RETT SYNDROME **GENETIC MEDICINES SUMMIT** 2023

rett syndrome research trust

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RETT SYNDROME
GENETIC MEDICINES SUMMIT 2023

CHELSEA COENRAADS

MONICA COENRAADS
Chief Executive Officer, RSRT

WELCOME

My daughter Chelsea was diagnosed with Rett syndrome in 1998. She was two years old. She was unable to walk, the single word in her vocabulary had disappeared, and she could no longer hold anything in her hands. She showed no interest in her surroundings or her toys and I wasn't sure she knew, or cared, who I was. I was consumed with fear, shock and dread.

As I started to explore the research landscape for Rett syndrome my heart sank even further as I learned the genetic cause was unknown, there were no animal models and no drug development efforts underway. I made a promise to Chelsea that I would make it my life's work to help accelerate the development of a cure for her and for hundreds of thousands like her.

Not having a scientific or medical background I had little appreciation for what that promise entailed. Fast forward 25 years and I cannot help but marvel at how far the Rett research landscape has come. Two gene therapy trials are underway as well as programs for base editing, prime editing, RNA editing and epigenetic approaches. We have animal models, biorepository, natural history data, efforts to develop objective clinical outcome assessments and specialized Rett syndrome clinics.

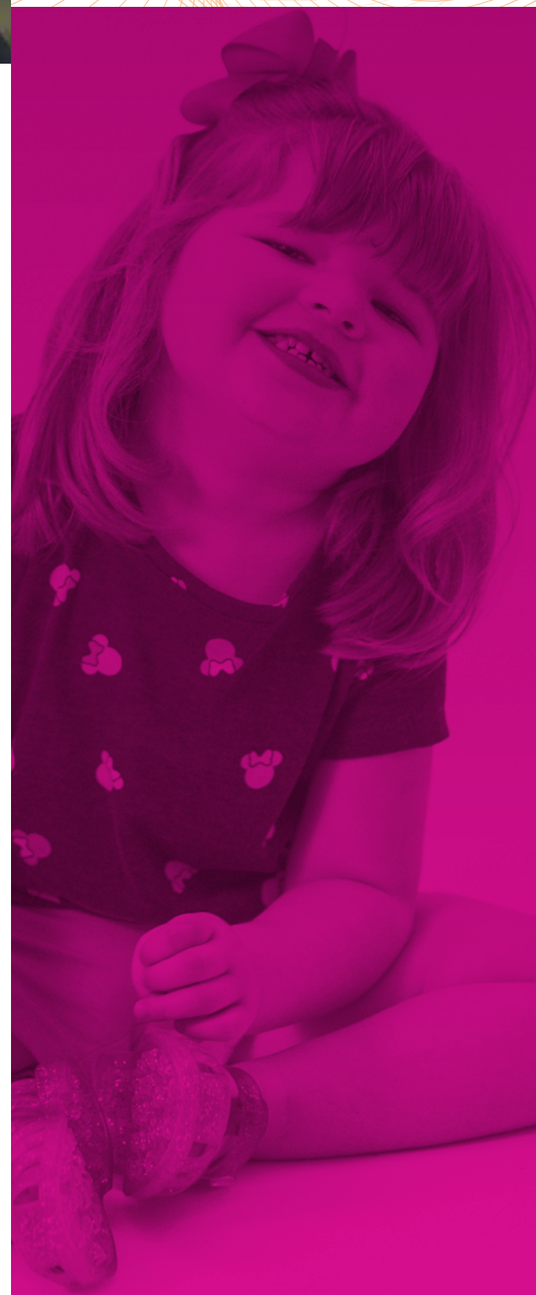
Despite all these advances, Chelsea, who is almost 27 years old, has yet to benefit. She is in a wheelchair, unable to speak or use her hands, she is fed through a tube and has suffered intractable seizures for over two decades. Rett tortures her daily with too many symptoms to list here. She is, however, so much more than a laundry list of symptoms. She is kind and loving and one of her brilliant smiles can immediately chase away stress and sadness.

On behalf of individuals with Rett who have no voice of their own, I welcome you, old colleagues and new ones, to this Summit. I hope the next several days will provide a stimulating and productive exchange of information and ideas that will help advance your Rett program or inspire you to add Rett to your company pipeline. My colleagues and I are here to help in any way we can.

Over the next few days you will have an opportunity to meet several children and adults with Rett syndrome and their parents. They are eager to interact with you so please feel free to introduce yourselves and ask any questions you like.

It is not an exaggeration to say that our children's futures are in your hands. I thank you for your time and effort to attend the Summit. I look forward to our interactions at this meeting and beyond.

Monica Coenraads
Chief Executive Officer, RSRT



ABOUT THE RETT SYNDROME RESEARCH TRUST

The Rett Syndrome Research Trust (RSRT) is a non profit organization with a highly personal and urgent mission: **achieving a cure for Rett syndrome as soon as possible**. Our focus is to advance genetic medicines that target the root cause of Rett syndrome. Since a one-size-fits-all approach rarely works in medicine, we are supporting every possible genetic modality, including gene therapy, base editing, prime editing, RNA editing, MECP2 re-activation.

We operate with a three pronged approach.

- 1 – Fund academic investigators to advance and de-risk programs to a stage they can be transitioned to industry.
- 2 – Generate resources that promote IND-enabling studies that are critical to advance programs into the clinic, and make them available to all.
- 3 – Develop high quality clinical research tools to improve the design and execution of clinical trials and support regulatory interactions.

Our internal research team at RSRT consists of drug developers that have over 90 years of collective experience. Their knowledge combined with RSRT's extensive network of advisors provides a valuable resource for academia and industry.

We are here to help you advance your Rett syndrome program. Meet with us and let's discuss how we can help.

Monica Coenraads, MBA

Chief Executive Officer, RSRT

Monica Coenraads's involvement with Rett syndrome began the day her then-two-year-old daughter was diagnosed with the disorder. A year later, in 1999, she co-founded the Rett Syndrome Research Foundation (RSRF) and held the position of scientific director during the eight years of the Foundation's drive to stimulate scientific interest and research in Rett syndrome, culminating with the groundbreaking work in 2007 which demonstrated the first global reversal of symptoms in preclinical models of the disorder. Monica launched the Rett Syndrome Research Trust in late 2008 to pursue the next steps from that milestone.

As chief executive officer she oversees all aspects of the organization, including day-to-day operations, strategic direction, fundraising, and communications. Together with her colleagues and with input from advisors and the scientific community at large, Monica sets and executes RSRT's research agenda.

Under Monica's leadership at RSRF and RSRT, over $100 million has been raised for Rett syndrome.

Monica has two honorary doctorate degrees, one from the University of Massachusetts Medical School, received in 2015, and one from the University of Edinburgh, received in 2019. She has an MBA with an emphasis in international business from the University of Connecticut.

Randall Carpenter, MD

Chief Medical Officer, RSRT

Dr. Carpenter is currently on the advisory boards of EU-AIMS and the Translational Neuroscience Center of Boston Children's Hospital, a research affiliate in the Department of Brain and Cognitive Sciences at MIT, and is a co-founder of Allos Pharma.

His career has focused on translating basic science discoveries into novel therapeutics for individuals with neuropsychiatric disorders. He co-founded Seaside Therapeutics in 2005 to develop therapeutics for individuals with intellectual disability and autism and served as President and CEO. Prior experiences include President and CEO at Sention, VP of Clinical Development and Regulatory Affairs at Adolor, and Director of Clinical Research at Astra Pain Control. While in industry, he led translational medicine teams responsible for eight INDs and dozens of FDA-compliant clinical trials. Prior to joining industry, he provided clinical care and held leadership positions in academic medical centers, in medical professional societies, and several peer-reviewed medical journals. He also performed basic science and translational research, and served as the principal investigator on dozens of FDA compliant clinical trials.

Jana von Hehn, PhD

Chief Scientific Officer, Head of Clinical Development, RSRT

Jana von Hehn leads RSRT's clinical research efforts to create novel resources to aid therapeutic development. She spearheads RSRT's digital biomarker effort focused on developing digital technologies for Rett symptom assessment. As Director of the Rett Syndrome Global Registry, she also oversees RSRT's CURETT (Combining Untapped Resources to Expedite Targeted Therapeutics) initiative, where parent experiences and medical records are collected in regulatory-grade datasets to support biopharma clinical development.

Prior to RSRT, Dr. von Hehn developed small molecule drugs at Marinus, Seaside, Sage, Karyopharm, and Concert Pharmaceuticals. With more than 15 years of drug development and clinical operations experience for disorders including Fragile X syndrome, autism, oncology, and autoimmune disorders, she has contributed to one FDA drug approval and is an inventor on a patent for a drug currently in development.

Dr. von Hehn earned her PhD in Genetics and Molecular Biology from Emory University.

Robert Deans, PhD

Chief Technology Officer and Head of Research, RSRT

Robert Deans is Chief Technology Officer and Head of Research at RSRT, applying his experience towards accelerating patient access to next generation gene therapies. Previously, Dr. Deans was CSO at Synthego, a genome engineering company automating a new era of cell and gene therapeutics. Prior to Synthego, Dr. Deans was founding CTO at BlueRock Therapeutics, creating iPSC based allogeneic cell therapeutics by harnessing pluripotent stem cell biology and gene editing tools. Before BlueRock, Dr. Deans was founding CSO at Rubius Therapeutics developing a platform of novel enucleated cell therapeutics from engineered hematopoietic stem cells. Dr. Deans was previously EVP at Athersys Inc, an adult stem cell therapeutics company now in late stage clinical development. Dr. Deans has more than 35 years of experience in adult stem cell therapeutics which includes HSC gene therapy and commercialization of progenitor cell therapeutics from bone marrow. Dr. Deans has been influential in stem cell and therapeutic societies in guiding translation and standardization of stem and progenitor cell practices.

TAYSHA GENE THERAPIES

Taysha is proud to stand alongside the Rett community as we work together to develop a gene therapy for those living with Rett syndrome.

We thank the Rett Syndrome Research Trust for their partnership.

Please join us for the **Gene Therapy Session** on September 14 from 8:15-9:30 a.m. to learn more about our investigational gene therapy for Rett syndrome.

Presentation from **Sukumar Nagendran, MD**, *President and Head of R&D*

Visit **www.tayshagtx.com** to learn more, or contact **medinfo@tayshagtx.com**.

SUMMIT PROGRAM

Thank you for joining our incredible assembly of basic science, pre-clinical, clinical, and regulatory experts. We are at a pivotal moment in drug development where the first genetic medicines have entered the clinic and the possibility of achieving cures for Rett is tangible. The goal of the Summit is to foster discussion, refine expectations, and identify best practices to expedite genetic medicine development. We hope the content of the meeting will be a rising tide to lift all boats and that the information shared will advance the field to move us closer to a future without Rett.

