Individualized Antisense Oligonucleotides for SCN2A Related Developmental Epileptic Encephalopathy

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Article

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Abstract

SCN2A variants are one of the most common genetic causes of intractable epilepsy in children, particularly in developmental and epileptic encephalopathies (DEEs) which can present with uncontrolled seizures at birth, accounting for 1-2% of all epileptic encephalopathies. There is significant genotype-phenotype heterogeneity in SCN2A-related disorders (SRD) which include neurologic symptoms of seizures, developmental delay, choreoathetosis, and autism spectrum disorder (ASD). A substantial fraction of causal variants is gain-of-functon(GOF) or mixed function, functionally associated with increased open-probability or greater sodium current flux. Individualized allele-selective antisense oligonucleotides (ASOs) were designed to target heterozygous intronic SNPs for decreased expression of mutant SCN2A transcript while preserving the wild-type copy. Efficacy measures were also individualized to phenotype. Improvements in seizure control, development, and quality-of-life were seen with no ASO-related adverse events. Haplotype phasing in a separate cohort of infants with SRD diagnosed by rapid whole genome sequencing identified 16% of patients with compatible SNPs. Allele-selective ASOs demonstrate potential to decrease seizures and impact neurodevelopment, providing a pathway for potential benefit in n-of-1 to n-of-more SRD patients.

INTRODUCTION

Epilepsy is one of the most common chronic neurological disorders of childhood associated with high disease burden, morbidity, and increased risk of mortality.[1] Channelopathies have emerged as a critical contribution to medically intractable seizures, affecting 30% of diagnosed patients and associated with increased incidence of sudden unexpected death in epilepsy. [1,2] DEE11 is a severe neurodevelopmental disorder caused by gain-of-function (GOF) and mixed function variants in the *SCN2A* gene, encoding the neuronal sodium channel NaV1.2 alpha subunit. Specific biophysical changes, including voltage sensing and ion flux alterations, result from individual missense variants, dependent on the location and amino acid substitution.[2] The phenotypic spectrum in *SCN2A*-related disorders (SRDs)encompasses intractable epilepsy, profound developmental impairment, communication and behavioral issues, movement disorders, autism spectrum disorder (ASD), and associated morbidity.[3] Current treatment options for DEE11 are symptomatic with sodium channel-blocking antiseizure medications (ASMs) that are often ineffective. Furthermore, because haploinsufficiency in *SCN2A* is one of the most common known genetic causes of autism[3], allele-selective ASO design is an important consideration forgenetic intervention in mixed gain and loss of function SRD variants.

Antisense oligonucleotides (ASOs) have emerged as effective disease-modifying therapies in several neurological disorders such as spinal muscular atrophy and amyotrophic lateral sclerosis and provide an opportunity to selectively correct effects of pathogenic DNA variants. In an *SCN2A* GOF mouse epilepsy model, an *SCN2A* gapmer ASO down-regulated mutant GOF transcripts, reduced seizures and extended life span.[5] A non-allele-selective ASO reduced seizures in a premature infant with early-onset SRD[6]. While promising, these strategies non-selectively targeted both copies of the gene, which in some

patients may have undesired consequences from reduction of both reference and pathogenic transcript. In contrast, allele-selective gapmer ASOs utilize specific single nucleotide polymorphisms (SNPs) to distinguish mutant from reference haplotypes, only reducing target RNA transcript without affecting expression of the healthy copy.[7] Such ASOs are often developed in the context of an individual patient, as the ASO design strategy is highly specific to the particular sequence of each allele. While individualized, allele-selective ASOs are relatively newly described as drugs,[7,8] diagnoses of toxic GOF and mixed function single-gene variants in DEEs have allowed the possibility of use of these drugs on larger scales than previously thought possible and are attractive for conditions for which targeting non-selectively could produce untoward risk, especially for essential genes like *SCN2A*.

METHODS

Patients and Study Design

Individualized ASOs were designed for two patients with intractable epilepsy with GOF and mixed GOF/LOF causal variants associated with DEE11. Whole genome sequencing identified SNPs on the reference haplotype allowing the design of ASOs targeting only the pathogenic haplotype. A distinct lead ASO was identified for each patient and assessed for allele selectivity in patients induced pluripotent stem cells (iPSCs) (Supplemental Figure 1). A single dose non-

Good Laboratory Practice (GLP) and repeat dose GLP toxicology studies in rodents were performed. Drug substance and drug products were manufactured under (Good Manufacturing Practice) GMP conditions. The SNPs used for allele-selectivity for Patient 1 and 2 had population allele-frequencies of 0.0002 and 0.18, respectively, suggesting other patients could potentially be treated with the ASO for Patient 2 (Supplemental Figure 2).

