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Antisense oligonucleotide therapy in an individual with KIF1A-associated neurological disorder

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KIF1A-associated neurological disorder (KAND) is a neurodegenerative and often lethal ultrarare disease with a wide phenotypic spectrum associated with largely heterozygous de novo missense variants in *KIF1A*. Antisense oligonucleotide treatments represent a promising approach for personalized treatments in ultrarare diseases. Here we report the case of one patient with a severe form of KAND characterized by refractory spells of behavioral arrest and carrying a p.Pro305Leu variant in *KIF1A*, who was treated with intrathecal injections of an allele-specific antisense oligonucleotide specifically designed to degrade the mRNA from the pathogenic allele. The first intrathecal administration was complicated by an epidural cerebrospinal fluid collection, which resolved spontaneously. Otherwise, the antisense oligonucleotide was safe and well tolerated over the 9-month treatment. Most outcome measures, including severity of the spells of behavioral arrest, number of falls and quality of life, improved. There was little change in the 6-min Walk Test distance, but qualitative changes in gait resulting in meaningful reductions in falls and increasing independence were observed. Cognitive performance was stable and did not degenerate over time. Our findings provide preliminary insights on the safety and efficacy of an allele-specific antisense oligonucleotide as a possible treatment for KAND.

Less than 10% of rare diseases have approved treatments[1]. Within rare diseases, there are nano-rare diseases with only 1–30 patients with the same pathogenic variant worldwide[2]. The rarity of these diseases often excludes them from drug development programs. While individually rare, these diseases collectively affect more than 263 million individuals worldwide[2]; thus, developing strategies to meet the needs of these patients is important[3]. The treatment reported in this study was made possible through the collaboration with the nonprofit n-Lorem Foundation, which aims to provide experimental antisense oligonucleotide (ASO)-based treatments to patients with diseases beyond the reach of traditional commercial drug programs because of their extremely low prevalence[2].

A splice-modulating ASO treatment was previously reported as a treatment for a child with Batten disease caused by biallelic loss-of-function (LOF) variants in *MFSD8* (ref. 4), highlighting the possibility of ASO-based treatment for personalized treatment. Another splice-modulating ASO was also well tolerated in a child with ataxia-telangiectasia[5]. Allele-specific ASO gapmers can be used for

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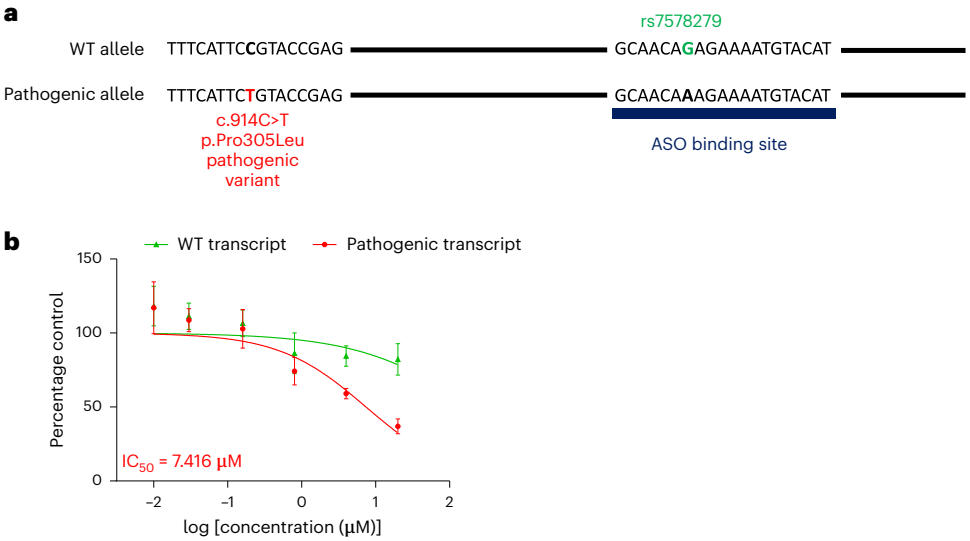

