N1C ANNUAL MEETING 2024

Individual Journeys, Shared Discoveries:
Partnering for Progress in Individualized Medicines

OCTOBER 5 | MONTREAL, QUEBEC

2025 Annual Meeting – Spring 2025 | Date & Location To Be Announced

WHO WE ARE

OUR STORY

In 2018, a seven-year-old girl named Mila with a fatal neurogenetic disease became the first person in the world to receive a drug tailored to a single patient. The drug was named milasen. In a remarkable collaboration between scientists, physicians, drug developers, foundations and regulators, this novel medicine was created, tested and delivered in just one year from her diagnosis. While Mila would eventually succumb to her condition, milasen suppressed her seizures and improved her quality of life. The 2019 publication of this effort in the New England Journal of Medicine drew international attention as the first example of individualized genomic medicine, setting a precedent and giving new hope to patients with conditions considered too rare to support treatments.

OUR MISSION

The N=1 Collaborative is a 501(c)3 non-profit organization building global collaborations to advance individualized medicines through developing an end-to-end pathway that is rigorous, reproducible, and openly available to researchers, clinicians, drug developers, families and advocacy groups.

OUR WORK

- Data Coordination Center
- Knowledge Base
- Connections Platform
- Donated Resource Center
- Annual Meeting

OUR COMMUNITY

- Academic researchers & clinicians
- Industry stakeholders & researchers
- Families & patients
- Nonprofit leaders including foundations and NGO's
- 700+ community wide contacts
- 7 workgroups meeting monthly
- 60 engaged volunteers
- 50+ resources, publications and seminars

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ACKNOWLEDGEMENT OF APPRECIATION

This meeting would not have been possible without the vision and leadership of the 2024 Organizing Committee. We would also like to express our appreciation to the groups and organizations below for their support in making this event's success possible:

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INVITED SPEAKERS & PANELISTS

Keynote Presentation

Regulatory Refresh: *How The Regulatory System Should Be A Driver For Change*

Dan O'Connor, MD
Director Regulatory and Early Access Policy
The Association of the British Pharmaceutical Industry

Dan O'Connor, MD, is the Director of Regulatory and Early Access Policy at The Association of the British Pharmaceutical Industry (ABPI). He joined the ABPI from the Medicines and Healthcare Products Regulatory Agency (MHRA) in 2023. At the MHRA, he was Deputy Director of the Innovation Accelerator and Regulatory Science. Dan has special interests in rare diseases, drug development, regulatory science, health innovation, patient engagement, drug repurposing, and preventative medicines. Within the rare disease community, Dan has been a central player in multiple local and international efforts to use the regulatory landscape to revolutionize patient access to therapies. Some of these key efforts include the Early Access to Medicines Scheme in the UK, the Rare Therapies Launch Pad (RTLP), and the International Rare Diseases Research Consortium (IRDiRC). He is an editor author of the first edition Oxford Specialist Handbook of Pharmaceutical Medicine and on the editorial board of the journals Expert Opinion on Orphan Drugs and Rare Disease and Orphan Drugs Journal.

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INVITED SPEAKERS & PANELISTS

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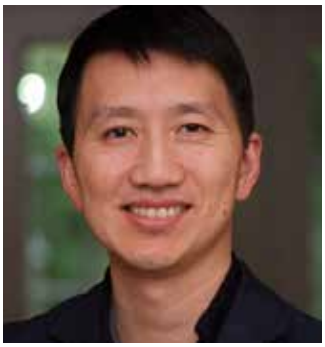