Research investigational new drug (INDs) applications were authorized by the FDA for investigator-initiated, open-label, single center, single patient (n=1) clinical trials for distinct pathogenic SRD variants with predefined safety and efficacy measures tailored to individual phenotype. The allele-selective ASOs were delivered intrathecally by lumbar injection in these two first-in-human clinical trials. Each trial was customized to the patient-specific SRD phenotype with predefined outcomes measures assessing seizures, behavior, communication, motor skills and quality-of-life. Following baseline assessments, ASOs were dose-escalated per respective individualized protocol schedule of activities. Dosing frequency was adjusted during the study guided by ongoing efficacy data.

The research studies were approved by ethics committees of the respective academic institutions and informed consent obtained from study participants.

RESULTS

Clinical Characteristics

Patient 1 was a 9-year-old male with a heterozygous *SCN2A* GOF pathogenic variant (c.5645 G>A, p.Arg1882Gln) and history of neonatal onset seizures. He had severe intellectual disability and ASD. He was nonverbal, could communicate with an assistive communication board and had good gross motor skills with normal gait. He had seizures medically refractory to >10 ASMs with ~30 seizures per month for years requiring rescue medications administered twice a week. He had recurrent status epilepticus requiring frequent emergency department visits. He was treated with cenobamate and phenytoin at baseline and required high dose phenytoin levels since infancy for sodium channel blockade.

Patient 2 was a 14-year-old male with a heterozygous pathogenic mixed GOF/LOF *SCN2A* variant (c.2558G>A, p. Arg853Gln) and history of infantile spasms at 8 months of age. He had history of multiple seizure types including myoclonic, focal, and tonic seizures which were medically refractory to >10 ASMs with referral to hospice at 2 years of age and near-daily seizures prior to study initiation. He had severe global neurodevelopmental delay and non-verbal at baseline, able to communicate by assistive communication board. He was non-ambulatory with prominent choreoathetosis and dyskinesias. He had significant chronic gastrointestinal issues regularly requiring suppository use.

A description of the individual genotypes and phenotypes of the patients is provided in Table 1. Research protocols and schedule of activities, including predefined treatment goals and measures individualized to phenotypes, are included in the Supplementary Appendix.

Seizures

Both patients had improvements in seizure control and quality-of-life, including prolonged periods of seizure freedom and reduction in concomitant medications.

Patient 1 experienced improvement in seizure control with <80 mg doses and a dosing interval of 60-75 days. Dosing interval was shortened due to recurrent seizures toward the end of the 90 day dosing interval. He demonstrated ~50% reduction in seizure frequency. This was maintained after weaning off phenytoin, a sodium channel blocker with significant side effects, on which he had been dependent since birth for seizure control (Figure 1A and 1B), suggesting evidence of target engagement by ASO for decreased abnormal mRNA expression and decreased presence of aberrant hyperactive sodium channels.

Patient 2 experienced seizure free days early in treatment (after 20 mg and 30 mg) but had bouts of seizures at 40 mg dosing. Due to waning efficacy seen 60 days post dosing, dosing interval was decreased to 60-90 days, resulting in periods of seizure control without emergency seizure rescue medications for >2-month intervals (Figure 1C and 1D).

Both patients had sustained seizure-free periods of consecutive weeks to months, and neither had ASO-related emergency room visits or hospital admissions.

Neurodevelopmental outcomes

Changes in neurodevelopmental skills were seen across multiple outcome domains in both patients. For Patient 1, there were improvements in Growth Scale Values (GSVs) across all subscales with an improved annualized rate of growth in some domains on the Bayley Scales of Infant and Toddler Development (BSID-4, Figure 2a). Patient 1 also showed clinically meaningful improvement on ORCA (Figure 2b), suggesting an increased rate of development in language and motor skills, notable at the patient's current age where slowing of the rate of cognitive growth with age and developmental would typically be expected. Aberrant, stereotyped and abnormal sensory behavior also improved (Supplemental Figure 3, a-c).

Patient 2 demonstrated clinically meaningful changes in communication (Figure 2c) and sustained trends toward reduction of maladaptive behaviors and irritability (Supplemental Figure 4b). Patient 2 also had significant reduction in ataxia and improvement in motor ability, including developmental motor milestone of independent gait at 15 years of age (Figure 2d, Supplemental Figure 4c).