Fig. 1 | ASO design and potency. a, The pathogenic c.914C>T p.Pro305Leu variant is in *trans* with a common benign SNP (rs7578279) at position chr2:240737847A–G (hg38). The ASO binds the mRNA from the allele carrying c.914C>T p.Pro305Leu at positions chr2:240737833–240737853 (hg38), which overlaps with the benign SNP on the WT allele. **b**, Dose–response of nL-KIF1-001 in vitro. Induced pluripotent stem cell-derived neurons were treated by free uptake with increasing concentrations of ASO for 5 days. RNA was isolated and *KIF1A* mRNA was evaluated using quantitative PCR with reverse transcription (RT–qPCR) (TaqMan). nL-KIF-001 showed an IC_{50} of 7.4 µM and high selectivity for the mRNA from the pathogenic allele over WT *KIF1A*. Each data point was derived from six independent biological replicates where *KIF1A* expression was normalized to cyclophilin and compared to untreated control. Data from these replicates are expressed as the mean value ± s.d.

allele-specific degradation of a transcript encoding a mutant protein without altering the transcript from the wild-type (WT) allele and could be a treatment approach for diseases caused by dominant negative mechanisms[6,7].

KIF1A-associated neurological disorder (KAND) is a neurodegenerative and often lethal ultrarare disease that has been associated with largely heterozygous de novo missense variants. The phenotypic features include brain and optic nerve atrophy, refractory epilepsy, cognitive impairment, spasticity and neuropathy[8]. Heterozygous variants leading to *KIF1A* haploinsufficiency are associated only with adult-onset hereditary spastic paraplegia without seizures or cognitive impairment, a phenotype significantly milder than that associated with missense variants[9], suggesting that gapmer ASO-mediated treatment might provide a bridging treatment approach for dominant negative missense variants in *KIF1A* until other treatment options are available.

KIF1A is a neuron-specific homodimeric kinesin involved in the anterograde transport of cargo along axonal microtubules. In mice, KIF1A is necessary for hippocampal synaptogenesis and learning[10]. Epilepsy of variable severity is reported in 42% (ref. 9) of individuals with KAND and is thought to result from altered synaptic function[11]. The p.Pro305Leu variant in KIF1A, identified in only eight individuals worldwide, was shown to act as a dominant negative variant able to bind to microtubules but displaying reduced processivity and velocity[12].

We report the use of an allele-specific phosphorothioate (PS) and phosphodiester (PO) 2′-methoxyethyl (MOE) gapmer individualized ASO in a 9-year-old female with KAND.

The participant is a 9-year-old female patient previously reported as case 3 by Kaur et al.[12]. She initially presented with global developmental delay. She has had progressive spasticity of the lower limbs and painful peripheral neuropathy. Her ambulation has deteriorated from being completely independent to frequently falling, with multiple bone fractures and requiring a wheelchair.

Before ASO treatment, the patient communicated using simple sentences with frequent pauses mid-sentence (Supplementary Video 1).

From 3 years of age, she has had spells of behavioral arrest concerning for seizures but without electrographic correlate on electroencephalogram (EEG). Her EEG recordings have shown diffuse slowing and disorganization in wakefulness and abundant epileptiform activation during sleep, with spikes present for 80% of the non-rapid eye movement sleep recording. The clinical episodes and interictal epileptiform activity were not responsive to medications, including lamotrigine, levetiracetam, lacosamide and benzodiazepine treatment.

This patient is heterozygous for a de novo pathogenic c.914C>T (p.Pro305Leu) variant in *KIF1A*; we refer to this allele as the 'pathogenic allele'. Using long-read sequencing for phasing, the c.914C>T variant was found to be in *trans* with the alternate allele for a common single-nucleotide polymorphism (SNP) (rs7578279) in intron 37 of 48 of *KIF1A* (NM_001244008.2). We refer to the allele carrying the rs7578279 variant and not carrying the pathogenic c.914C>T variant as the WT allele.