Timothy Yu
Boston Children's Hospital

MEETING AGENDA

8:00 – 9:00	Registration & Networking Coffee
9:00 – 9:10	Welcome from N=1 Collaborative *Timothy Yu, MD, PhD & Jillian Belgrad, MD*
9:10 – 9:30	Parent Perspective *Yiwei She, Leo's Mom & TNP02 Foundation*
9:30 – 10:20	Keynote Presentation ***Dan O'Connor, Association of the British Pharmaceutical Industry*** ***Regulatory Refresh:*** *How The Regulatory System Should be a Driver for Change*
10:20 – 10:45	Networking Break
10:45 – 11:45	Community Updates *N=1 Collaborative | Winston Yan, MD, PhD & Hugh Hempel, N1C Executive Director* *n-Lorem | Julie Douville, PhD* *1 Mutation, 1 Medicine (1M1M) | Willeke van Roon-Mom, PhD*
11:45 – 12:30	Emerging Researcher Talks ***Individualized Antisense Oligonucleotide Therapy for a Patient with Posterior Column Ataxia with Retinitis Pigmentosa (PCARP)*** *Boxun Zhao, Postdoctoral Research Fellow | Manton Center for Orphan Disease Research* ***Personalized Splice-modulating Antisense Oligonucleotide Therapy for PEX1-related Zellweger Spectrum Disorder (ZSD)*** *Robert Thompson, Genetics and gene therapy Fellow | Boston Children's Hospital/MGH* ***Antisense oligonucleotides targeting linked-SNPs provide allele-specific knockdown to a dominant-negative SPTAN1 pathogenic variant in a complex genetic region*** *Christiana Wang, PhD Student | Baylor College of Medicine* ***N-of-1 for N-of-Many: comprehensive, scalable development of patient-customized splice modulation ASOs for Ataxia Telangiectasia*** *Clemens Lochmann, M.Sc., PhD Student | Hertie-Institute for Clinical Brain Research* ***Individualised Exon Skipping Antisense Oligonucleotide Therapy for CHD2-Related Neurodevelopmental Disorders*** *Jack Morgan, PhD Student | Dutch Center for RNA Therapeutics | Leiden University Medical Center* ***First In Class ASO Targeting IGHMBP2 Cryptic Splice Variant: Efficacy and Safety*** *Caroline Johnson, Clinical | Vanda Pharmaceuticals Inc.*
12:30 – 1:30	Networking Lunch

MEETING AGENDA

1:30 – 3:00 Best Practices

ASO Design & Safety
Jonathan Watts, PhD, UMass Chan Medical School

Genetics
Marlen Lauffer, PhD, Leiden Univ Medical Center & David Cheerie, MSc, University of Toronto

Getting to the Clinic
Scott Demarest, Children's Hospital Colorado

Late Breaking Clinical Update
Michelle Hastings, PhD, Univ of Michigan Medical School & Yael Shiloh-Malawsky, MD, UNC School of Medicine

3:00 – 3:15 Refreshment Break

3:15 – 4:00 Engaging Industry Panel
Nianwei Lin
iXCells Biotechnologies

David Butler
Hongene Biotech

Rebecca Miles
ReiNA Consulting LLC

Cat Lutz
The Jackson Laborites

Jeremy Little
ChemGenes

4:00 – 5:30 N of 1 to N of Many
Beyond ASO's
Fyodor D. Urnov, PhD, UC Berkeley

N of 1 to N of Many
Timothy Yu, MD, PhD, Boston Children's Hospital

Clinical Trial Design
Richard Finkel, MD, St Jude Children's Hospital

Panel Q&A with presenters above, Dan O'Connor and Julia Vitarello

5:40 – 6:10 Patient & Family Panel
***Lauren Dempsey**, Sick Kids Advisory Board*
***Giacomo Mancini**, Charles River Laboratory*
***Yiwei She**, Leo's Mom & TNP02 Foundation*
***Stephanie Telesca**, KCNC1 Foundation*
***Julia Vitarello**, Mila's Miracle Foundation*

6:10 – 6:15 Closing Remarks

6:16 – 8:00 Reception
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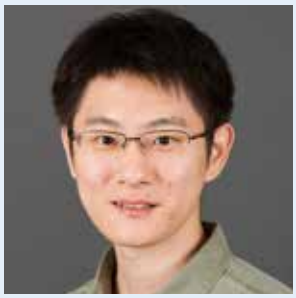
FLASH TALK PRESENTERS