"The best way to predict the future is to create it." – Alan Kay, PhD

WEDNESDAY, SEPTEMBER 13

8:00 – 8:15 am

Welcome & Opening Remarks

Monica Coenraads, Rett Syndrome Research Trust

8:15 – 9:15 am

MECP2 Basic Science

Understanding the Molecular Basis of Rett Syndrome
Adrian Bird, University of Edinburgh

Dissecting the Readout of Cell-type-specific DNA Methylation by MeCP2
Harrison Gabel, Washington University

9:15 – 10:15 am

Advances in Delivery of Therapeutics to the Brain

A Novel AAV Capsid that Crosses the Blood Brain Barrier though Engineered Interactions with a Human Protein
Ben Deverman, Broad Institute of MIT and Harvard

Identifying Novel LNPs by Combining High Throughput Screening and Single Cell Multiomic Readouts
Kalina Paunovska, Nava Therapeutics

10:15 – 10:45 am — BREAK

10:45 am **– 12:00** pm

Accessing the Central Nervous System & Rett Syndrome
Thomas Carroll, Sheffield Teaching Hospitals NHS Foundation Trust

Discussants
John Maraganore, Ben Deverman, Kalina Paunovska, Thomas Carroll, David Lockhart, Patrick Finn

12:00 – 1:00 pm — LUNCH

1:00 – 2:00 pm

MECP2 Basic Science

"Seq-ing" Pathogenic Insights into Rett Syndrome
Joe Zhou, University of Pennsylvania

Investigating the Immediate Consequences of Rapid MeCP2 Protein Degradation
Lisa Boxer, National Cancer Institute

2:00 – 3:30 pm

Pre-Clinical Resources

Development of a Preclinical Testing Resource for Mouse Models of Rett Syndrome
John Sinnamon, Rett Syndrome Research Trust

Humanized Mouse and Marmoset Models for Rett Syndrome Research
Guoping Feng, Massachusetts Institute of Technology

Mecp2 Dosage-sensitive Proteomes as Putative Disease Biomarkers
Victor Faundez, Emory University

The RSRT Biorepository
Jana von Hehn, Rett Syndrome Research Trust

3:30 – 4:00 pm — BREAK

4:00 – 5:00 pm

Discussion of Pre-Clinical Resources

Discussants
PJ Brooks, Guoping Feng, John Sinnamon, Victor Faundez, Jana von Hehn, Danielle Brooks, Mustafa Sahin

5:00 – 6:30 pm — POSTER SESSION / COCKTAILS

6:30 pm — DINNER

THURSDAY, SEPTEMBER 14

8:00 – 8:15 am

Introduction

A Comparison of Genetic Therapies and their Relevance to Rett Syndrome
Jonathan Watts, University of Massachusetts Chan Medical School

8:15 – 9:30 am

Gene Therapy

First-in-human Use of TSHA-102 Gene Therapy for Rett Syndrome: Where We Stand and How We Got Here
Sukumar Nagendran, Taysha Gene Therapies

NGN-401: A Self-regulating Gene Therapy Product for Rett Syndrome
Stuart Cobb, University of Edinburgh, Neurogene

Discussants
Seng Cheng, Peter Marks, Stuart Cobb, Sukumar Nagendran, Guoping Feng, Elizabeth Berry-Kravis

9:30 – 10:00 am — BREAK

10:00 – 11:30 am

Therapeutic Gene Editing

Base Editing and Prime Editing: Correcting Mutations that Cause Genetic Disease in Cells, Animals, and Patients
David Liu, Broad Institute, Harvard University, HHMI

Discussants
Fyodor Urnov, David Liu, Peter Marks, Winston Yan, Brett Staahl, Jeremy Duffield

11:30 am **– 12:30** pm

RNA Editing

RNAfix® – A Programmable RNA Editing Platform that Expands the Potential of RNA Therapeutics
David Huss, Shape Therapeutics

Adapting AIMer-based RNA Editing Technology for Application in CNS
Michael Byrne, Wave Life Sciences

12:30 – 1:30 pm — LUNCH

1:30 – 3:00 pm

Directed RNA Editing for the Repair of MECP2 Mutations that Cause Rett Syndrome: Optimization of Guide Strands for Difficult-to-edit Sites
Peter Beal, University of California, Davis

Development of Mice Modeling Missense Variants Introduced by RNA Editing to Restore MECP2 Function in Patients with Nonsense Mutations
John Sinnamon, Rett Syndrome Research Trust

Repair of Loss-of-function Mutations of MECP2 mRNA by Pentatricopeptide Repeat (PPR) Protein-mediated U-to-C Single Nucleotide Editing
Osamu Nakanishi, EditForce

3:00 – 3:30 pm

New Approaches on the Horizon

Rescuing Rett Syndrome Pathology through Astrocyte Dependent MeCP2 Reactivation
Gaia Skibinski, Herophilus

Epigenome Editing of MECP2 to Rescue Rett Syndrome Neurons
Shawn Liu, Columbia University

Novel tRNA Medicines, Stop Codon Disease, and Premature Termination Codons in Rett Syndrome
Theonie Anastassiadis, Alltrna

3:30 – 4:00 pm — BREAK

Continues

THURSDAY, SEPTEMBER 14

4:00 – 5:00 pm

Discussion of Biologic Approaches

Discussants
Fyodor Urnov, Peter Marks, Peter Beal, Stuart Cobb, Erik Sontheimer, David Huss, Elizabeth Berry-Kravis, Eric Marsh

5:00 – 6:15 pm

Evolving Regulatory Landscape for Biologic Therapeutics in Orphan Diseases

Peter Marks, CBER, FDA

Discussants
Frank Sasinowski, Peter Marks, Toby Ferguson, PJ Brooks, Fyodor Urnov, Andrew Mulberg, Eric Marsh, Elizabeth Berry-Kravis

6:30 pm — **COCKTAILS / DINNER**

FRIDAY, SEPTEMBER 15

8:00 – 8:30 am

Introduction

Modulating MeCP2 Levels: A Potential Therapeutic Strategy for Rett Syndrome
Huda Zoghbi, Baylor College of Medicine, HHMI

8:30 – 9:30 am

Validating Clinical Outcome Assessments

Incorporating Clinical Outcome Assessments in Rare Disease Clinical Trials
Lindsey Murray, Critical Path Institute

Discussants
Lindsey Murray, Randy Carpenter, Christy Zigler, Elizabeth Berry-Kravis, Paul Wang

9:30 – 10:30 am

Digital Biomarkers

Digital Biomarkers for Rett Syndrome: Sleep, Activity and Circadian Rhythms
Gari Clifford, Emory University, Georgia Institute of Technology

AI-Powered Radio Signals Paving the Way for Objective Biomarkers in Rett Syndrome
Dina Katabi, Massachusetts Institute of Technology

Pilot Study of the MC-10 Bio-Stamp and Emerald Device in the Rett Population
David Lieberman, Boston Children's Hospital

10:30 – 11:00 am — **BREAK**

11:00 am **– 12:00** pm

EEG and Evoked Potentials: A Potential Objective Measure of Rett Syndrome Severity
Eric Marsh, Children's Hospital of Philadelphia

The Hunt for Endophenotypes in Rett Syndrome
John Foxe, University of Rochester Medical Center

Using Digital Health Technologies to Advance Clinical Drug Development
Kate Lyden, VivoSense

12:00 – 12:30 pm — **WORKING LUNCH**

12:30 – 1:45 pm

Discussion of Digital Biomarkers

Discussants
Chris Leptak, Gari Clifford, Dina Kitabi, David Lieberman, Eric Marsh, John Foxe, Kate Lyden, Lindsey Murray

1:45 – 2:45 pm

Utilizing Real World Data in Therapeutic Development

Using Patient-centric EMR Data for Robust Rare Disease Research and Drug Development
Nasha Fitter, Ciitizen

Combining Untapped Resources to Expedite Targeted Therapeutics (CURETT) Initiative: A Vision
Cary Fu, Vanderbilt University Medical Center
Bernhard Suter, Baylor College of Medicine / Texas Children's Hospital

Discussants
Jana von Hehn, Nasha Fitter, Cary Fu, Bernhard Suter

2:45 – 3:00 pm

Vision for the Future

Jana von Hehn, Rett Syndrome Research Trust

OUR SPEAKERS, MODERATORS & PANELISTS

Theonie Anastassiadis, PhD

Chief Innovation Officer, Alltrna
Senior Principal, Flagship Pioneering

Theonie is a senior principal at Flagship Pioneering, where she conceives, builds, and grows the science, intellectual property, and business strategy that form the foundation of Flagship's next breakthrough startups. She co-founded Alltrna and serves as its Chief Innovation Officer.

Theonie is a Business Advisory Board member of the Harvard Institute for RNA Medicine and a member of the Bioscience & Investor Inclusion Group (BIIG) Diverse Talent Network Group.

Prior to joining Flagship, Theonie completed her PhD in cell and molecular biology at the Perelman School of Medicine at the University of Pennsylvania. Her research focused on replication fork dynamics in the context of cancer development and therapeutics.

Theonie has received several awards and has been granted multiple fellowships for her academic work, including an NIH NRSA Predoctoral Fellowship. During her graduate studies, Theonie held multiple leadership positions on Executive and Curriculum Committees. She also completed a Wharton Business Foundations Specialization and was a mentor at the yearly Larta Institute NIH CAP FeedForward Sessions.

Theonie's work has resulted in multiple pending patents and publications, including articles in Nature Biotechnology, Molecular Cell, and Journal of Biological Chemistry.

Peter A. Beal, PhD

Professor, Department of Chemistry, Director of NIH funded Chemical Biology Graduate Program, University of California at Davis

For over 25 years, work in the Beal laboratory has advanced understanding of the structures and mechanism of action for the ADAR enzymes responsible for adenosine to inosine RNA editing in humans. In addition, his group has led in the development of structure-guided methods for optimizing chemically modified oligonucleotides for recruitment of RNA-binding proteins including ADARs. Beal teaches organic chemistry at the undergraduate level and several classes in nucleic acids chemistry and chemical biology at the graduate level. He has authored over 100 peer-reviewed publications in the field of RNA chemical biology and mentored over 50 PhD and MS degree students.

Elizabeth Berry-Kravis MD, PhD

Professor of Pediatrics and Neurological Sciences, Rush University Medical Center

Elizabeth Berry-Kravis is a Professor of Pediatrics and Neurological Sciences at Rush University Medical Center in Chicago. She established the Fragile X Clinic and Research Program in 1991, through which she has provided care to over 800 patients with fragile X syndrome (FXS). She has studied medical issues, epilepsy and psychopharmacology in FXS, and has been a leader in translational research in FXS for 20 years, including development of clinical outcome measures and biomarkers, natural history studies, newborn screening, and particularly clinical trials of new targeted treatments in FXS, for which she has been PI or Co-PI of 27 trials, both industry and investigator sponsored. Her laboratory studies the cellular role of fragile X mental retardation protein (FMRP), relationship between FMRP and clinical function, and optimization of genetic testing methods.

More recently she has expanded clinical and translational work to other neurodevelopmental disorders in addition to FXS, including autism spectrum disorders and single gene models of ASD, Phelan McDermid syndrome, Rett syndrome, and Angelman syndrome. She also is working on translational research in rare neurogenetic disorders including Niemann-Pick type C, Battens disease, pantothenate kinase-associated neurodegeneration, and creatine transporter deficiency. She is on Advisory and/or Review Boards for the FRAXA Research Foundation, National Fragile X Foundation, Phelan McDermid Syndrome Foundation, International Rett syndrome Foundation, Angelman Syndrome Foundation, Foundation for Angelman Syndrome Therapeutics, Combined Brain, N=1 Collaborative, n-Lorem Foundation and the GATHER Foundation.

She has received the NFXF Jarrett Cole Clinical Award, FRAXA Champion Award, NFXF William and Enid Rosen Research Award, March of Dimes Jonas Salk Research Award, American Academy of Neurology Sidney Carter Award in Child Neurology, John Merck Fund Sparkplug Award, the FRAXA Ingenuity Award, the FAST Innovation Award, and the inaugural Martha Bridge Denckla Award from the Child Neurology Society for work in cognitive disorders of children.

Adrian Bird, PhD

Buchanan Chair of Genetics, University of Edinburgh

Adrian Bird holds the Buchanan Chair of Genetics at the University of Edinburgh. After obtaining his PhD in Edinburgh and postdoctoral experience at Yale and Zurich Universities he joined an MRC Unit and later moved to Vienna, returning to Edinburgh in 1990. His research focuses on DNA methylation and other epigenetic processes, including the molecular mechanisms underlying neurological disorders. His group discovered the MeCP2 protein, established its likely mechanism of action and developed the first animal model of Rett syndrome. In 2007 his lab provided pre-clinical evidence that Rett syndrome is likely to be curable.

Lisa Boxer, PhD

Stadtman Investigator at the Laboratory of Genome Integrity, National Institutes of Health

Lisa Boxer is a Stadtman Investigator in the Laboratory of Genome Integrity at the National Institutes of Health. Her lab studies chromatin and epigenetics in brain development and disease, with a focus on DNA methylation and MeCP2. She received her PhD in Biology from Stanford University in 2015. Her thesis research in Dr. Paul Khavari's lab focused on transcriptional regulation of epidermal differentiation. She then performed postdoctoral training with Dr. Michael Greenberg at Harvard Medical School. As a postdoctoral fellow, she developed multiple new approaches to study how MeCP2 regulates neuronal gene expression. Dr. Boxer joined the National Institutes of Health as a Stadtman Investigator in 2022.

Danielle Brooks, PhD

Pharmacology / Toxicology Team Lead, Center for Biologics Evaluation and Research, FDA

Danielle Brooks is a Team Lead in the Division of Pharmacology / Toxicology 1 in the Office of Therapeutic Products in the Center for Biologics Evaluation and Research at the FDA. She received her PhD in Biomedical Sciences with a concentration in Cancer and Developmental Biology at The University of Tennessee Health Science Center in Memphis, TN. Following her graduate training, Dr. Brooks completed her post-doctoral training in the Women's Malignancies Branch of the National Cancer Institute. In 2017, she was selected for a fellowship in the NCI-FDA Interagency Oncology Task Force Fellowship program where she participated in product quality research and review of cellular therapies in the Cellular and Tissues Therapies Branch. At the completion of her fellowship in 2018, Dr. Brooks joined the Pharmacology / Toxicology Branch where she now focuses on overseeing the review of preclinical toxicology and pharmacology data to support the safety of cell and gene therapies, tissue-engineered products, devices and combination products.