The drug named nL-KIF1-001 is the 19-sodium salt of a 20-base (20-mer) oligonucleotide that binds the reference sequence in *cis* with the *KIF1A* pathogenic variant (Fig. 1a). The drug is injected intrathecally and dose escalation was done in 20-mg increments (Extended Data Table 1).

The dose–response analysis demonstrated a half-maximal inhibitory concentration (IC_{50}) of 7.4 µM. There was no significant reduction of mRNA level from the WT allele at any concentration tested, highlighting the high selectivity of nL-KIF1-001 for the mRNA from the pathogenic allele over the WT allele (Fig. 1b).

The first intrathecal (IT) administration was complicated by an epidural cerebrospinal fluid (CSF) collection at the injection site causing back pain that necessitated an external 4% lidocaine patch, acetaminophen and increased doses of gabapentin for 10 days, and leading to inability to walk for 7 days. The patient fully recovered 11 days after ASO administration once the CSF collection resorbed, which was confirmed by repeat magnetic resonance imaging. Because it was unclear if or how much of this first dose went into the epidural space, the 20-mg dose was repeated 1 month later.

During the 9-month study, no other adverse events were observed. Clinical signs of increased intracranial pressure were specifically monitored and none were seen during treatment. Laboratory tests for safety assessments (Extended Data Table 1) remained within normal during the study. The patient is still being treated.

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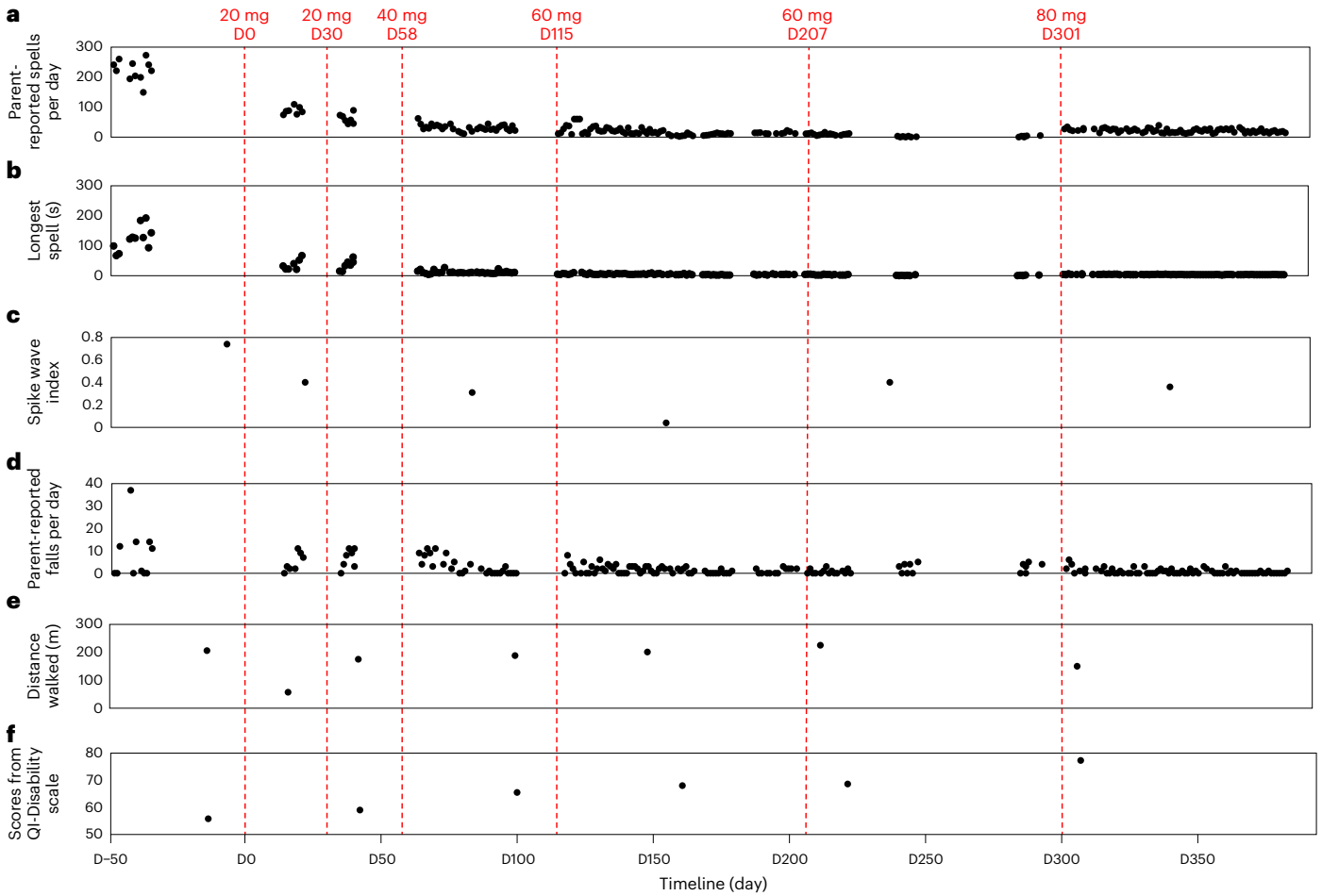