Individualized Antisense Oligonucleotide Therapy for a Patient with Posterior Column Ataxia with Retinitis Pigmentosa (PCARP)

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Abstract

Splice-switching antisense oligonucleotides (ASOs) have emerged as a promising therapeutic modality, enabling the development of genetically tailored treatments for ultra-rare conditions affecting as few as individual patients. Here, we describe the development of an investigational therapeutic strategy for a child with posterior column ataxia with retinitis pigmentosa (PCARP), a rare neurodegenerative disorder characterized by progressive vision loss and pain insensitivity.

Our patient came to medical attention at 20 months old with recurrent injuries and a suspected insensitivity to pain in her extremities. She presented with mild motor delays, severe pain insensitivity, and progressive visual impairment due to retinitis pigmentosa diagnosed at the age of two. Genome sequencing revealed compound heterozygous mutations in the FLVCR1 gene: a paternally inherited missense mutation (c.1193A>G; p.Tyr398Cys) and a maternally inherited ~3 kb ISG20L2 pseudogene insertion in intron 8. RNA sequencing and RT-PCR analysis confirmed that the effect of the pseudogene insertion was to activate a novel intronic splice site, creating a truncating pseudoexon that rendered the transcript non-functional. This mutation's mechanism of pathogenicity was very similar to the gain-of-splicing SVA insertion in a patient with CLN7 Batten disease, who demonstrated beneficial clinical responses to treatment with a customized ASO (Kim & Hu et al., 2019, NEJM).

Our patient continues to suffer ongoing vision loss. Given 1) the absence of existing PCARP treatments, 2) the critical role of her remaining vision in preventing her high risk for injury due to pain insensitivity, and 3) the demonstrated feasibility of ASO therapy to the eye, we pursued the development of tailored ASOs for our patient. Through rigorous testing, we successfully developed ASOs capable of 1) correcting FLVCR1 mis splicing and 2) restoring functional protein in patient-derived cells. This proof-of-concept work supported the submission of an IND application to the FDA, in collaboration with n-Lorem, to start investigational intravitreal ASO treatment to forestall vision loss. Three doses have been well tolerated to date. While clinical efficacy readouts are expected to require several years of observation due to the slowly progressive nature of this disease, parallel experiments in patient-derived retinal organoid models confirm robust efficacy of ASO treatment.

This case expands the scope of N-of-1 ASO approaches to conditions that can be treated via intravitreal injection, opening doors for individualized ASO treatments for rare ocular diseases. It underscores the importance of comprehensive genomic analysis, rational drug design and development, and ethical considerations in investigational case studies.

FLASH TALK PRESENTERS

Personalized Splice-modulating Antisense Oligonucleotide Therapy for PEX1-related Zellweger Spectrum Disorder (ZSD)

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Abstract

Zellweger Spectrum Disorder (ZSD) is a severe genetic disorder caused by autosomal recessive variants in PEX genes which code for peroxins, a class of proteins that are critical for the biogenesis and proper functioning of peroxisomes. These mutations disrupt peroxisomal function, resulting in impairment of peroxisomal fatty acid metabolism, accumulation of very long chain fatty acids (VLCFAs), pristanic acid, di- and tri-hydroxy-cholestenoic acid and pipecolic acid and deficiency of plasmalogens (Wanders et al, 2016). These lead to progressive, debilitating neurological and multi-organ system disease, resulting in significant morbidity and death in infancy and childhood. There are no disease modifying treatments currently available. Clinical management focuses on symptomatic or supportive therapy (Braverman et al, 2016).