Philip J. (P.J.) Brooks, PhD

Acting Director, NCATS' Division of Rare Diseases Research Innovation, National Institutes of Health

Philip J. (P.J.) Brooks is the acting director of NCATS' Division of Rare Diseases Research Innovation. Brooks represents NCATS in the NIH-wide Gene Therapy Working Group, the Regenerative Medicine Innovation Project and the International Rare Diseases Research Consortium (IRDRC). He also is the working group co-coordinator for the NIH Common Fund program on Somatic Cell Genome Editing, one of the leaders of the Platform Vector Gene Therapy (PaVe-GT) pilot project and the co-chair of the Bespoke Gene Therapy Consortium.

In May 2022, Brooks was selected as the recipient of the 2022 Sonia Skarlatos Public Service Award by the American Society of Gene & Cell Therapy for consistently fostering and enhancing the field of gene and cell therapy.

Brooks received his doctorate in neurobiology from The University of North Carolina at Chapel Hill. After completing a postdoctoral fellowship at The Rockefeller University, he became an investigator in the NIH intramural program, where he developed an internationally recognized research program focused on two distinct areas: the molecular basis of alcohol-related cancer, and rare neurologic diseases resulting from defective DNA repair, including xeroderma pigmentosum, Cockayne syndrome and Fanconi anemia.

Michael Byrne, PhD

Vice President In Vivo and CNS Biology, Wave Life Sciences

Dr. Byrne has been with Wave Life Sciences, Inc. for six years where he serves as Vice President of In Vivo and CNS biology. In that time his focus has been on assessing the utility of stereopure oligonucleotides as potential therapeutics for rare genetic diseases through the development of appropriate animal models and drug discovery efforts. Prior to joining Wave, Dr. Byrne spent 9 years at RXi Pharmaceuticals where he led RXi's self-delivering siRNA oligonucleotides to the clinic for treatment of retinal scarring associated with late-stage AMD. Dr. Byrne received his PhD from Northeastern University in Boston, MA where he studied microRNA expression patterns and impact on embryo development.

Thomas Carroll

Consultant Neurosurgeon, Sheffield Teaching Hospitals NHS Foundation Trust

Thomas Carroll is a Consultant Neurosurgeon and Clinical Director of Neuroscience at Sheffield Teaching Hospitals NHS Foundation Trust, UK. Thomas also has some personal experience in neuro/learning disability, including having been Chair of the Board of Governors of one of Sheffield's special schools. He was a co-founder and previous chair of the UK cancer genetic rare disease charity Fanconi Hope and involved in the instigation of a gene therapy development programme for Fanconi Anaemia.

Seng H. Cheng, PhD

Senior Vice President of Research and Product Development, Alexion, AstraZeneca Rare Disease

Dr. Cheng obtained his BSc and PhD degrees in Biochemistry at the University of London and trained as a postdoctoral fellow in the field of tumor biology at the National Institute of Medical Research, U.K.

He was Group Vice President of Genetic Diseases Science at Genzyme for 25 years and prior to joining Alexion, he was the Chief Scientific Officer of the Rare Disease Research Unit at Pfizer and before that, the Global Head of Research of Rare Diseases at Sanofi where he led the translational research and clinical development of therapies for patients with rare diseases. Dr. Cheng is experienced in bringing research initiatives from discovery through to early clinical development. He has co-authored 282 research articles and reviews and is a named co-inventor on 64 issued US patents in the area of biotechnology.

As Senior Vice President of Research and Product Development at Alexion, he is charged with building upon the foundation of rare disease programs (rare immunologic, hematologic, metabolic, and neurologic diseases) that are resident in the Rare Disease Therapeutic Area using a variety of extant and emerging technology platforms, including genetic therapies.

Gari Clifford, PhD

Chair of Biomedical Informatics, Emory University
Professor of Biomedical Engineering, Georgia Tech University

Gari Clifford was elected as a Fellow of the IEEE for contributions to machine learning applications in cardiovascular time series, and has been developing and applying machine learning in the medical domain for 25 years, with a focus on open science through his leadership of the annual PhysioNet Challenges. His application areas span neuropsychiatric health, sleep, cardiovascular disease and maternal-fetal health. He has a specific interest in working with marginalized communities, particularly in Central America where he works with his anthropologist partner, Prof. Rachel Hall-Clifford, and with whom he jointly founded the Co-design Lab for Health Equity and Safe+Natal.

Stuart Cobb, PhD

Lab Head and Professor of Translational Neuroscience, Patrick Wild Centre and Simons Initiative for the Developing Brain, Centre for Discovery Brain Sciences, University of Edinburgh
Chief Scientific Officer, Neurogene Inc.

Stuart Cobb heads a translational research laboratory that is focused on developing genetic therapies for severe neurological and neurodevelopmental disorders, notably Rett syndrome. His research aims to address the tractability of severe brain disease to genetic rescue and to develop innovative therapeutic solutions for clinical translation. His research highlights include contributing to original genetic rescue studies in Rett syndrome, and his group was the first to report the ameliorative effect of gene therapy in mice modeling the disorder. His laboratory has developed a number of novel gene therapy approaches optimized for efficacy and safety. This includes the development of regulated expression cassettes and RNA based approaches. He was a founding member of the Rett Syndrome Research Trust Gene Therapy Consortium.

Since late 2018 he has been Chief Scientific Officer at Neurogene, a genetic therapy company focusing on rare neurological disease, including a clinical stage Rett syndrome program.

Ben Deverman, PhD

Senior Director of Vector Engineering, Institute Scientist, Broad Institute of MIT and Harvard

Ben Deverman is the Senior Director of Vector Engineering and an Institute Scientist at the Broad Institute of MIT and Harvard. His laboratory in the Stanley Center for Psychiatric Research at the Broad Institute develops and applies novel high-throughput screens and data-driven protein and genome engineering techniques to create more efficient and targeted AAV vectors to advance gene therapy and support scientific discovery. In prior work at the California Institute of Technology, Ben and colleagues identified numerous capsids, including AAV-PHP.B and an enhanced variant, AAV-PHP.eB, that made efficient brain-wide gene delivery possible for the first time. Ben earned a PhD in Molecular Cell Biology from Washington University and was trained as a postdoc in neuroscience at the California Institute of Technology.

Jeremy Duffield, MD, PhD, FRCP

Chief Scientific Officer, Prime Medicine

Jeremy Duffield, MD, PhD, FRCP, is the Chief Scientific Officer of Prime Medicine. He has many years of drug discovery experience at Vertex Pharmaceuticals and Biogen Inc. preceded by a distinguished career in academic medicine.

Dr. Duffield has held several leadership roles, with focus in the fields of human genetics, innate immunity and regenerative medicine. He served as Global Head of Human Biology at Vertex Pharmaceuticals and as Vice President of Business Development where he and his team played important roles in discovering and advancing candidates to clinical studies in rare diseases including cystic fibrosis, a1-antitrypsin deficiency, sickle cell disease, FSGS and muscular dystrophies. Several candidates are now approved therapies. He was instrumental in building Vertex Cell and Genetic Therapies.

At Biogen, Dr. Duffield served as Senior Research Fellow and Vice President with responsibilities in early research programs, as joint Head of Innate Immunity and Regenerative Medicine therapeutic area, and as Head of the Biogen Post-Doctoral program. There he contributed to advancing integrin inhibitors, TNF superfamily inhibitors and IRAK inhibitors to clinical evaluation for pulmonary fibrosis and autoimmune diseases.

Prior to joining the leadership at Biogen, Dr. Duffield had a distinguished academic career on the faculty at University of Washington and Harvard Medical School as Head of the National Institutes of Health/National Center for Advancing Translational Sciences/American Heart Association-funded Laboratory for Innate Immunity and Regeneration. His laboratory used contemporary genetic methods to study cell and molecular function in innate immune and vascular cells in disease. Inventions from the laboratory contributed to the creation of several biotech companies, two of which advanced candidates now in late clinical trials. Additionally, Dr. Duffield practiced Internal Medicine and Nephrology at Massachusetts General Hospital until 2019.

Dr. Duffield served on NIH study sections, several company scientific advisory boards, is a member of the American Society of Clinical Investigation and received many scientific awards including the ASN-AHA Young Investigator Award and the NIH Early Career Investigator/Scholar Award.

Dr. Duffield received his BA and MD (BM, BCh) from Oxford University and a PhD in Immunology from the University of Edinburgh in the laboratory of Sir John Savill.

Victor Faundez, MD, PhD

Professor and Vice Chair, Department of Cell Biology, Emory University School of Medicine

Dr. Victor Faundez is Professor and Vice Chair of the Department of Cell Biology at the Emory University School of Medicine. He obtained his MD and PhD in Catholic University, Santiago, Chile to then perform post-doctoral studies at the Department of Biochemistry and Biophysics UCSF as a Fogarty-NIH fellow. He joined Emory University in 2000. His research interest resides in the mechanisms of rare neurological childhood disorders using proteomics approaches in mouse models of Rett syndrome and other rare childhood neurological disorders, such as 22q11.2 microdeletion syndrome and CDKL5 deficiency disorder. His scholarly contributions can be perused at www.faundezlab.org. In addition to his research, Dr. Faundez has been an active member of the Emory community participating in key educational and service activities. His teaching has been recognized twice with the School of Medicine Dean's Teaching Award. He was also twice awarded the Phi Beta Kappa Society Excellence in Teaching Award, and he is a recipient of the Graduates in Neuroscience Faculty of the Year Award.

Guoping Feng, PhD

Poitras Chair Professor of Neuroscience, MIT

Dr. Feng is the Poitras Chair Professor of Neuroscience, a member of the Yang Tan Collective, and Associate Director of the McGovern Institute for Brain Research, Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology. He is also the Director of Model Systems and Neurobiology at the Stanley Center for Psychiatric Research at Broad Institute of MIT and Harvard. Dr. Feng's research is devoted to understanding the mechanisms regulating the development and function of synapses in the brain and how synaptic dysfunction may contribute to brain disorders. Using genetically engineered animal models, Dr. Feng's laboratory combines cutting-edge technologies and multidisciplinary approaches to unravel the neurobiological mechanisms of neurodevelopmental and psychiatric disorders. Dr. Feng's lab is also a leading lab in generating genetic tools for neuroscience and brain disorder research.

Toby Ferguson, MD, PhD

Head Neuromuscular and Movement Disorders Development Unit, Biogen

Toby is a neuromuscular neurologist and neuroscientist who joined Biogen in 2013. His professional experience has focused on developing treatments for neuromuscular disease, with a focus on ALS and SMA as well as a more recent focus on movement disorders. He is currently the Head of the Neuromuscular and Movement Disorders Development Unit at Biogen. He plays a key role in developing clinical trials across his therapeutic areas and in driving clinical and preclinical strategy within neurodegenerative disease more broadly. His group also works closely with the preclinical scientific and biomarker teams as well as with external collaborators to identify novel disease targets and to develop the needed tools for efficient clinical development.

At Biogen, he has advanced multiple programs into the clinic for ALS, SMA, Myotonic Dystrophy, and Parkinson's disease. He has also helped to successfully develop Qalsody (tofersen) an ASO indicated for the treatment SOD1 ALS. He is dedicated to the successful development of meaningful therapeutics for neurologic disease and strongly believes that collaboration across industry, academia, and advocacy organizations is crucial to developing meaningful therapies.

Prior to Biogen, Toby had a clinical neuromuscular neurology practice and a lab focused on peripheral axon injury and regeneration at Shriners Research Center and Temple University in Philadelphia. Toby trained in neurology and neuromuscular neurology at the University of Pennsylvania. He obtained an MD and PhD (Neuroscience) at the University of Florida.

Patrick Finn, PhD

Vice President of Preclinical Development, Rare Disease Research, Moderna

Patrick Finn is a dedicated and enthusiastic scientist with over 15 years of experience in the biotech industry primarily focusing on developing drugs for patients with rare diseases. He currently serves as Vice President of Preclinical Development and Rare Disease Research at Moderna. His work focuses on identifying and characterizing mRNA drug candidates from discovery to clinical development.

Throughout his career, he has held various positions at Genzyme Corporation, Sanofi, and now Moderna. Dr. Finn holds a PhD in Cellular and Molecular Physiology from Tufts University School of Medicine. He has a strong track record of publications and is a co-inventor on several patents. His research interests include lysosomal storage diseases, muscle biology, metabolism.

