences in blood–brain barrier penetration. Other possibilities for failure include (1) insufficient treatment duration for an effect, (2) selection of insensitive outcome measures, or (3) challenges related to protocol variances including adherence, accurate completion of seizure diaries, and missing data from questionnaires.

This study has implications for future trials of compounds that promote read-through compounds in treating CNS disorders. In addition to variability in lipid solubility, genetic, and other individual factors may affect this. CNS pharmacokinetic studies with brain levels in large mammals may help refine dosing in humans. Small

animal models may help predict likelihood of success in human studies. We did not assess ataluren in a rodent model of nonsense-mediated DS or CDD, nor did we measure changes in SCN1A or CLDK1 expression levels, which if possible, should be considered in future CNS trials. However, compounds that rescue the phenotype in rodent models of CNS disorders may fail to show efficacy in human studies (Fragile X trials with mGluR5 antagonists, GABA receptor agonist and glutamate agonists).<sup>20</sup> Future trials should carefully consider patient age ranges (i.e., there may be different temporal windows to alleviate neuronal dysfunction in different CNS disorders), consideration of periods of disease progression or regression based on natural history studies, refined clinical trial design and duration, outcome measures that are sensitive and specific for relevant phenotypic changes, and biomarkers that reflect CNS penetration and molecular efficacy.

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

### Conflict of interest

Orrin Devinsky has equity interests in Qstate Biosciences, Tevard Biosciences, Regal Therapeutics, and Script Biosciences, Privateer Holdings, Tilray, Receptor Life Sciences, Empatica, Engage, Egg Rock/Papa & Barkley, Rettco, SilverSpike, and California Cannabis Enterprises (CCE). Orrin Devinsky receives grant support from NINDS, NIMH, MURI, CDC, and NSF. He is an investigator for PTC Therapeutics, Inc., Stoke Therapeutics, Marinus, Ovid and GW Pharmaceuticals. Daniel Friedman receives salary support for consulting and clinical trial-related activities performed on behalf of The Epilepsy Study Consortium, a nonprofit organization. Dr. Friedman receives no personal income for these activities. NYU receives a fixed amount from the Epilepsy Study Consortium toward Dr. Friedman's salary. Within the past 2 years, The Epilepsy Study Consortium received payments for research services performed by Dr. Friedman from: Axcella, Biogen, Cerevel, Crossject, Engage Pharmaceuticals, Eisai, Pfizer, SK Life Science, Xenon, and Zynerba. He has also served as a paid consultant for Eisai and Neurelis Pharmaceuticals. He has received travel support from Medtronics, Eisai and the Epilepsy Foundation. He receives research support from the CDC, NINDS, Epilepsy Foundation, Empatica, Epitel, UCB, Inc and Neuropace unrelated to this study. He serves on the scientific advisory board for Receptor Life Sciences. He holds equity interests in Neuroview Technology and Receptor Life Sciences.

### References

1. Olson HE, Demarest ST, Pestana-Knight EM, et al. Cyclin-dependent kinase-like 5 deficiency disorder: clinical review. *Pediatr Neurol* 2019;97:18–25.
2. Dravet C, Bureau M, Oguni H, et al. Severe myoclonic epilepsy in infancy: Dravet syndrome. *Adv Neurol* 2005;95:71–102.
3. Chieffo D, Battaglia D, Lettori D, et al. Neuropsychological development in children with Dravet syndrome. *Epilepsy Res* 2011;95:86–93.
4. Genton P, Velizarova R, Dravet C. Dravet syndrome: the long-term outcome. *Epilepsia* 2011;52(Suppl 2):44–49.
5. Shmueli S, Sisodiya SM, Gunning WB, et al. Mortality in Dravet syndrome: a review. *Epilepsy Behav* 2016;64:69–74.
6. Welch EM, Barton ER, Zhuo J, et al. PTC124 targets genetic disorders caused by nonsense mutations. *Nature* 2007;447:87–91.
7. Du M, Liu X, Welch EM, et al. PTC124 is an orally bioavailable compound that promotes suppression of human CFTR-G542X nonsense allele in CF mouse model. *Proc Nat Acad Sci USA* 2008;105:2064–2069.
8. Ghosh S, Ganesan R, Amrani N, Jacobson A. Translational competence of ribosomes released from a premature termination codon is modulated by NMD factors. *RNA* 2010;16:1832–1847.
9. Lim BC, Hwang H, Chae JH, et al. SCN1A mutational analysis in Korean patients with Dravet syndrome. *Seizure* 2011;20:789–794.
10. Bahi-Buisson N, Bienvenu T. CDKL5-related disorders: from clinical description to molecular genetics. *Mol Syndromol* 2012;2:137–152.
11. Gidal BE, Pitterle ME, Spencer NW, Maly MM. Relationship between valproic acid dosage, plasma concentration and clearance in adult monotherapy patients with epilepsy. *J Clin Pharm Ther* 1995;20:215–219.
12. Kong R, Laskin OL, Kaushik D, et al. Ataluren pharmacokinetics in healthy Japanese and Causasian subjects. *Clin Pharmacol Drug Dev* 2019;8:172–178.
13. Ghodke-Puranik Y, Thorn CF, Lamba JK, et al. Valproic acid pathway: pharmacokinetics and pharmacodynamics. *Pharmacogenet Genomics* 2013;23:236–241.
14. Hirawat S, Welch EM, Elfring GL, et al. Safety, tolerability, and pharmacokinetics of PTC124, a nonaminoglycoside nonsense mutation suppressor, following single- and multiple-dose administration to healthy male and female adult volunteers. *J Clin Pharmacol* 2007;47:430–444.
15. McDonal CM, Campbell C, Torricelli RE, et al. Ataluren in patients with nonsense mutation Duchenne muscular dystrophy (ACT DMD): a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 2017;390:1489–1498.

16. Bushby K, Finkel R, Wong B, et al. Ataluren treatment of patients with nonsense mutation dystrophinopathy. *Muscle Nerve* 2014;50:477–487.
17. Linde L, Kerem B. Nonsense-mediated mRNA decay and cystic fibrosis. *Methods Mol Biol* 2011;741:137–154.
18. Du M, Liu X, Welch EM, et al. PTC124 is an orally bioavailable compound that promotes suppression of the human CFTR-G542X nonsense allele in a CF mouse model. *Proc Natl Acad Sci USA* 2008;105:2064–2069.
19. Spiegel RJ, Bedwell DM, Keeling KM, et al. Ataluren as a potential new treatment for nonsense mutation mucopolysaccharidosis type 1 (nmMPS1). 13th International Symposium on Mucopolysaccharidoses and Related Diseases, August 2014, Brazil (abstract).
20. Erickson CA, Davenport MH, Schaefer TL, et al. Fragile X targeted pharmacotherapy: lessons learned and future directions. *J Neurodev Disord* 2017;9:7.