Fig. 2 | Clinical outcomes from 50 days before the first dose to day 360. The dates of dosing are represented by the dashed red lines. **a**, Number of spells of behavioral arrest as reported by the parents. **b**, Duration of the longest spells of behavioral arrest as reported by the parents. **c**, SWI as determined using overnight EEG. **d**, Number of falls per day as reported by the parents. **e**, Distance walked during the 6-min Walk Test. **f**, QoL determined using the Quality of Life Inventory-Disability (QI-Disability) scale. Blanks correspond to the absence of data. Because of their high frequency, the parents were unable to accurately count the number of seizures on days 18, 198 and 203.

At run-in (the 50 days before the first dose), surveys completed by the parents reported between 100 and 290 spells of behavioral arrest per day in the 4 months before the first dose, each lasting from 14 to 240 s. Events were characterized clinically by behavioral arrest with unresponsive staring, sometimes with eye rolling and sometimes leaning to the side and falling. While clinical observation strongly suggested epileptic seizures and her interictal EEG demonstrated epileptiform abnormalities indicating a risk for seizures, the events themselves were not accompanied by consistent electrographic changes on the EEG. Throughout, the EEG showed generalized slowing. The posterior dominant rhythm, the hallmark of normal electrocerebral organization in wakefulness, was rarely seen and slow for her age, which is consistent with diffuse cerebral dysfunction (Extended Data Fig. 1). At baseline, her sleep EEG showed abundant multifocal spikes with a spike wave index (SWI) of 74% (Fig. 2).

The daily number of spells of behavioral arrest reported by the parents dropped after the first dose and was less than 30 in the week after the most recent dose of 80 mg. In addition to lower spells of behavioral arrest frequency, the longest was considerably shorter (Fig. 2). There were three days (days 18, 198 and 203) when the spells of behavioral arrest were too frequent to be counted. After initiating the ASO, the EEG demonstrated improvements with greater organization after the second dose that persisted, with a reduction in the frequency of epileptiform spikes (SWI = 36% by day 343, 42 days after the sixth dose) (Fig. 2).

At baseline, walking was severely impaired due to spasticity; the parents reported an average of 26.2 falls per day (s.d. = 19.8) in the 4 months before the first dose. The rare days without falls before dosing corresponded to the days when she was in her wheelchair and not ambulating. Improvement in gait was noted in the month after the first dose, and the number of falls decreased to a maximum of seven per day, with many days without falls (mean = 0.75 and s.d. = 1.19) (Fig. 2) despite an increase in overall activity and ambulation.