Nasha Fitter, MBA

Vice President of Patient Data Platform, Ciitizen

Nasha Fitter is a leader in the rare disease space through her work on utilizing real world evidence to accelerate treatments. She is currently Vice President of Patient Data Platform at Ciitizen. She is also the mother of a child with the rare disease, FOXG1 Syndrome, and co-founded and leads the FOXG1 Research Foundation.

Formerly, Nasha was the founder/CEO of education-tech startup Schoolie, which was acquired in 2016 by GreatSchools. She also was a Director at Microsoft Corp where she held various management and product development positions.

Nasha is also on the public board of the ACMG Foundation for Genetic and Genomic Medicine and has an MBA from Harvard Business School.

John Foxe, PhD

Kilian J. and Caroline F. Schmitt Chair in Neuroscience, University of Rochester Medical Center

John Foxe is an Irish Neuroscientist with a history of basic research studies into the neurophysiology of multisensory integration and attention. He works to translate new understanding generated in his basic research program to advance understanding of the neuropathology underlying a number of neurodevelopmental and psychiatric conditions. Special emphasis is placed on the identification of endophenotypes (neuromarkers) of disease, in linking these biomarkers to the underlying genotype, and in associating these markers with clinical manifestations. He has worked extensively in neurodevelopmental disorders such as Autism, Rett syndrome, and other rare diseases of neurodevelopment, successfully gathering large-scale neuroimaging and neurophysiological datasets from these vulnerable and often difficult-to-test populations. He is Editor-in-Chief of *The European Journal of Neuroscience*, the official journal of the Federation of European Neuroscience Societies (FENS). He has published over 340 peer-reviewed papers (H-Index = 102).

Cary Fu, MD

Assistant Professor, Department of Pediatrics, Vanderbilt University Medical Center

Dr. Cary Fu is a pediatric epileptologist and assistant professor in the Department of Pediatrics at Vanderbilt University Medical Center in Nashville, Tenn. He also serves as medical director of the Rett syndrome clinic at Vanderbilt Children's Hospital. Dr. Fu works closely with the Rett syndrome program at the Vanderbilt Kennedy Center and was involved with the NIH-sponsored Rett and related disorders natural history study. Dr. Fu has also served as a co-investigator on several clinical trials for Rett syndrome, MECP2 duplication syndrome, and Angelman syndrome. He earned his MD from the University of Missouri School of Medicine, completed a residency in pediatric neurology at the University of Alabama at Birmingham, and completed a fellowship in clinical neurophysiology and epilepsy at Vanderbilt University Medical Center. After his fellowship, Dr. Fu spent three years engaged in post-doctoral research in the Department of Neurology at Vanderbilt University Medical Center prior to joining the faculty.

Harrison Gabel, PhD

Associate Professor, Department of Neuroscience, Washington University

Harrison Gabel received his BA in Molecular Biology from Princeton University and his PhD in Genetics from Harvard University. He is currently an associate professor in the Department of Neuroscience at Washington University in St Louis. Since establishing his laboratory in 2015, Dr. Gabel's research group has combined genetic, genomic, and biochemical approaches in mice and human cellular models to identify and dissect molecular mechanisms of essential gene-regulatory pathways in the brain. A major goal of this work is to understand how disruption of transcriptional regulation can lead to Rett syndrome and related neurodevelopmental disorders that are caused by mutation of DNA methylation pathway genes. In ongoing studies, Dr. Gabel's group is investigating the molecular and cellular consequences of gene mutation in these disorders and developing experimental systems to explore new candidate therapeutic approaches.

David J. Huss, PhD

Chief Scientific Officer, Shape Therapeutics

David J. Huss obtained his PhD in Molecular, Cellular and Developmental Biology from The Ohio State University where he established himself as a card-carrying T cell immunologist. During his PhD, exposure to both basic research and clinical and translational medicine ignited David's interest to pursue a career in the biotech industry.

In his professional career, David has held roles along the entire drug development lifecycle from discovery research to post-approval sales and marketing. David is currently Chief Scientific Officer at ShapeTX and has driven all aspects of the business, including financings, scientific strategy, and business development. His team is developing programmable RNA medicines. Their innovations have caught the attention of pharma and venture capitalists alike. Under David's leadership, ShapeTX raised $150M and secured a $3B research collaboration with Roche.

Prior to ShapeTX, David led a T cell engineering team at Juno Therapeutics. Before Juno, he led preclinical research programs at Biogen and contributed important scientific insights into the Daclizumab and Tecfidera mechanisms of action, resulting in four peer-reviewed publications.

Dina Katabi, PhD

Thuan and Nicole Pham Professor of Electrical Engineering and Computer Science, MIT

Dina Katabi is the Thuan and Nicole Pham Professor of Electrical Engineering and Computer Science at MIT, and the director of MIT's Center for Wireless Networks and Mobile Computing. She is a recipient of the MacArthur Genius Award, and a member of the National Academy of Engineering, the National Academy of Sciences, and the American Academy of Arts and Sciences. Dr. Katabi received her PhD and Master's degrees from MIT, and her BS from Damascus University. Her research focuses on innovations in digital health, wireless sensing, and applied machine learning. Dr. Katabi's research has been recognized with the Association for Computing Machinery (ACM) Prize in Computing, the ACM Grace Murray Hopper Award, two ACM SIGCOMM and one ACM SIGMOBILE Test of Time Awards, the Faculty Research Innovation Fellowship, a Sloan Research Fellowship, the NBX Career Development Chair, and the National Science Foundation CAREER Award. Her students twice received the ACM Doctoral Dissertation Award in computer science and engineering. Her work was also recognized with the IEEE William R. Bennett Prize, three ACM SIGCOMM Best Paper awards, a Networked Systems Design and Implementation Best Paper award, and a TR10 award. Several startups have been spun out of Katabi's lab, including PiCharging and Emerald.

David Lieberman, MD, PhD

Assistant Professor of Neurology, Boston Children's Hospital

David Lieberman, MD, PhD, is a child neurologist at Boston Children's Hospital. Dr. Lieberman completed his medical and doctorate degrees in Neuroscience at Stanford University School of Medicine. He continued his training at Johns Hopkins University School of Medicine, completing an internship in Pediatrics and a residency in Pediatric Neurology. Dr. Lieberman remained at Johns Hopkins as faculty for a number of years before moving back to the west coast to take a faculty position in the Neurosciences Department at UC San Diego in 2007. Dr. Lieberman moved to Boston in 2015 and serves as the Director of the Comprehensive Rett Syndrome Program at Boston Children's Hospital where he follows more than 200 patients with Rett syndrome and Rett-related disorders. Dr. Lieberman has served as a site PI for a number of interventional studies in Rett syndrome.

David R. Liu, PhD

Richard Merkin Professor and Director of the Merkin Institute of Transformative Technologies in Healthcare, Vice-Chair of the faculty at the Broad Institute of MIT and Harvard, the Thomas Dudley Cabot Professor of the Natural Sciences at Harvard University, HHMI

Liu's research integrates chemistry and evolution to illuminate biology and enable next-generation therapeutics. His major research interests include the engineering, evolution, and in vivo delivery of genome editing proteins such as base editors and prime editors to study and treat genetic diseases; the evolution of proteins with novel therapeutic potential using phage-assisted continuous evolution (PACE); and the discovery of bioactive synthetic small molecules and synthetic polymers using DNA-templated organic synthesis and DNA-encoded libraries. Base editing—the first general method to perform precision gene editing without double-stranded breaks, and a *Science* 2017 Breakthrough of the Year finalist—as well as prime editing, PACE, and DNA-templated synthesis are four examples of technologies pioneered in his laboratory. These technologies are used by thousands of labs around the world and have enabled the study and treatment of genetic diseases. Five base editing clinical trials are already underway to treat leukemia, hypercholesterolemia, sickle-cell disease, and beta-thalassemia, and the first clinical benefit of a base edited therapeutic in a T-cell leukemia trial has been reported.

Liu graduated first in his class at Harvard College in 1994. During his doctoral research at UC Berkeley, Liu initiated the first general effort to expand the genetic code in living cells. He earned his PhD in 1999 and became assistant professor of chemistry and chemical biology at Harvard University in the same year. He was promoted to associate professor in 2003 and to full professor in 2005. Liu became a Howard Hughes Medical Institute investigator in 2005 and joined the JASONs, academic science advisors to the U.S. government, in 2009. In 2016 he became a Core Institute Member and Vice-Chair of the Faculty at the Broad Institute of MIT and Harvard, and Director of the Chemical Biology and Therapeutics Science Program.

Liu has published more than 250 papers and is the inventor on more than 110 issued U.S. patents. Liu has been elected to the U.S. National Academy of Sciences, the U.S. National Academy of Medicine, and the American Association for the Advancement of Science. He is the 2022 King Faisal Prize Laureate in Medicine. He has earned several University-wide distinctions for teaching at Harvard, including the Joseph R. Levenson Memorial Teaching Prize, the Roslyn Abramson Award, and a Harvard College Professorship. His research accomplishments have earned distinctions including the Ronald Breslow Award for Biomimetic Chemistry, the American Chemical Society David Perlman Award, *ACS Chemical Biology* Award, the American Chemical Society Pure Chemistry Award, the Arthur Cope Young Scholar Award, the NIH Marshall Nirenberg Lecturer, and awards from the Sloan Foundation, Beckman Foundation, NSF CAREER Program, and Searle Scholars Program.

In 2016, 2019, 2020, and 2021 he was named one of the Top 20 Translational Researchers in the world by *Nature Biotechnology*, and was named one of *Nature*'s 10 researchers in world. Liu was named as a leading global thinker by the *Foreign Policy* Leading Global Thinkers in 2017, to the STATUS List of 46 leaders in health, medicine, and science by *STAT News*, and as one of the "Most Influential People in Biopharma in 2023" by *Fierce Biotech*. Liu is the founder or co-founder of several public and private biotechnology and therapeutics companies, including Beam Therapeutics, Prime Medicine, Editas Medicine, Pairwise Plants, Chroma Medicine, Exo Therapeutics, Resonance Medicine, and Nvelop Therapeutics.

Outside of the lab, Liu pursues a variety of hobbies at the intersection of science and art, including photography around the world, raising large bonsai trees indoors under LED light canopies he designed, creating art from wood, metal, stone, and/or electronic components, and painting.

Shawn Liu, PhD

Joan and Paul Marks Assistant Professor, Department of Physiology and Cellular Biophysics, Columbia University

Shawn Liu is a Joan and Paul Marks Assistant Professor in the Department of Physiology and Cellular Biophysics at Columbia University. Shawn developed a series of epigenome editing tools to systematically investigate the functional significance of epigenetic modifications in neurological disorders (Liu et al., Cell, 2016; Liu et al., Cell, 2018). Established in 2020, Shawn's laboratory aims to decode the epigenome in physiology and diseases. His lab combines molecular tool development with genetic and genomic approaches to explore epigenetic mechanisms underlying normal physiological functions such as the molecular substrates of memory (Shpokayte et al., Nature Communication Biology, 2022), and to uncover the epigenetic basis of diseases to accelerate the development of therapeutics such as Fragile X syndrome (Liu et al., Cell, 2018) and Rett syndrome (Qian et al., Science Translational Medicine, 2023).

David J. Lockhart, PhD

President & Chief Scientific Officer, ReCode Therapeutics

Dr. Lockhart is an accomplished biotech executive with more than 25 years of experience across all phases of drug discovery, drug development and technology development.

Prior to becoming president and CSO of ReCode Therapeutics in January 2022, he served as CEO and president of the company since March 2020. Prior to serving as CEO and president of ReCode, he was CEO and president of its predecessor, TranscripTx from 2014 to 2020. Prior to TranscripTx, he was the Chief Scientific Officer at Amicus Therapeutics from 2006 through 2013. He led a team at Amicus that brought multiple rare disease programs into clinical trials.

Previously, he co-founded Ambit Biosciences, serving as Chief Scientific Officer and president for five years, during which the company developed a novel kinase profiling technology and new kinase inhibitors for cancer and other diseases. Prior to Ambit, Dr. Lockhart served as Director of Genomics at the Genomics Institute of the Novartis Research Foundation (GNF). Dr. Lockhart began his biotech career at Affymetrix, where he was the primary inventor and developer of gene expression profiling with DNA microarrays.