Other domains

Improvement in quality-of-life was observed across other domains. Patient 2 had required frequent suppositories for severe gastrointestinal symptoms which were tracked with the Bristol Stool Scale (Supplemental Figure 4c). Post-ASO, this patient required minimal suppository use and normalizing bowel movements, suggesting that ASO treatment positively modulated autonomic dysfunction.

Safety

No ASO-related adverse events or serious adverse events were reported in either patient (Supplemental Table 1). Both ASOs were well tolerated with no abnormal biochemical or hematological labs and no significant changes in EKG or EEG from baseline throughout the study.

DISCUSSION

Individualized allele-selective ASOs for *SCN2A* DEE11 variants reveal positive safety and efficacy signals with clinically meaningful improvements across multiple domains in children with differing genotypes and phenotypes. These results support that NDD phenotypes may remain at least partially treatable beyond early development, as seen in ASO trials for Angelman syndrome.[9] Of note, the ASOs were effective both for GOF and mixed function pathogenic variants and in the setting of distinct *SCN2A*-associated phenotypes, including DEE11 and ASD.

Dose optimization posed specific complexities due to concurrent use of sodium channel-blocking ASMs, risk of seizure exacerbation from excessive sodium channel reduction, potential circuit-level effects, and long-term neural adaptation to underlying pathophysiology.

These n-of-1 trials required pre-defined individualized treatment goals, and patients demonstrated gains beyond typical developmental windows, providing substantial insights regarding evolving and effective trial design and implementation of outcome measures.

In combination with rapid WGS, which allows genetic diagnosis of DEE11 within days of first-time seizures, targeted disease-modifying therapies have the potential to not only decrease seizures but also impact neurodevelopment. The allele-selective ASO developed specifically for Patient 2 has the potential to be used in patients with other causal *SCN2A* variants if they are heterozygous for the same SNP on the reference haplotype, as this could confer the needed selectivity to downregulate expression from the pathogenic haplotype. We analyzed a cohort of 19 SRD probands that were identified through rapid WGS at Rady Children's Institute for Genomic Medicine between 2018-2024, diagnosed from 2 weeks-of-age up to 12 years of age [10]. 16% in our cohort were identified to have the correct SNP configuration amenable to ASO treatment, supporting the potential to enable use of reference sequence-targeting ASOs for additional patients, allowing extension of "n-of-1" ASOs to "n-of-many" for dominant SRDs.

CONCLUSION

Open-label investigational individualized ASO therapy demonstrate positive safety and efficacy in two patients with different causal GOF and mixed GOF/LOF *SCN2A* variants and phenotypes, with improvement in quality-of-life, reduction in seizures and concomitant medications, decrease in autistic behaviors, and improvement in neurodevelopmental outcomes with disease-modifying effect, offering new treatment paradigms for more patients with SRD and other monogenic DEEs.

Declarations

The patients' legal guardians provided consent for participation in research and publication of study findings, which include photograph(s) and/or videos and/or case history and/or identifiable details within the text ("Material") to be published.

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Disclosures

L.M., H.P., J. D., C.P., S.G. and S.T.C. are employees of the n-Lorem Foundation. F.B. is an employee of Ionis Pharmaceuticals. S.P. is an employee of Praxis Precision Medicines. J.G.G. is a paid consultant for Ionis Pharmaceuticals and n-Lorem Foundation.