There was improvement in quality of speech, with longer sentences, less dysarthric speech and improved prosody and intonation. Her level of attention was improved, with faster response time to tasks and improved ability to follow multistep commands. The motor exam showed improvement in pulling to stand, initially needing assistance and later able to stand up from sitting on the floor with a modified Gower distance. There were improvements in hand tremor amplitude, dysmetria on finger-to-nose testing and ataxia when walking. Spasticity persisted in both lower extremities with progressive scissoring.

There was minimal change in the distance walked during the 6-min Walk Test (Fig. 2). During the study, we assessed fine motor skills with the nine-hole peg test performed after the fifth and sixth doses, which showed mild improvement with the dominant hand. However, the assessment was complicated by a lack of participant concentration at one time point. The Gross Motor Function Measure-66 score also showed mild improvement over the assessment period (56.9 at run-in

and 61.2 after the fifth dose). Neurofilament light chain in the CSF was stable over time.

The patient was screened for visual and motor confounds on each subtest of the Differential Ability Scales-Second Edition with training trials; she successfully passed items to proceed with each subtest[13]. Her cognitive performance was generally stable over three time points. Run-in evaluation at day −158 indicated average verbal abilities ($\bar{X}$ = 98), low nonverbal abilities ($\bar{X}$ = 77) and very low spatial abilities ($\bar{X}$ = 34). Her performance was comparable at day 227, with low end of average verbal abilities ($\bar{X}$ = 87), low nonverbal abilities ($\bar{X}$ = 74) and very low spatial abilities ($\bar{X}$ = 50). Her memory performance was also low ($\bar{X}$ = 70, s.d. = 3.78) across time points. Her overall intellectual composite was stable ($\bar{X}$ = 65, s.d. = 2.6) (Extended Data Fig. 2).

Over the course of treatment, the parents reported an improvement in engagement, connectivity, awareness, speech fluency and complexity, and ability to interact in group activities. There was an increase in the quality of life (QoL) score from 55.8 at baseline to 77.3 at day 308 (Fig. 2)[14]. At the last evaluation, there were still unmet needs, including pain in the feet, difficulty stepping, gastroesophageal reflux and behavioral outbursts.

Determining the optimal dose and frequency for an ASO in an *n* of 1 treatment requires a combination of frequent evaluations and objective and subjective assessments to evaluate efficacy. In this study, the spells of behavioral arrest and fall diaries were used to assess clinical impact. EEGs provided further objective evidence of impact on neurophysiological function, as reflected by improved organization and reduced epileptic irritability.

The ASO did not address all manifestations of KAND. Residual neuropathy suggests that there are still unmet treatment challenges. Completing the seizure and fall diaries is burdensome for parents and caregivers and not all days were captured, particularly when the participant was away from home. For future studies, wearables may reduce this burden. Standardized assessment of spasticity should also be added.

The measure of genetic constraint for the *KIF1A* gene against LOF variants (pLI of 1) in the general population suggests that *KIF1A* haploinsufficiency is not without consequence[15]. However, people with heterozygous LOF variants have a much less severe phenotype than missense variants. The current ASO strategy has provided evidence that it is clinically possible to adequately modulate KIF1A, leading to substantial improvement in QoL. However, this treatment will not affect all symptoms (for example, visual symptoms due to optic nerve atrophy) due to the route of administration.

Developing and implementing *n* of 1 treatments has many challenges, especially the considerable work needed for each case and ASO. While acknowledging the benefits of the US Food and Drug Administration (FDA) guidance documents for individualized ASO[16–19] to facilitate the discovery and development pathway, as well as the investigator-initiated new drug (IND) application, this process still requires substantial expertise, such as ASO design, methodology, toxicology, regulation and clinical management. A close partnership with the patients and families is required to set expectations, ensure protocol compliance and discuss risks and benefits. The n-Lorem Foundation has submitted 12 INDs as of 15 March 2024 for different indications, target organs and including individualized outcome measures specific for each patient. The n-Lorem Foundation has built the needed infrastructure to support *n* of 1 programs from ASO discovery to patient treatment, which represents a large-scale effort for patients with nano-rare mutations who have no other treatment opportunities.