Dr. Lockhart received his PhD in Chemistry from Stanford University and was a postdoctoral fellow at the Whitehead Institute in the Biology Department at MIT. He has published more than 80 peer-reviewed scientific articles on pharmacological chaperones, mutation-specific selection of patients for clinical trials, clinical studies with pharmacological chaperones, genomics, kinase technology, kinase inhibitor discovery, and the use of genomic tools to address important biological and biomedical problems. He is also the inventor on more than 40 issued U.S. patents

Kate Lyden, PhD

Chief Science Officer, VivoSense

Kate Lyden, PhD is the Chief Science Officer for VivoSense, a science and analytics company that develops and validates digital clinical measures and provides end-to-end services for their delivery in regulated clinical trials. In this role, Kate leads a team of scientists dedicate to developing and delivering real-world digital measures that are trusted and valued by all stakeholders.

Kate has an extensive and diverse background in the development and implementation of wearable sensor methodologies across clinical, academic, and industry settings. Her research has focused on synchronizing continuous measures of real-world physical behavior with physiological signals and patient reported outcomes to build contextually rich datasets that inform patient focused drug development and clinical care.

In addition to her work at VivoSense, Kate maintains adjunct faculty positions at The University of Massachusetts and Colorado State University, has authored many scientific publications on the objective measurement of human behaviors, and is a frequent contributor on this topic in academic, industry, and clinical working groups.

John Maraganore, PhD

Former Founding CEO, Alnylam Pharmaceuticals

Dr. John Maraganore served as the founding CEO and a Director of Alnylam from 2002 to 2021, where he built and led the company from early platform research on RNA interference through global approval and commercialization of the first four RNAi therapeutic medicines, ONPATTRO,® GIVLAARI,® OXLUMO,® and Leqvio.® The fifth RNAi therapeutic, AMVUTTRA,® was approved in mid-2022. At Alnylam, he also led the company's value creation strategy, building over $25B in market capitalization and forming over 20 major pharmaceutical alliances. He continues to serve on the Alnylam Scientific Advisory Board.

Prior to Alnylam, he was at Millennium Pharmaceuticals, Inc., where he was responsible for the company's product franchises in oncology, and cardiovascular, inflammatory and metabolic diseases, in addition to leadership of M&A, strategy, and biotherapeutics functions. Before Millennium, he held scientific and business roles at Biogen, Inc. where he invented and led the discovery and development of ANGIOMAX® (bivalirudin) for injection. Previously, he was a scientist at ZymoGenetics, Inc. and the Upjohn Company.

Dr. Maraganore is currently a Venture Partner at ARCH Venture Partners, a Venture Advisor at Atlas Ventures, an Executive Partner at RTW Investments, a Senior Advisor for Blackstone Life Sciences, and an Advisor for M28. He is also member of the Board of Directors of publicly traded companies, including Agios Pharmaceuticals, Beam Therapeutics, Kymera Therapeutics, ProKidney Corp., and Takeda Pharmaceuticals. He is also on the Board of a number of private companies — including Aerium Therapeutics, Aera Therapeutics (Chair), Aitia (Chair), Hemab Therapeutics (Chair), Orbital Therapeutics (Executive Chair), and Versanis Bio. As the principal of JMM Innovation, LLC, John serves as a strategic advisor to a number of innovative biotechnology companies, including mentorship of CEOs in their mission to advance science and innovation for patients.

Dr. Maraganore is on the Board of the Biotechnology Innovation Organization, or "BIO," where he was Chair from 2017 to 2019 and is Chair Emeritus. In addition, he serves on the Board of the Termeer Foundation — committed to continuing the legacy of the late Henri A. Termeer, as Chair of the n-Lorem Foundation Advisory Council — committed to meeting the needs of patients with nano-rare diseases, on the Advisory Board of Ariadne Labs — advancing global health system innovations, and as an advisor to Nucleate — a student-led organization facilitating the formation of pioneering life sciences companies.

Dr. Maraganore received his BA, MS and PhD in biochemistry and molecular biology at the University of Chicago.

Peter Marks, MD, PhD

Director, Center for Biologics Evaluation and Research, FDA

Peter Marks received his graduate degree in cell and molecular biology and his medical degree at New York University and completed Internal Medicine residency and Hematology/Medical Oncology training at Brigham and Women's Hospital in Boston. He has worked in academic settings teaching and caring for patients and in industry on drug development and is an author or co-author of over 100 publications. He joined the FDA in 2012 as Deputy Center Director for CBER and became Center Director in 2016. Over the past several years he has been integrally involved in the response to various public health emergencies, and in 2022 he was elected a member of the National Academy of Medicine.

Eric Marsh, MD, PhD

Attending Pediatric Neurologist, Division of Neurology at Children's Hospital of Philadelphia (CHOP)
Associate Professor of Neurology, Perelman School of Medicine at the University of Pennsylvania

Dr. Marsh specializes in diagnosing and treating children with developmental epilepsies (including infantile spasms syndrome), brain malformations, and other neurogenetic conditions. He runs the neurogenetics, Rett, and CDKL5 clinics at CHOP. His clinical research focuses on Rett syndrome, Dravet syndrome, Lennox-Gastaut syndrome, CDKL5 syndrome, and related disorders, and is involved in clinical trials for novel therapeutics for these disorders. Dr. Marsh also has a basic and translational science lab where he studies mutations in genes that cause epilepsy and how developmental disorders lead to the early epileptic encephalopathies using mice as models of these conditions. Dr. Marsh earned his MD and PhD from the New York University School of Medicine, and completed his pediatric residency at Bellevue Hospital. His residency and fellowship in pediatric neurology and clinical research were completed at CHOP.

Andrew E. Mulberg, MD

Senior Vice President, Regulatory Affairs, Quality Assurance and Quality Control, Neurogene Inc.

Andrew is currently an executive pharmaceutical leader leading strategy and operational aspects of drug and biological development for NDA and BLA submissions for pediatric and adult rare diseases, cystic fibrosis, gastroenterology and hepatology. He is currently Senior Vice President, Regulatory Affairs at Neurogene Inc, a company devoted to gene therapy approaches to management of neurodegenerative and neurodevelopmental disorders in children and adults. Formerly, he served Senior Vice President, Global Regulatory Affairs at Amicus Therapeutics from 2016-2020 and was responsible for the approval of Galafold (migalastat) for the treatment of Fabry disease in adults. He has been involved in the registration planning for AT-GAA, a novel enzyme replacement therapy for Pompe disease in infants, children and adults. He served as Division Deputy Director of Gastroenterology and Inborn Errors Products, Center for Drug Evaluation and Research (CDER), U.S. Food and Drug Administration (FDA) from 2010 to 2016. Before joining FDA, Andrew was Portfolio Leader in Established Products responsible for worldwide leadership of Internal Medicine products at Johnson and Johnson from 2000 to 2010.

Andrew is a graduate of Columbia College of Columbia University and of the Mount Sinai School of Medicine. He completed his residency in Pediatrics at the Children's Hospital of Philadelphia followed by a Pediatric Gastroenterology Clinical Fellowship and a Post-Doctoral Fellowship in Cellular and Molecular Physiology at New England Medical Center. Andrew is Adjunct Professor of Pediatrics at the University of Maryland School of Medicine and has served as Attending, Pediatric Gastroenterology and Nutrition at Cooper University Hospital in New Jersey caring for children with gastrointestinal diseases. He is Principal Editor of Pediatric Drug Development: Concepts and Applications published in 2011 and 2013. He is a member of Alpha Omega Alpha Honor Medical Society, American Gastroenterological Association and the North American Society for Pediatric Gastroenterology and Nutrition.

Lindsey Murray, PhD, MPH

Executive Director, Rare Disease Clinical Outcome Assessment (COA) Consortium, Critical Path Institute

Lindsey Murray, PhD, MPH, is Executive Director of the Rare Disease Clinical Outcome Assessment (COA) Consortium. Dr. Murray has more than 15 years of experience in health outcomes research. She specializes in quantifying the patient's perspective of health, illness, and treatment through COA development, psychometric testing, and the design and analysis of clinical trials involving COAs. Prior to joining C-Path, Dr. Murray was an integral part of the EXacerbations of Chronic Pulmonary Disease Tool (EXACT®) - Patient-Reported Outcome Initiative team, taking over as Director of the EXACT PROgram. As Director, she had oversight of all EXACT licensing, translations, and analysis work being conducted on the EXACT and its derivative measure, the Evaluating Respiratory Symptoms in Chronic Obstructive Pulmonary Disease (E-RS®: COPD). Dr. Murray's research activities also included serving as principal investigator to develop patient-reported outcome measures in several rare diseases, including neurotrophic keratosis and hypertrophic cardiomyopathy. She was also involved in adapting the E-RS: COPD for use in idiopathic pulmonary fibrosis (E-RS: IPF) and asthma-COPD overlap syndrome.

Dr. Murray holds a PhD from George Washington University in epidemiology. She received her MPH from George Washington University in epidemiology with a certificate in health promotion. Previously, Dr. Murray received a BA from the University of Virginia in Charlottesville, Virginia, where she double majored in anthropology and biology with a minor in German literature and language studies.

Sukumar Nagendran, MD

President and Head of Research & Development, Taysha Gene Therapies, Inc.

Sukumar Nagendran is the President and Head of R&D at Taysha Gene Therapies, Inc., a clinical-stage gene therapy company focused on developing and commercializing AAV-based gene therapies for the treatment of monogenic rare diseases of the central nervous system (CNS). He is a physician leader with more than 30 years of experience in key functional areas, including gene therapy development, clinical development strategy, medical affairs and diagnostics of therapeutic products. Before joining Taysha, Dr. Nagendran served as Chief Medical Officer and President of R&D at Jaguar Gene Therapy. Prior to that, Dr. Nagendran served as Chief Medical Officer of AveXis, Inc. where he oversaw the development of Zolgensma for the treatment of spinal muscular atrophy, the first one-time systemic gene therapy approved in the U.S. Dr. Nagendran has also held key leadership positions at Quest Diagnostics, Pfizer, Novartis, Daiichi Sankyo, and Reata Pharmaceuticals.

Prior to transitioning to the biotech industry, Dr. Nagendran practiced internal medicine, with a focus on diabetes and cardiovascular disease. He currently serves on the Board of Directors of SalioGen Therapeutics, Solid Biosciences, Cove, Medocity, Project Healthy Minds, and Taysha Gene Therapies.

He holds an undergraduate degree in Biochemistry from Rutgers University and earned his MD from Rutgers Medical School, and he trained in Internal Medicine at Mayo Clinic, Rochester.

Dr. Nagendran is a Mayo Alumni Laureate and founding member of the Robert Wood Johnson Legacy Society. He is also the sponsor for the Jerry Mendell award for Translational Science at the American Society of Gene and Cell Therapy which recognizes the extensive work required to bring gene and cell therapies to clinical trial, and the Fonseca-Nagendran Scholar award at the American Diabetes Association to enhance research in minority populations.

Osuma Nakanashi, PhD

Chief Scientific Officer, EditForce, Inc.

Osuma Nakanashi received a PhD in biochemistry at the University of Tokyo in 1994. He has more than 20 years of experience at pharma companies including Nihon Schering and Takeda Pharmaceuticals working on drug discovery research, especially in oncology. Nakanashi is now working on drug discovery in RNA-targeting therapeutics applying EditForce's proprietary pentatricopeptide technology. Life-time research interests include transmembrane signaling, splicing, epigenetics and disease biology.

Kalina Paunovska, PhD

Co-founder, Nava Therapeutics

Kalina Paunovska is the co-founder of Nava Therapeutics, an early-stage biotech startup in Cambridge, MA specializing in lipid nanoparticle (LNP) delivery and gene therapy. Nava Therapeutics emerged from postdoctoral work combining high-throughput LNP screening with single cell RNA sequencing that Kalina developed alongside Curtis Dobrowolski and James Dahlman. Kalina completed her PhD in Biomedical Engineering at Georgia Tech in spring 2020, under the supervision of James Dahlman; her doctoral studies focused on LNP discovery and high-throughput, barcoding-based LNP screening in vivo.

Mustafa Sahin, MD, PhD

Professor of Neurology, Harvard Medical School
Rosamund Stone Zander Chair, Boston Children's Hospital

Mustafa Sahin is a developmental neurobiologist and a pediatric neurologist at Boston Children's Hospital and Harvard Medical School. He received his ScB degree from Brown University, and his MD and PhD from Yale School of Medicine. He completed a pediatrics residency at Children's Hospital of Philadelphia and a child neurology residency at Boston Children's Hospital. Dr. Sahin is a Professor of Neurology at Harvard Medical School and the Rosamund Stone Zander Chair at Boston Children's Hospital. At Boston Children's, Dr. Sahin is the Director of the Translational Research Program and the Rosamund Stone Zander Translational Neuroscience Center. He also chairs the Clinical Translational Research Executive Committee (CTREC). He is the co-PI of the Intellectual and Developmental Disabilities Research Center (IDDRC) at BCH. He directs a national consortium to study biomarkers and comparative pathobiology of TSC, PHTS and Phelan McDermid Syndrome, three genetic disorders associated with autism and intellectual disability.