We identified a 2 year old male with ZSD due to recessive mutations in PEX1. PEX1 encodes for a member of the AAA ATPase family that pairs with PEX6 to form an essential complex required for peroxisome assembly. This individual bore a paternally inherited frameshift mutation (PEX1 c.2916delA, p.Gly973fs), and a maternally inherited intronic variant in between exons 6 and 7 (PEX1 c.1359+601A>G) that we predicted to cause mis-splicing of the PEX1 gene. RT-PCR and RNA-sequencing of patient fibroblasts confirmed activation of a cryptic pseudoexon in between introns 6 and 7, resulting in loss of PEX1 transcript and protein. Patient fibroblasts also exhibited impaired peroxisomal import as well as biochemical abnormalities (elevated C26:0 LPC, decreased total plasmalogen levels) pathognomonic for ZSD.

Given the progressive nature of his severe neurologic symptoms and the lack of existing treatments, this case was deemed a strong candidate disease for consideration of personalized ASO therapy. We designed and tested antisense oligonucleotides (ASOs) to block the cryptic splice donor site. Administration of these ASOs to patient fibroblasts and induced pluripotent stem cell lines increased the expression of properly spliced PEX1 mRNA by two to three-fold. ASO treatment also resulted in increased protein levels of both PEX1 and its binding partner PEX6. Finally, ASO treatment rescued defective peroxisomal import and corrected ZSD-associated biochemical abnormalities (reducing abnormal elevation of C26:0 LPC and normalizing abnormally depressed total plasmalogen levels) – thus providing potential clinically relevant biomarkers for a potential clinical n=1 trial. Unfortunately, before such a trial could be initiated, the index patient passed away due to progressive respiratory compromise. Nonetheless, these results provide a pilot template for potential future personalized ASO therapies for PEX1-associated Zellweger Spectrum Disorder.

FLASH TALK PRESENTERS

Antisense oligonucleotides targeting linked-SNPs provide allele-specific knockdown to a dominant-negative SPTAN1 pathogenic variant in a complex genetic region

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Abstract

Developmental and epileptic encephalopathy 5 (DEE-5) is an ultra-rare neurodevelopmental disorder (NDD) caused by monoallelic, pathogenic variants in SPTAN1, which encodes αII-spectrin. Individuals with the c.6908_6916dup (p.D2303_L2305dup) variant present with infantile epilepsy with refractory seizures, microcephaly, and significant intellectual and developmental disabilities. Individuals harboring the duplication variant mentioned above typically succumb to the disorder by six years of age. Previous studies have demonstrated a dominant negative consequence of the variant with aggregation of αII with β-spectrins in patient-derived fibroblasts. We sought to explore the potential of RNaseH-dependent, antisense oligonucleotide (ASO)-mediated allele-specific degradation as a therapeutic strategy.

We enrolled 5 children with DEE-5 caused by the duplication variant from 4 families. Patient-derived neural stem cells (NSCs) and neurons (Ns) were generated from peripheral blood mononuclear cells or fibroblasts. ASOs directly targeting the duplicated region of the gene failed to effectively knockdown the expression or discriminate between the normal and duplicated alleles, likely because the duplicated sequence prevents the variant-specific nucleotides within the ASO from being centered and causing self-dimerization. Thus, as an alternative approach, we performed genome sequencing, identified candidate heterozygous exonic and intronic linked single nucleotide polymorphisms (SNPs) in each individual, and designed targetable gapmer ASOs. SNPs with high population frequency and supporting sequence features allow for a broader application of our strategy for other pathogenic variants within this gene. Several ASOs targeting the linked-SNPs showed promising allele-specific mRNA knockdown verified through ddPCR and RNA-seq. An exonic-SNP-targeting ASO resulted in knockdown efficiency of ~95% by ddPCR of the variant allele. The corresponding RNA-seq demonstrated gene TPM lowered by 30.75% with the two linked-exonic-SNP fractions dropping by 20% and 18%. Similarly, an intronic-SNP-targeting ASO knocked down the variant allele by ~46%, and RNA-seq revealed 21.82% gene TPM lowering with the SNP fractions dropping by 13% and 17%.