Our findings support the use of an allele-specific ASO as a possible treatment for KAND, and we are planning to treat other patients. Because of phenotypic variability in this condition according to variant and over the lifespan, each patient will require individualized outcome measures tailored to their age, cognitive function and disease stage, including visual impairment. A future goal is to start treatment as early as possible to prevent irreversible damage.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41591-024-03197-y.

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Methods

Ethics

Consent from the parents was obtained for the treatment and for the publication of the data, including the videos of the patient. A written informed consent was obtained from the parents. After authorization by the US FDA to commence treatment under an IND protocol (IND no. 161670), administration of the ASO was conducted, with concurrence of the Columbia University institutional review board (protocol no. AAAS9161).

Drug design

ASOs were designed to promote selective degradation of the mutant *KIF1A* transcript through recruitment of RNase H1 to the RNA–ASO heteroduplex.

The initial screen targeted 77 heterozygous sites in the patient's *KIF1A* genomic sequence using 468 gapmer ASOs. The lead ASO was identified from the primary screen for which several ASOs were designed to each polymorphism. The lead ASO was named nL-KIF1-001 and bound the reference sequence in *cis* with the *KIF1A* pathogenic variant (Fig. 1a). This approach allowed for differential binding and reduction of pre-mRNA from the pathogenic allele, while sparing pre-mRNA from the reference allele.

nL-KIF1-001 sodium is the 19-sodium salt of a 20-base (20-mer) oligonucleotide. The oligonucleotide general structure is a 5-10-5 MOE gapmer and includes five nucleotides at the 5′ end and five nucleotides at the 3′ end that are modified at the 2′-O of ribose with an MOE group. The nL-KIF1-001 sodium sequence is written as 5′-AUoGoUoACATTTTCTTTGoUoUGC-3′.

The underlined nucleotides contain the MOE modification, while 'o' indicates PO linkages. All other linkages are PS. All cytosine bases were modified at the 5′ position with a methyl group, as were the uracil bases in the MOE nucleotides. The ten nucleotides in the middle all contained 2′-deoxyribose.

In vitro potency and allele selectivity were determined according to a dose–response of nL-KIF1-001 in induced pluripotent stem cell-derived neurons from the patient's fibroblasts using free uptake at concentrations of 0.01, 0.03, 0.16, 0.80, 4.0 and 20.0 μM for 5′ (Fig. 1b). The expression level from each allele was then measured using tagged probes ('WT transcript' tagged in green; 'pathogenic transcript' tagged in red). *KIF1A* mRNA was quantified using RT–qPCR.

Toxicity

The potential toxicity of nL-KIF1-001 was assessed in an 8-week single intracerebroventricular dose study in mice, an 8-week single IT dose study in rats, and good laboratory practice-compliant, 13-week, once monthly IT dose study in rats.

As quality control, multiple release tests were performed, including tests for appearance (lyophilized white to off-white cake), identity (within 2 Da of an expected mass of 7,070.0 Da), identification (synthesis report matched the expected sequence), purity (determined as 98.2% pure by high-performance liquid chromatography), moisture content (reported as 0.71%), sodium content (5.75%), bacterial endotoxin (0.032 EU mg^{-1}), pH (7.46) and bioburden (total aerobic microbial count and total yeast and mold counts less than 100 colony-forming units g^{-1} each).

Dose selection

The selection of appropriate clinical doses was based on previous experience in IT ASO clinical trials with 2′-MOE mixed backbone gapmers. A starting dose of 20 mg for this patient was proposed based on the similarity of potency of this ASO to others evaluated at this pediatric age, in this chemical class, using this design and route of administration, in addition to the severity of the patient's condition. Dose escalation was done in 20-mg increments at the physician's discretion.