Frank J. Sasinowski, MS, MPH, JD

Director, Hyman, Phelps & McNamara

Frank J. Sasinowski, MS, MPH, JD, by assisting sponsors and patient organizations in developing new medicines, has helped secure FDA approval for hundreds of new drugs, including more than 100 new molecular entities, often for serious and rare diseases. Frank was involved in 6 of the most recent 8 drugs FDA approved by way of its accelerated approval process. Frank is involved in many cell and gene therapies and aided significantly on the first approved systemic gene therapy, Zolgensma.

Frank joined FDA in 1983 as regulatory counsel in the Center for Drugs and Biologics, where he was key to implementing both the 1983 Orphan Drug law and the 1984 Hatch-Waxman law. In 1987, he left the FDA as Deputy Director of health policy in Commissioner's office and joined Hyman, Phelps & McNamara.

In its March 2012 issue, the Drug Information Journal published Frank's seminal analysis on therapies for rare disorders: "Quantum of Effectiveness Evidence in FDA's Approval of Orphan Drugs Cataloguing FDA's Flexibility in Regulating Therapies for Persons with Rare Disorders." Other papers of his on Accelerated Approval and Orphan Drugs are regularly cited FDA, industry and academia as well. Thanks to his extensive FDA experience, both from within the Agency and from a sponsor's perspective, as well as his passionate advocacy for integrating the voice of the patient in developing medicines, Frank possesses a nuanced and deep understanding of the drug regulatory process.

Since 2014, Frank has been an Adjunct Professor of Neurology at the University of Rochester School of Medicine. His work has been widely recognized by industry and political leaders. For example, Frank was asked by both political parties to testify at the May 2014 inaugural congressional hearing on the 21st Century Cures law. In August 2018 Frank was appointed to the Board of Directors for the Alliance for Regenerative Medicine (ARM) Foundation for Cell and Gene Medicine. In May 2013, the National Organization for Rare Disorders (NORD) awarded Frank its first ever Lifetime Achievement Award. In 2000, Frank was elected to NORDS's Board of Directors, where he served as Chair and as Vice Chair & was on its Board until 2016. In 2017 Frank joined the Board of the Everylife Foundation for Rare Diseases where he currently is its Vice Chair. In October 2012, President Obama recognized Frank's contributions to the President's Council of Advisors on Science and Technology (PCAST) report "Propelling Innovation in Drug Discovery, Development and Evaluation." Frank is a founding Board member of the IndoUSrare patient organization, representing those in India and in the US with rare conditions. Frank has also served on the Board of Directors of the United States Pharmacopeia (USP) and has served on the board of several biotechnology companies.

John Sinnamon, PhD

Consultant, Rett Syndrome Research Trust

John received his PhD from Stony Brook University in Neuroscience where his work focused on post-transcriptional regulation in the nervous system. He then joined the lab of Dr. Gail Mandel in the Vollum Institute at Oregon Health and Science University, where as a post-doctoral fellow he developed a site-directed RNA editing approach for Rett syndrome and was promoted to research assistant professor. In 2022, John joined Pfizer as a Principal Scientist where he works on developing nucleic acid based therapeutics.

Gaia Skibinski, PhD

Senior Vice President of Biology, Herophilus

Gaia is the SVP of biology at Herophilus and specializes in target and therapeutic discovery using patient derived stem cells and organoid biology. She read Biological Sciences at Magdalen College at Oxford University and earned a PhD at the Institute of Neurology in London where she identified *CHMP2B*, a novel gene for Frontotemporal Dementia. During her postdoctoral training at the Gladstone Institutes, she established a range of human and rodent neuron models of Parkinson's disease. She utilized these models to investigate the pivotal roles played by LRRK2 and alpha-synuclein during the degenerative process and facilitated discovery of potential therapeutic targets. The endeavor flourished into a highly successful Parkinson's disease program that revolved around complex *in vitro* models, assays and screening platforms. As a program leader at the Gladstone Institutes, she successfully secured and executed successful program collaborations with industry partners including Amgen, Biogen, Merck, Google Accelerated Science and Verily.

At Herophilus, a tech-enabled neurotherapeutics company, she heads up the biology group. As one of the early employees, she played a pivotal role in co-building the Herophilus human cerebral organoid platform from its inception. She also spearheaded the development and implementation of robust scientific strategies for the discovery programs focused on Rett syndrome, Alzheimer's disease and Schizophrenia.

Erik J. Sontheimer, PhD

Pillar Chair in Biomedical Research, University of Massachusetts Chan Medical School

Erik J. Sontheimer is the Pillar Chair in Biomedical Research and Professor at the University of Massachusetts Chan Medical School, where he is also Vice Chair of the RNA Therapeutics Institute (RTI). He earned his PhD from Yale University in 1992, followed by postdoctoral research as a Jane Coffin Childs Fund Fellow at the University of Chicago. He then joined the faculty at Northwestern University where he continued his work on the roles of RNA molecules in gene expression, including pre-mRNA splicing mechanisms, RNA interference pathways, and CRISPR immune systems in pathogenic bacteria. Among other advances, in 2008 his group reported that CRISPR systems can function via DNA destruction, and they first described CRISPR's potential for RNA-guided genome engineering.

He has received a CAREER Award from the National Science Foundation, a New Investigator Award in the Basic Pharmacological Sciences from the Burroughs Wellcome Fund, a Basil O'Conner Award from the March of Dimes, a Scholar Award from the American Cancer Society, a Distinguished Teaching Award from the Weinberg College of Arts and Sciences at Northwestern, the Nestlé Award from the American Society for Microbiology, the Mid-Career Award from the RNA Society, and election to the American Academy of Microbiology. In 2014 he co-founded Intellia Therapeutics, Inc. for the development of clinical applications of CRISPR gene editing. That same year he also moved to the RTI at UMass Chan Medical School, where he is continuing his research on the uses of RNA molecules in biomedical research and the treatment of human disease.

From 2021-2023 he co-chaired the Board of Scientific Counselors at the National Cancer Institute, and he currently serves as a member of the Scientific Advisory Boards at Intellia Therapeutics as well as Tessera Therapeutics.

Brett T. Staahl, PhD

Vice President of External Innovation and Co-Founder, Scribe Therapeutics

Brett T. Staahl is the Vice President of External Innovation and Co-Founder at Scribe Therapeutics. Previously, he was a Postdoctoral Fellow in the lab of Jennifer Doudna where he pioneered the use of CRISPR ribonucleoproteins, developed robust methods to deliver the proteins to various cell types both in vivo and ex vivo, and showed how they could safely and effectively be used to inactivate disease-causing genes and slow neurodegeneration. Brett has over 20 scientific publications in top-tier journals, several patents issued and pending, and received numerous research grants and awards including the F. Hoffmann-La Roche Postdoctoral Fellowship.

Prior to completing his postdoctoral work at the University of California, Berkeley, Brett received his BS in Molecular, Cellular and Developmental Biology from the University of Colorado, Boulder, and his PhD in Developmental Biology from Stanford University, where he studied the genetic circuitry underlying development of the mammalian nervous system and discovered de novo function-altering mutations in the genes of sporadic Amyotrophic Lateral Sclerosis (ALS) patients.

Bernhard Suter, MD

Assistant Professor, Department of Pediatrics, Texas Children's Hospital, Baylor College of Medicine

Dr. Bernhard Suter is the medical director of the Blue Bird Circle Rett Center at Texas Children's Hospital and director of the CDKL5 Center of Excellence. He is an associate professor in the Department of Pediatrics and Neurology at Baylor College of Medicine. Dr. Suter is certified by the American Board of Psychiatry and Neurology in neurology and epilepsy and also has research interests in gait analysis and movement disorders in neurodevelopmental disorders. Dr. Suter participated in the NIH-sponsored Rett and related disorders natural history study and has served as an investigator in several clinical trials for Rett syndrome, CDKL5 deficiency disorder, and MECP2 duplication disorder. Originally from Germany, he underwent his medical training in Bonn, Germany, at the Rheinische Friedrich-Wilhelms Universtät. Subsequently, he completed a post-doctoral fellowship in developmental neurobiology at Massachusetts General Hospital and Harvard Medical School, prior to completing his residency in pediatrics and pediatric neurology at Baylor College of Medicine / Texas Children's Hospital and joining the faculty.

Fyodor Urnov, PhD

Professor of Molecular Therapeutics, University of California, Berkeley
Scientific Director, Innovative Genomics Institute

Fyodor Urnov is a Professor of Molecular Therapeutics at UC Berkeley and a Scientific Director at its Innovative Genomics Institute (IG). He co-developed the toolbox of human genome and epigenome editing and led the team that developed a strategy for genome editing in the hemoglobinopathies, sickle cell disease and beta-thalassemia, that has yielded sustained clinical benefit for subjects in several ongoing clinical trials. At the IGI Fyodor directs efforts to develop scalable CRISPR-based approaches to treat diseases of the immune system, sickle cell disease, neurodegeneration, and neuroinflammation. His recent op-ed in the New York Times describes a major goal for the field of genome editing, and a key focus of Fyodor's work at the IGI — expanding access to CRISPR therapies for N=1 genetic disease.

Paul Wang, MD

Deputy Director, Clinical Research, Simons Foundation

Paul Wang is trained as a developmental-behavioral pediatrician, and has worked in academia, industry, and the non-profit sectors. Over the past 20 years, his career has focused on drug development, especially for autism and related genetic disorders. He designed and directed some of the largest randomized, controlled trials ever conducted in these conditions. In his current position, Paul focuses on trial design for autism, the development and validation of outcome measures for these trials, and biomarker strategy for autism drug development. He frequently provides consultation on these issues to academic investigators and to industry. Paul has served in leadership positions for the American Academy of Pediatrics and other professional pediatric organizations, and currently sits on the federal Interagency Autism Coordinating Committee.

Jonathan Watts, PhD

Professor at the RNA Therapeutics Institute of UMass Chan Medical School

Jonathan Watts is Professor at the RNA Therapeutics Institute of UMass Chan Medical School (Worcester, MA). He obtained his PhD in nucleic acid chemistry from McGill University and did postdoctoral work in biochemistry and pharmacology at UT Southwestern Medical Center. Current work in the Watts lab is mainly focused on the optimization of oligonucleotide therapeutics for use in the brain and lung, and on advancing genome editing approaches toward therapeutic use. The Watts group works on both platform technology and disease applications, and has contributed to the development of two drugs that have reached patients on a compassionate use basis.

Winston X. Yan, MD, PhD

Co-Founder, Head of Translational Strategy, Arbor Biotechnologies

Winston X. Yan, MD, PhD, is a physician scientist and genome engineer motivated by using genetic medicines to treat patients, particularly those with rare genetic disease for which no treatments exist. He is a Co-Founder and Head of Translational Strategy at Arbor Biotechnologies, and the Founding President of the N=1 Collaborative, working in both biotech and academic/nonprofit settings to enable new treatments. Winston completed his undergraduate degree in Physics magna cum laude at Harvard College, MD from Harvard Medical School (Health Sciences & Technology track), and PhD from Harvard University working with Feng Zhang at the Broad Institute on early CRISPR genome editing technology development, prior to co-founding Arbor with David Scott, David Walt, and Feng Zhang in 2016.

Zhaolan (Joe) Zhou, PhD

Professor of Genetics and Neuroscience, University of Pennsylvania Perelman School of Medicine
Director of the Preclinical Models Core at the Intellectual and Developmental Disabilities Research Center (IDDRC), Children's Hospital of Philadelphia

Zhaolan (Joe) Zhou is a Professor of Genetics and Neuroscience at the University of Pennsylvania Perelman School of Medicine and Director of the Preclinical Models Core at the Intellectual and Developmental Disabilities Research Center (IDDRC) at Children's Hospital of Philadelphia. After receiving a BS degree from Nankai University and PhD from Harvard University, he carried out postdoctoral training at Harvard Medical School. Since joining the faculty at University of Pennsylvania, his research program focuses on understanding the pathophysiology of neurodevelopmental disorders with known genetic causes, such as Rett syndrome and CDKL5 deficiency disorder (CDD), autism spectrum disorders with complex genetics, and major depressive disorder with environmental insults. In the past few years, he has led a research team that developed the first allelic series of mouse models recapitulating genetic mutations found in Rett syndrome and CDD, engineered genetically modified mice to interrogate stress-related neuroepigenetics, and identified robust and quantitative biomarkers. Through a combined genetic, genomic, physiological, and behavioral approaches, the Zhou laboratory aims to better understand disease pathophysiology and develop therapeutic strategies to treat those neurodevelopmental and neuropsychiatric conditions.