Our preliminary work shows promising potential of allele-specific ASOs targeting linked-SNPs for managing an ultra-rare NDD. Functional studies, including a mouse model for in vivo testing and electrophysiology, are in progress to facilitate therapy development.

N-of-1 for N-of-Many: comprehensive, scalable development of patient customized splice modulation ASOs for Ataxia Telangiectasia

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Abstract

Gene-targeted therapies present a promising approach for thus far untreatable, rare neurological diseases (RNDs). Current regulatory frameworks and drug development fall short on RNDs where only a few or only one patient exists worldwide. Splice modulation could serve as a disease-overarching ASO strategy to target super-rare, or even n-of-1 mutations. Scalability for multiple splice mutations of one disease or even multiple splice mutation diseases alike could facilitate regulatory and experimental aspects. This project aims to develop a platform approach for the preclinical development of n-of-1 ASOs by showcase of a severe early-onset brain disease: Ataxia telangiectasia (AT).

Methods: Development of a comprehensive preclinical, scalable n-of-1 ASO platform for AT. Aggregation of a cohort 290 fully genotyped AT patients, followed by systematic identification of ASO-amenable ATM mutations with successive design of nusinersen analogous ASOs. Effective ASOs were determined by RNA splice restoration assays, followed by an immunoblot panel to investigate restoration of ATM and phosphorylation of key downstream targets in patient-derived fibroblasts. The platform currently comprises 6 ATM mutations (11 cell lines: 6 in compound-heterozygous, 2 in homozygous, 3 in heterozygous state), representing two different types of ASO strategies: targeting deep

intronic cryptic splice mutations leading to inclusion of cryptic exons (n=3 different variants), and exonic splice mutations resulting in cryptic donor sites (n=3 different variants). A recently established clinical benchmark ASO for AT, atipeksen, targeting an exonic cryptic splice ATM variant (c.7865C>T), as well as homozygous cell lines of several A-T variants targeted here were included as part of the platform approach, all serving as a reference to size the effect sizes of the outcomes of all conducted assays.

Results: 36 of 290 A-T patients (12.4%) carried deep-intronic or exonic cryptic splice variants possibly or probably amenable to splice modulation. Deep-intronic cryptic splice targeting. For c.2639-384A>G, 4 ASOs showed restoration of ATM activity of up to 75% of wildtype levels. For c.1236-404C>T, 3 ASOs led to RNA splice and up to 50% ATM restoration. Exonic cryptic splice targeting. For c.967A>G and c.8565T>G, RNA assays revealed effective ASOs. The ATM kinase assay revealed restoration of 20% ATM for the c.967A>G mutation, >5% ATM for atipeksen targeting c.7865C>T, and >5% ATM for c.8565T>G. Validation and implementation of in-vitro toxicity experiments are ongoing, including clinically known toxic ASOs as positive controls (reverse translation).

Conclusions: These findings provide proof-of-concept for establishing a unified platform approach to develop personalized ASO treatments for a severe early-onset RND: it allows to target ultra-rare, or even private mutations in a programmable and scalable fashion applicable to multiple splice mutations of one disease or even multiple splice mutation diseases alike.

Individualised Exon Skipping Antisense Oligonucleotide Therapy for CHD2-Related Neurodevelopmental Disorders

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Abstract

Chromodomain helicase DNA binding domain 2 (CHD2) encodes an ATP-dependent chromatin remodelling enzyme that plays a crucial role in the regulation of gene expression by altering chromatin structure and accessibility. CHD2 is implicated in various cell processes such as DNA repair, replication and transcription, with significant importance in the development and function of the nervous system. Given its essential role in neuronal development and gene regulation, pathogenic variants can lead to significant consequences. CHD2-related neurodevelopmental disorders encompass a large spectrum of conditions. These disorders are most often characterised by early-onset developmental and epileptic encephalopathy (DEE) as well as intellectual disability. CHD2 haploinsufficiency has emerged as the causative factor behind these neurodevelopmental abnormalities, for which no treatment is currently available.