More than 10,000 patients have been treated with PS 2′-MOE ASOs in clinical trials and commercially. After IT administration, PS 2′-MOE ASOs distribute broadly throughout the spinal cord and central nervous system and are broadly pharmacologically active. To date, intrathecally administered mixed PS and PO backbone 2′-MOE ASOs have been generally well tolerated and safe over a range of doses (10–120 mg) and schedules (monthly, every other month and quarterly). Drug-related adverse events have been limited, with transient radiculopathy that was self-limited observed most frequently when HTT-Rx was administered monthly at a 120-mg dose and reduced in incidence with an every-other-month dosing. Other adverse events noted that may be drug-related included an increase in CSF protein levels. It is important to note that PS 2′-MOE ASOs differ substantially in their potential toxicities compared to PS 2′-locked nucleic acid ASOs, which have been associated with adverse events after IT administration.

Manufacture of nL-KIF1-001

nL-KIF1-001 was manufactured by the ChemGenes Corporation according to good manufacturing practices. nL-KIF1-001 was reconstituted in Elliotts B Solution and filtered through a 0.22-μm polyvinyldene fluoride membrane by the Columbia research pharmacy at the concentrations required to meet the dose level requirements. The dose was administered in a volume of 10–14 ml. Before dosing, the formulated nL-KIF1-001 was kept at room temperature for no longer than 4 h.

Study design

The objectives of the study were to evaluate the safety and efficacy of nL-KIF1-001. The *n* of 1 open-label study was designed as a dose escalation study, starting at 20 mg and increasing incrementally by 20 mg, up to a potential maximum dose of 80 mg (Extended Data Table 1).

Clinical efficacy was measured using a battery of parent-reported outcomes and clinical measures. The parents were asked to report the number of falls, number of spells of behavioral arrest and the length of the longest spells of behavioral arrest in a daily diary. Standardized motor evaluations (6-min Walk Test), clinical neurological exams, QI-Disability scale and overnight EEGs were obtained at baseline and between each dosing. The 6-min Wak Test measures how far a person can walk in 6 min; a higher score correlates with higher function. The QI-Disability scale is a 32-item Likert scale specifically developed to assess the QoL of children with intellectual disability. This 100-point scale focuses on the following six domains: physical health; positive emotions; negative emotions; social interaction; leisure and the outdoors; and independence. Higher scores correlate with a better QoL. The Differential Ability Scales-Second Edition is a measure of general cognitive abilities and was performed at baseline and day 225.

The primary endpoint was a change in frequency of spells of behavioral arrest compared to baseline. Secondary endpoints were change in mobility using the 6-min Walk Test and the mean number of falls each day over time.

The experiments were not randomized and the investigators were not blinded to allocation during the experiments and outcome assessment. No compensation was provided to the patient and her family.

The ASO was administered via IT injection under sedation. From baseline to the last dose of ASO, the antiepileptic medical treatment was unchanged. Safety assessment is detailed in Extended Data Table 2 and Supplementary Table 1.

Clinical data were gathered in a dedicated REDCap server (v.13.4.2).

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

To protect the privacy of the patient, the phenotypic data generated during the current study are available upon request from the corresponding author (wendy.chung@childrens.harvard.edu) within a month on request and completion of a data transfer agreement. Source data are provided with this paper.

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Author contributions
A.Z., J.C., J.M.B., T.T.S., R.J.F., D.U., C.H.K., J.M. and W.K.C. contributed to the clinical management of the patient. S.G., J.D., L.M., J.G.G. and S.T.C. created the ASO. All authors were involved in the writing of the paper.

Competing interests
W.K.C. is on the Board of Directors at Prime Medicine and Rallybio. The other authors declare no competing interests.

Additional information
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Correspondence and requests for materials should be addressed to Wendy K. Chung.