Christina K. Zigler, PhD, MSEd

Assistant Professor, Department of Population Health Sciences, Duke University School of Medicine

A psychometrician and statistician by training, Dr. Zigler uses rigorous, patient-centered methods to develop and evaluate clinical outcome measures. Her primary interest is in designing tools for children with rare diseases so that their voices and the voices of their families can be prioritized in research.

Dr. Zigler was part of the team that developed the Observer-Reported Communication Ability (ORCA) measure through a partnership with the Foundation for Angelman Syndrome Therapeutics to develop the measure for individuals with Angelman syndrome. She also was a co-investigator on a project funded by the Rett Syndrome Research Trust to evaluate the measure for individuals with Rett syndrome. Currently, Dr. Zigler is principal investigator of a five-year study to expand the ORCA measurement model for twelve other neurodevelopmental disorders with similar communication impacts, funded by the U.S. Food & Drug Administration.

Dr. Zigler received her PhD in Research Methodology from the University of Pittsburgh and her MSEd in counseling psychology from the University of Miami. She has been involved in research for over 15 years and has published applied work in rheumatology, pediatrics, human engineering, veterans' affairs, and rehabilitation science. Her current research interests include using mixed methods to explore meaningful changes in patient-reported outcome scores, small sample size statistical methods, and anchoring vignettes.

Huda Zoghbi, MD

Distinguished Service Professor of Pediatrics, Molecular and Human Genetics, Neurology, and Neuroscience, Baylor College of Medicine
Investigator, Howard Hughes Medical Institute
Founding Director, The Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital

Dr. Zoghbi's expertise ranges from neurodevelopment to neurodegeneration. She and Dr. Harry Orr discovered that Spinocerebellar Ataxia type 1 is caused by expansion of a polyglutamine tract. Her subsequent studies demonstrating that such expansion leads to accumulation of the mutant protein in neurons has had profound ramifications since many late-onset neurodegenerative disorders involve similar accumulations of disease-driving proteins. She also discovered that mutations in *MECP2* cause the postnatal neurological disorder Rett syndrome and revealed insight into mechanisms driving neuronal dysfunction. Her team highlighted the sensitivity of the brain to MeCP2 levels, and is currently pursuing therapeutic strategies to help people suffering from Rett and *MECP2* duplication syndrome.

Dr. Zoghbi has trained over 110 scientists and physician-scientists. She has been committed to educating the next generation of scientists and to creating collaborative opportunities. She is a member of multiple professional organizations and boards. She has been elected to the National Academy of Medicine, the National Academy of Sciences, and the American Academy of Arts and Sciences. Dr. Zoghbi's honors include the Pearl Meister Greengard Prize from Rockefeller University; the Breakthrough Prize in Life Sciences; the Canada Gairdner International Prize; and the Kavli Prize in Neuroscience.

SPEAKER ABSTRACTS

Understanding the Molecular Basis of Rett Syndrome

Adrian Bird, University of Edinburgh

Cytosine bases in genomic DNA can be modified post-synthetically by methylation which affects local protein-DNA interactions. The nuclear protein MeCP2 binds to methylated sites, potentially allowing it to interpret this "epigenetic" mark. Several clinical disorders are caused by *MECP2* mutations, including the profound neurological disorder Rett syndrome. Evidence suggests that a root cause of Rett syndrome is failure of MeCP2 to globally restrain neuronal gene expression in a DNA methylation-dependent manner. Our recent studies shed light on the relationship between MeCP2 and heterochromatin and have provided insight into the corepressor NCoR and its partner proteins. We also elucidated the mechanistic consequence of a poorly understood class of Rett syndrome mutations with variable phenotypic penetrance, going on to develop a bespoke genome editing approach with therapeutic potential.

Dissecting the Readout of Cell-type-specific DNA Methylation by MeCP2

Harrison Gabel, Washington University

Understanding the molecular functions of MeCP2 across the diverse cell types that make up the nervous system can provide critical foundational knowledge to support Rett syndrome therapeutic development. Recent studies have made substantial progress in identifying the mechanisms by which MeCP2 binds DNA methylation to regulate transcription. However, systematic analysis of these mechanisms in distinct neuronal populations is essential to better understand the impact of MeCP2 mutation on nervous system function. I will present our latest studies dissecting MeCP2-mediated gene regulation in isolated neuronal cell types. I will describe results showing how cell-type-invariant and cell-type-specific patterning of neuronal non-CG DNA methylation converge to drive overlapping and distinct gene regulation by MeCP2 across cell types, and discuss the potential impact of loss of this regulation in Rett syndrome.

A Novel AAV Capsid that Crosses the Blood Brain Barrier though Engineered Interactions with a Human Protein

Ben Deverman, Stanley Center for Psychiatric Research, Broad Institute of MIT and Harvard

In my talk I will overview our recent work to reprogram the tropism of AAV capsids by introducing new receptor interactions. Unlike conventional in vivo capsid selections in other species, our high-throughout, direct human receptor binding screens generate AAVs that cross the blood brain barrier and deliver genes throughout the CNS via predefined mechanisms of action and have more predictable translational potential. I will present data on a novel capsid that is a promising candidate vehicle for the next generation of gene therapies for neurodevelopmental disorders.

Identifying Novel LNPs by Combining High Throughput Screening and Single Cell Multiomic Readouts

Kalina Paunovska, Nava Therapeutics

High-throughput barcoding-based LNP screening is a useful tool for the identification of new classes of lipids that can deliver to extra-hepatic cell types. Recently, we published a new screening platform that combines high-throughput LNP screening with single cell RNA sequencing. This platform, known as SENT-Seq and published in Nature Nanotechnology in 2022, has since been licensed to Nava Therapeutics, a therapeutics company dedicated to identifying and developing novel LNPs for the treatment of genetic diseases. Using SENT-Seq, we were able to identify LNP candidates that deliver to rare cell types in novel tissues. Herein, we will discuss the capability of LNP screening and what it can be used for, show data related to what SENT-Seq can accomplish, and show comparative validation data using mRNA-based reporters in both mice and NHPs.

Accessing the Central Nervous System and Rett Syndrome

Thomas Carroll, Sheffield Teaching Hospitals NHS Foundation Trust

A summary is presented about key aspects of interventional techniques in actual clinical use to access the central nervous system. The emphasis is on using what is currently available in hospital practice to facilitate momentum towards first-in-human Rett syndrome clinical trials. Surgical steps for insertion of an external ventricular drain and associated complications will be summarized. The presentation will also discuss perceived hurdles around procedure-associated risk, where might Rett syndrome sit in the context of other neurosurgical procedure indications, and the sort of admission pathway that might be expected for a Rett patient.

"Seq-ing" Pathogenic Insights into Rett Syndrome

Zhaolan (Joe) Zhou, Departments of Genetics and Neuroscience, Epigenetics Institute, University of Pennsylvania

Mutations in the X-linked *MECP2* gene cause Rett syndrome (RTT), a progressive neurodevelopmental disorder with unique clinical presentation. The monogenic cause of RTT, the dominant prevalence in heterozygous females, and the progressive nature of RTT manifestation have prompted us to focus on three major questions: what else to learn from RTT genetics; what drives RTT pathogenesis in heterozygous females; and what underlies the progressive feature of RTT. To address these questions, we first took a knock-in approach and generated multiple mouse models recapitulating common RTT mutations, such as MeCP2 T158M, T158A, R106W and R306C. Unexpectedly, we found that several missense mutations impair the binding of MeCP2 to chromatin but concomitantly reduce MeCP2 protein stability. Through transgenic studies *in vivo*, we observed significant therapeutic potentials by enhancing MeCP2 T158M expression or stabilizing T158M protein, thus revealing new avenues for therapeutic development. To elucidate the molecular basis of RTT pathogenesis, we next engineered additional knock-in mice for Cre-dependent biotin tagging of endogenous MeCP2, thereby allowing nuclear transcriptome profiling in Cre-defined cell types along disease progression, while overcoming X-linked cellular heterogeneity in heterozygous females. We found that MeCP2 dysfunction leads to progressive accumulation of subtle changes in gene expression, resembling a "snowball". Importantly, these nuclear transcriptome changes occur in a cell autonomous manner, highlighting a critical role of MeCP2 in maintaining precise gene transcription persistently overtime. We propose the use of "MeCP2 sensitive genes" instead of "MeCP2 target genes" in describing similar gene expression studies in the future.

Investigating the Immediate Consequences of Rapid MeCP2 Protein Degradation

Lisa Boxer, National Institutes of Health

Rett syndrome is caused by mutations in the methyl-DNA-binding protein MeCP2, but the function of MeCP2 has puzzled researchers for years because the severe disease symptoms associated with MeCP2 mutations are accompanied by only subtle molecular and cellular changes. These include small-magnitude changes in the expression of many genes (both up- and down-regulation), some changes in mRNA splicing, slight changes in chromatin compaction, and a slight decrease in total cellular RNA levels and nuclear size. However, most studies of MeCP2 function have been in constitutive loss-of-function models, such as knock-out mice, which are valuable models of Rett syndrome, but the changes observed likely represent a mix of primary and secondary consequences of MeCP2 loss. Thus, it is currently unknown which of these changes are primary consequences of MeCP2 loss and which are secondary consequences of long-term neurological impairment.

To distinguish the primary and secondary consequences of MeCP2 loss, we generated a mouse line with a degradation tag (dTAG) on MeCP2, which enables rapid and specific degradation of the MeCP2 protein upon treatment with the small molecule dTAG-13. By performing experiments at a series of timepoints after MeCP2 degradation, we aim to determine which molecular and cellular changes occur first and the subsequent order of events. Importantly, we chose the rapid protein degradation approach over a genetic approach because the MeCP2 protein has a very long half-life (~2 weeks). Our experiments show that the MeCP2 protein can be degraded within 30 minutes of dTAG-13 treatment in neurons cultured from the MeCP2-dTAG mice. Furthermore, we developed an approach to degrade MeCP2 within two hours of dTAG-13 injection in vivo in the adult mouse brain. In ongoing work, we are characterizing molecular and cellular changes, including gene expression, histone modifications, and nuclear size, at a series of timepoints after MeCP2 degradation in cultured neurons and in the mouse brain. These experiments are identifying the immediate consequences of acute MeCP2 loss and potentially inform discovery of treatments for Rett syndrome.

Development of a Preclinical Testing Resource for Mouse Models of Rett Syndrome

John Sinnamon, Rett Syndrome Research Trust

Small animal models of human disease are essential for the development of new therapeutics, however, the cost associated with licensing, importing and characterizing these models can be prohibitive. We describe the ongoing development of a novel resource in collaboration with the Jackson Laboratory, a world leader in mouse development, for testing novel therapies to treat Rett syndrome. Newly generated models of common Rett causing mutations have been created on an isogenic background, without IP restriction and will be characterized using a pipeline of behavioral experiments that can be used to test the efficacy and safety of novel therapies. Further, using this resource we will be testing four newly available Rett mouse lines to mice modeling the introduction of compensatory point mutations that can be introduced by RNA editing. These experiments will test the possibility of utilizing RNA editing as a therapeutic approach for patients with these common mutations.

Humanized Mouse and Marmoset Models for Rett Syndrome Research

Guoping Feng, Broad Institute of MIT and Harvard

Over the past decade, genome editing technologies have made unprecedented advances. As a result, new gene therapy approaches now hold realistic promise for treating and curing a large number of human diseases including Rett syndrome. To facilitate the translation of animal model studies to clinical applications, we developed humanized mouse models for some of the most common Mecp2 mutations found in Rett patients. In these mouse models, DNA sequences of exon 4 of the Mecp2 gene were humanized. Thus, DNA and RNA-editing based gene therapies developed and tested in these humanized mouse models can be readily transferred to human cell validation and clinical development. In addition, we developed a common marmoset model of Rett syndrome with Mecp2 R270X mutation. Common marmoset is a small non-human primate with brain structure and functions much closer to that of humans. We hope that these new animal models will facilitate the testing of therapeutic approaches for Rett syndrome.