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Figures

Figure 1

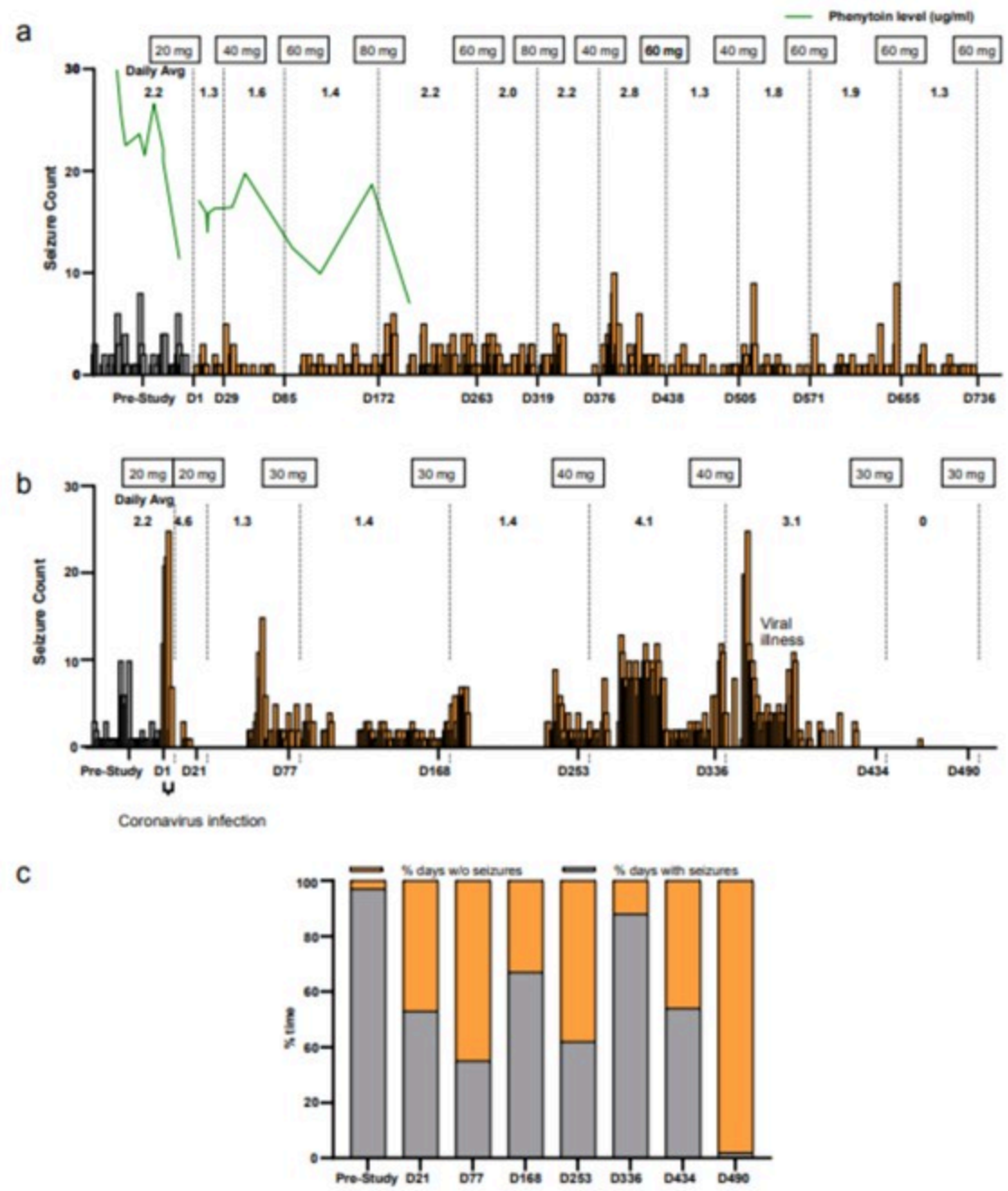

Figure 1

Analysis of changes in daily countable motor seizure frequency (CMSF) across time.

(a) Decrease in Patient 1 CMSF was observed after ASO initiation. To date, patient 1 received 12 doses of ASO over 24 months. His pre-treatment daily average seizure count was 2.2, which reduced to between 1.3 and 1.6 in the first 6 months of treatment (D1 to D172) and between 1.3 and 1.9 in the last 9 months (since D438). Of note, the patient previously required high doses of phenytoin since birth (green line representing phenytoin levels), which was fully weaned off in June 2024 (~Day 310), suggesting ASO efficacy with reduced sodium channel blocker

requirement. (b) Decrease in Patient 2 CMSF was observed after ASO initiation. To date, Patient 2 received 9 doses over 18 months, with an average of 2.0 daily average seizure count pretreatment to 0 average after 14 months of treatment. (c) Patient 2 percent of seizure free days.