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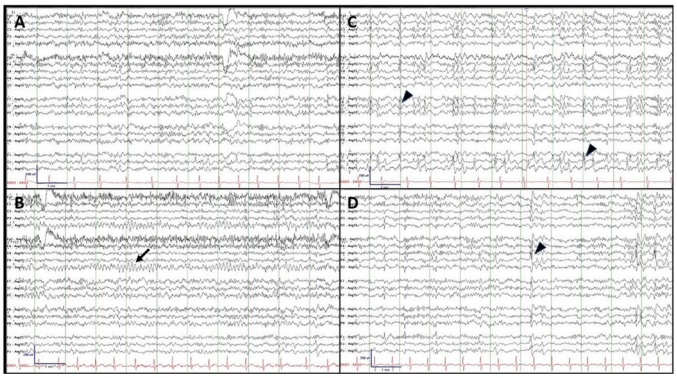

Extended Data Fig. 1 | Run-in and post-treatment electroencephalography. Run-in (**a, c**) and post-treatment (**b, d**) electroencephalography (EEG). Awake EEG (**a, b**) shows diffuse slowing and excess beta frequency activity related to benzodiazepine therapy. A posterior dominant rhythm (PDR) of 9 Hz is appreciated (arrow) in B, recorded after the third dose. Sleep EEG (**c, d**) shows abundant spikes (arrowheads) in the temporal and parietal regions at the onset of sleep. A modest improvement in spike incidence is shown in D, recorded after the second dose.

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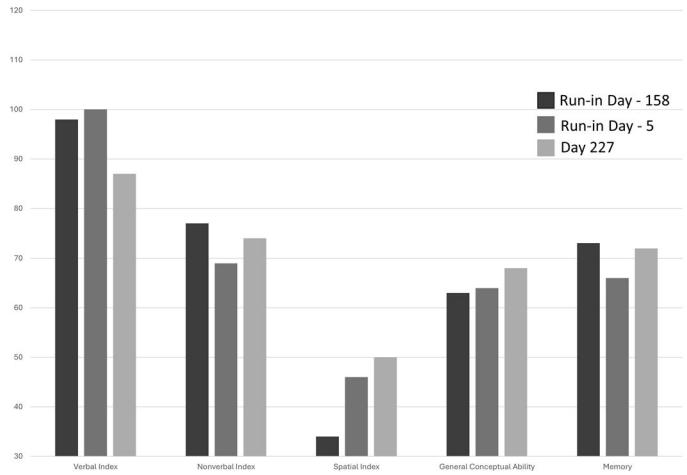

Extended Data Fig. 2 | Cognitive performance over the course of the study. Cognitive performance over the course of the study as evaluated by the differential ability scales-second edition (DAS-II).

https://doi.org/10.1038/s41591-024-03197-y

Extended Data Table 1 | Actual dosing regimen and dose escalation

Dose	Day	Injected volume	Notes
20mg	0	15	Large epidural collection at the site of injection. Dose effectively delivered to the central nervous system is thought to be low.
20mg	30	10	
40mg	58	10	
60 mg	115	14	
60 mg	207	14	
80 mg	301	14	

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Extended Data Table 2 | Clinical laboratory tests for safety assessments

Serum Chemistry	Hematology	Urinalysis	Cerebrospinal fluid analysis
Albumin Blood Urea Nitrogen (BUN) Calcium Bicarbonate Chloride Creatinine Glucose Magnesium Phosphate Potassium Sodium Total Bilirubin Total Protein Alanine Aminotransferase (ALT) Alkaline Phosphatase (ALP) Aspartate Aminotransferase (AST) Creatinine Clearance	Hematocrit (Hct) Hemoglobin (Hgb) Red Blood Cell Count (RBC) White Blood Cell Count (WBC) WBC differential Absolute Neutrophil Count (ANC) Platelets Mean Corpuscular Volume (MCV) Mean Corpuscular Hemoglobin (MCH) Mean Corpuscular Hemoglobin Concentration (MCHC)	Color Clarity/turbidity pH Specific gravity Glucose Ketones Nitrites Leukocyte esterase Bilirubin Urobilinogen Blood Protein RBCs WBCs	Cell count Total protein Glucose