Here we present the case of a young girl carrying a nonsense variant (NM_001271.4: c.3021dupT), leading to the generation of a stop codon (p.E1008*) in exon 24 of CHD2 that has not been reported previously. According to the recently established N1C consensus guidelines for assessment of variant eligibility towards exon skipping treatments, this exon is a likely eligible candidate for exon skipping. In this study, we aim to assess the feasibility of a individualised exon skipping antisense oligonucleotide (ASO) to remove the exon the harbouring nonsense variant, thereby restoring the reading frame and potentially protein function. To elucidate the effect of skipping exon 24 of CHD2, we will assess protein stability and function through CRISPR/Cas9-mediated deletion of exon 24 in iPSC-derived neuronal cultures. Subsequently, ASOs will be designed and tested on cell lines harbouring the c.3021dupT variant to determine rescue of protein function.

In addition to our exon skipping approach, several other groups are investigating targeted augmentation of nuclear gene output (TANGO) as a therapeutic approach to CHD2-related disorders. The long -non-coding RNA CHASERR has been implicated in the repression of CHD2 expression. By downregulating CHASERR or by implementing other TANGO approaches, these groups aim to upregulate expression of the wild-type allele, thereby ameliorating the haploinsufficiency phenotype associated with variants in CHD2. As such, we also aim to compare the effectiveness of our exon skipping strategy to that of the TANGO approaches.

First In Class ASO Targeting IGHMBP2 Cryptic Splice Variant: Efficacy and Safety

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Abstract

Charcot-Marie-Tooth disease Type 2S (CMT2S) is a rare autosomal recessive Charcot-Marie-Tooth disease subtype. Variants in immunoglobulin mu□binding protein 2 (IGHMBP2) may result in abnormal RNA processing leading to alpha□ motor neuron degeneration, causing CMT2S.

A patient was reported with pathogenic IGHMBP2 variants. Whole genome sequencing revealed a paternally inherited cryptic splice site variant (c.1235+894 C>A) in intron 8. Resulting transcript undergoes nonsense□mediated decay. Our objective was to rescue IGHMBP2 by targeting this splice site with a novel antisense oligonucleotide (ASO).

Patient fibroblasts were incubated for 48-hours with ASO (1μM). Motor neurons (MNs) were generated from patient fibroblasts. CMT2S-MN physiology was characterized through patch-clamp electrophysiology and neuromuscular junctions (NMJs) were examined. MNs were incubated with ASO and treatment effects were quantified.

IGHMBP2 increased (~50-70%) in treated-fibroblasts (WB antibody Sigma SAB2106426). qPCR confirmed increased ratio of restored wild-type transcript to cryptic exon-containing transcript (~1.8-fold), and RNA sequencing confirmed 1.3log2-fold (adjusted p-value<0.002) IGHMBP2 increase. There were limited off-target effects in-silico. CMT2S-MN electrophysiology revealed early hyperexcitability. CMT2S-NMJs revealed a higher fatigue index (FI) than WT-NMJs.

CMT2S-NMJs showed quick fatigue, simulating muscle weakness. We demonstrate NMJ functioning rescue following treatment, captured by decreases in FI and decay and chaotic responses. Potential toxicity was evaluated in a 3-month rat study. Transient clinical observations were noted and resolved within 24-hours, with no neurobehavioral changes. The no-adverse-event-level was the highest dosage.

Precision medicine is instrumental in designing treatments for genetic disorders. There have been increasing reports of CMT2S cases caused by IGHMBP2 variants, and subsequent analyses have revealed deep intron 8 IGHMBP2 pathogenic variants to be a hotspot of aberrant splicing. Future analyses include developing our ASO to cover this hotspot region to expand therapeutic intervention potential and treat more individuals with CMT2S caused by intron 8 IGHMBP2 pathogenic variants. Our case exemplifies WGS-based diagnoses and research capabilities allowing for the design of personalized ASO-based treatments.