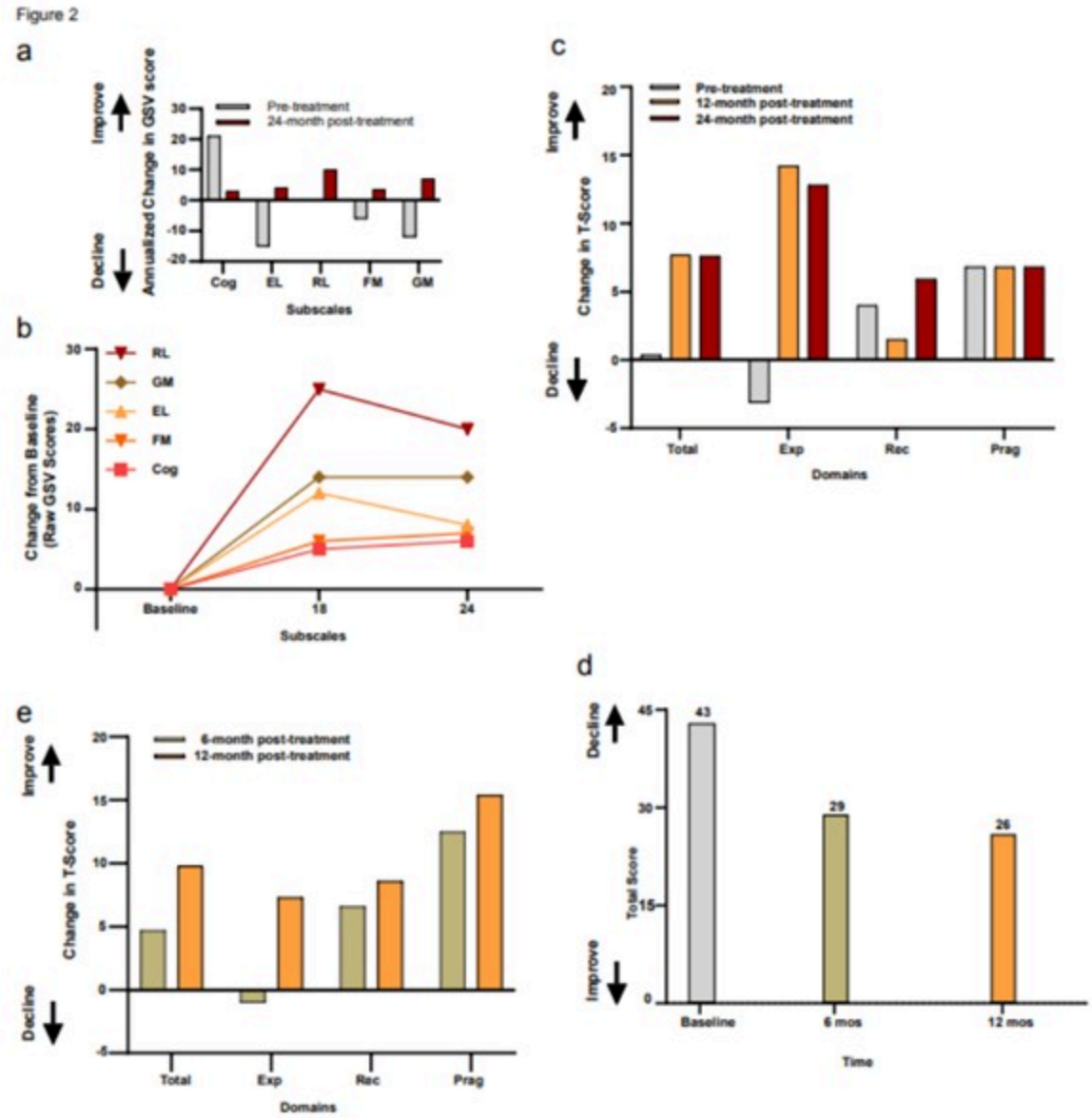

Figure 2

Analysis of changes in neurodevelopmental and communication measures across time. Improvements in neurodevelopmental skills were seen across multiple outcome domains in both patients.

(a) Annualized rate of change in different BSID-4 domains pre- and post-treatment showing improvement in rate in all domains except cognitive; (b) Actual GSV change from baseline (right) over time; Cog = cognitive, EL = expressive language, RL = receptive language, FM = fine motor, GM = gross motor. For Patient 1, improvements in Growth Scale Values (GSVs) were seen

across all subscales with an improved annualized rate of growth in some domains on the Bayley Scales of Infant and Toddler Development; (c) ORCA change from baseline pre- and post-treatment in Patient 1;

Exp = expressive, Rec = receptive, Prag = pragmatic. Patient 1 also showed clinically meaningful improvement on ORCA (Figure 2 Panel B); (d) ORCA change from baseline in Patient2, Exp = expressive, Rec = receptive, Prag = pragmatic. Patient 2 also had clinically meaningful changes in ORCA. This suggests an increased rate of development in language and motor skills, which is notable at the patient's current age, where slowing of the rate of cognitive growth with age and developmental would typically be expected. Aberrant, stereotyped and abnormal sensory behavior have also significantly improved. (e) Dyskinetic Cerebral Palsy Functional Impact Scale (D-FIS) at baseline and over-time. Patient 2 had reduction in ataxia and improvement in motor ability, including developmental milestone of independent gait at 15 years of age.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- nL00333TreatmentProtocolSCN2AV512February2024CLEANpatient1.pdf
- nL00001SCN2AProtocolV1019June2025CLEANpatient2.pdf
- SUPPLEMENTALAPPENDIX.docx