Mecp2 Dosage-sensitive Proteomes as Putative Disease Biomarkers

Victor Faundez, Emory University

Defects in the Mecp2 gene profoundly affect the brain transcriptome. However, the extent to which these transcriptome modifications inform the brain proteome in Mecp2-null animals remains mostly unexplored. Our study focused on the proteome to discover analytes with potential as outcome measures. We observed that the correlation between transcriptomes and proteomes varied across tissues of Mecp2-null mice at a presymptomatic age. Transcriptome-proteome correlations ranged from approximately 0.7 in the liver to no correlation across different brain regions. The brain proteome in Mecp2-null mice was rescued by adenoviral restitution of Mecp2 expression driven from its promoter. Levels of protein modules rescued by Mecp2-gene therapy correlated with the degree of phenotypic rescue in the same animals. Moreover, adenoviral overexpression of Mecp2, using the Tau promoter, reversed the directionality of proteome changes observed in the Mecp2-null brain. Finally, we validated the biomarker potential of brain proteins responsive to Mecp2 gene dosage in the cerebrospinal fluid (CSF) proteome of Mecp2-null mice. We selected CSF as a clinically accessible biosample. Twenty-five percent of the mouse CSF Mecp2-sensitive proteins matched those in mutant brain. These results establish a paradigm for the identification of analytes with translational potential in human genetic disorders of neurodevelopment.

The RSRT Biorepository

Jana von Hehn, Rett Syndrome Research Trust

RSRT has established a fully unencumbered biorepository of Rett syndrome patient cell lines. A collection of fibroblasts and induced pluripotent stem cell (iPSC) lines are available to industry and academia upon request. Primary fibroblast lines isolated from skin biopsies are mosaic while iPSCs, generated by Harvard Stem Cell Institute from blood, are paired clonally-isolated isogenic lines. iPSC lines are karyotyped and assessed for MECP2 allelic expression and X inactivation.

Since the biorepository launched in 2019, the lines have been requested by over 75 institutions around the globe. Industry represents about one-third of requests while two-thirds are academic. The most common missense and nonsense mutations are represented in the iPSC collection, and a variety of other mutation types are also available in the fibroblast collection. The lines have enabled Rett research from molecular characterizations to CRISPR-based approaches and beyond. Future directions include expanding the biorepository to include CSF. RSRT is grateful to the families who have made this resource possible.

A Comparison of Genetic Therapies and their Relevance to Rett Syndrome

Jonathan K. Watts, RNA Therapeutics Institute, UMass Chan Medical School

Gene-targeted therapies are becoming increasingly prevalent in the treatment of genetic diseases. With options from gene silencing to splice switching, from viral gene replacement therapy to RNA editing and several technologies for gene editing, each has advantages and disadvantages for addressing some or all of the mutations that cause Rett syndrome and related diseases.

In this session introduction, we will examine the status and the strengths and weaknesses of these various therapeutic options. We will look at the versatility for treating different Rett-causing mutations, their safety and ability to provide appropriately regulated MeCP2 levels, and their potential to bring therapeutic efficacy for some or all of the Rett population. Finally we will look at the challenges associated with delivery of the molecules required for each approach and the path toward clinical use.

First-in-human use of TSHA-102 Gene Therapy for Rett Syndrome: Where We Stand and How We Got Here

Sukumar Nagendran, Taysha Gene Therapies

Following a single intrathecal injection of TSHA-102, a 20-year-old woman recently became the first person ever to undergo gene therapy for Rett syndrome (RS). This event was the culmination of decades of basic research on the genetic basis of RS, and many years of developing and testing RS gene therapy strategies in animal models.

RS arises from loss-of-function in the X-linked *MECP2* gene, encoding MeCP2, a chromatin regulatory protein essential for normal cognitive and motor development. RS represents a challenging case for gene therapy, in part because of the narrow therapeutic window of expression of MeCP2. Two-fold overexpression of the endogenous gene (*MECP2* duplication syndrome) produces neurologic impairment no less severe than RS. Because X-chromosome inactivation in RS produces a chimeric pattern of normal and MeCP2-deficient brain cells, unregulated expression of a *MECP2* transgene is not a viable strategy for gene therapy in this disease.

TSHA-102 is an adeno-associated virus 9- (AAV9-) recombinant vector driving expression of a therapeutic transgene in cells of the CNS. Design of TSHA-102 was based on insights into MeCP2 protein structure, leading to creation of a truncated form of the transgene (*miniMECP2*) that can be accommodated within an AAV9 vector. Later innovations led to inclusion of a microRNA autoregulatory element designed to allow cell-by-cell control of miniMeCP2 expression, thus permitting therapeutic expression of the transgene in cells lacking MeCP2 function while avoiding toxic overexpression elsewhere in the brain.

Mouse studies showed that early postnatal restoration of MeCP2 function with TSHA-102 prevented the early lethality seen in *Mecp2*$^{-/y}$ males, a well-established model of RS. This treatment also restored the animals' normal growth trajectory and delayed onset of characteristic gait and behavior phenotypes that mimic clinical signs and symptoms of RS. In wild-type animals, this same early treatment caused no significant mortality or behavioral effects. Coupled with evidence that TSHA-102 treatment is nontoxic in wild-type rats and cynomolgus monkeys, this finding offered reassurance that TSHA-102 might be used safely in girls and women with RS. Work in these animal models also suggested a human-equivalent dosage range wherein TSHA-102 is expected to be safe and therapeutically effective, thus paving the way for dose-finding clinical trials.

The first such trial, the REVEAL Adult study, is now underway in Canada, enrolling women with RS. Preliminary findings on the prespecified safety and efficacy outcomes for the first enrolled participant will be presented at this meeting, in a poster by Rossignol et al. Here, I will share videos in which Dr. Elsa Rossignol (the primary Investigator in this study) and Dr. Jenny Downs (an independent clinical reviewer working with REVEAL) describe their clinical observations of this participant.

The initial quantitative and qualitative findings on Participant 1 have been reviewed by the Independent Data Monitoring Committee, who determined that the study should proceed. Participant 2 is scheduled to receive TSHA-102 treatment later this year, at the same dose used for Participant 1.

NGN-401: A Self-regulating Gene Therapy Product for Rett Syndrome

Stuart Cobb, Neurogene Inc., University of Edinburgh

Gene therapy is a promising therapeutic approach for the treatment of Rett syndrome but is faced with the challenge that *MECP2* is a dosage-sensitive gene. Neurogene developed an investigational gene therapy, NGN-401, designed to deliver a full-length human *MECP2* transgene under the control of Neurogene's EXACT self-regulating technology. Preclinical data from relevant animal models has demonstrated that NGN-401 delivers a narrow range of MeCP2 expression levels, enabling functional rescue of translational phenotypes with no notable toxicity. NGN-401 was further evaluated in nonhuman primates expressing physiological levels of MeCP2 in all cells and thus representing a high bar for evaluation of safety. In a 6-month GLP study in juvenile female nonhuman primates, NGN-401 was well-tolerated at >4x the clinical dose. Studies also confirmed that intracerebroventricular administration maximizes vector biodistribution to key brain regions underlying Rett syndrome pathobiology. RTT-200 is an open-label Phase 1/2 multi-center clinical trial to dose female pediatric subjects with genetically confirmed typical RTT. The study participants will be administered a single dose of NGN-401 via intracerebroventricular injection. Patients will be closely monitored for safety and early efficacy measures will also be evaluated.

Base Editing and Prime Editing: Correcting Mutations that Cause Genetic Disease in Cells, Animals, and Patients

David Liu, Broad Institute, Harvard University, HHMI

In this lecture I describe the development and therapeutic application of two precision gene editing technologies that install or correct targeted mutations without requiring double-strand DNA breaks, thereby minimizing undesired consequences of chromosomal cleavage. We developed base editors, proteins that directly perform chemistry on individual DNA bases in living cells to install or correct mutations at targeted positions in genomic DNA. We recently engineered CRISPR-free, all-protein base editors that enabled the first purposeful changes in the sequence of mitochondrial DNA in living cells. By integrating base editors with *ex vivo* and *in vivo* delivery strategies that deliver therapeutic proteins, we rescued animal models of human genetic diseases including sickle-cell disease, progeria, and spinal muscular atrophy (SMA). Single-AAV base editing systems enhance the safety and practicality of *in vivo* base editing. Our development of engineered virus-like particles (eVLPs) provide additional in vivo delivery methods for gene editing proteins that minimize off-target editing and the risk of oncogenic DNA integration. Base editors are in at least five clinical trials to treat diseases including familial hypercholesterolemia, sickle-cell disease, beta-thalassemia, and T-cell leukemia, resulting in the first report of clinical benefit from a base edited therapeutic. I will also describe prime editors, engineered proteins that directly write new genetic information into a specified DNA site, replacing the original sequence, without requiring double-strand DNA breaks or donor DNA templates. Prime editing can mediate any base substitutions, deletions, and/or insertions of up to ~200 base pairs in living cells *in vitro* and *in vivo*, and has been applied to directly install or correct pathogenic alleles that previously could not be corrected in therapeutically relevant cells. We recently illuminated the cellular determinants of prime editing outcomes, and used the resulting insights to develop new prime editing systems with substantially higher editing efficiencies and product purities. The combination of prime editing and site-specific recombinases enable programmable gene-sized (>5 kb) integration and inversion at loci of our choosing in human cells. Prime editing has recently been used to rescue animal models of genetic diseases including sickle-cell disease, metabolic liver diseases, and genetic blindness. Base editing and prime editing enable precise target gene correction, in addition to target gene disruption, in a wide range of organisms with broad implications for the life sciences and therapeutics.

RNAfix® – A Programmable RNA Editing Platform that Expands the Potential of RNA Therapeutics

David Huss, Shape Therapeutics

Programmable RNA base editing that harnesses endogenous adenosine deaminase acting on RNA (ADAR) has great potential as a versatile gene therapy approach, without the need to deliver a foreign protein or protein-encoding transgene. ADAR deaminates RNA adenosines into inosines, which are read by the cellular machinery as a guanosine, creating a functional A-to-G base change. Because RNA adenosines play critical roles in many biological processes, RNA editing can be used to correct pathogenic mutations, control RNA splicing, and modulate protein expression and function, thereby expanding the potential of RNA therapeutics.

RNAfix® is a proprietary RNA editing platform that combines high throughput screening with generative machine learning to engineer gRNAs that interact with a disease-relevant target transcript, recruit endogenous ADAR and specifically edit the target adenosine. For durable and potential one-time treatments, RNAfix gRNAs are delivered in genetically-encoded form to drive sustained, high-level expression in target cells. This delivery approach enables the use of promoter engineering and AAV vector design to tailor gRNA expression and stability for each disease target.

The central nervous system (CNS) expresses high levels of ADAR1 and ADAR2, making it a particularly attractive target organ for therapeutic application of RNAfix. However, published results to date have relied on delivery of hyperactive forms of ADAR to achieve efficient in vivo RNA editing in the CNS. Here we demonstrate novel gRNA design combined with increased gRNA expression and stability unlock high efficiency RNA editing with endogenous ADAR in cell-based neuronal models and the CNS of adult mice. In differentiated SH-SY5Y cells, human NSC-derived neurons and mouse primary neurons we achieved >90% target base editing efficiencies. These robust editing results translated to in vivo mouse models where we achieved 40% to >90% target base editing, depending on the gRNA design, AAV serotype and route-of-administration. These results demonstrate the power of the RNAfix platform and pave the way for its application in many neurological diseases.

Adapting AIMer-based RNA Editing Technology for Application in CNS

Michael Byrne, Wave Life Sciences

We have previously shown that endogenous ADAR enzymes (adenosine deaminases acting on RNA) can be directed to perform sequence-specific RNA editing in liver by using chemically modified stereopure oligonucleotides called AIMers. Here, we describe further development and optimization of AIMer design, which enhances editing potency, supports editing across the CNS, and enables the restoration of MECP2 protein expression in neuronal cell culture models of Rett syndrome. Initially using ubiquitous housekeeping transcripts as surrogate targets, we developed AIMers that directed substantial RNA editing in human iPSC-derived neurons and astrocytes. Following administration into transgenic mice that express human *ADAR1*, we demonstrate 25% to 65% RNA editing across the entire CNS, which was sustained to 16-weeks post-single injection. Administration of AIMers in non-human primates via intrathecal injection resulted in around 50% RNA editing in the spinal cord and up to 30% in the brain. To assess the ability to edit a clinically relevant target, we designed stereopure AIMers to edit the *MECP2* R168X mutation, the most frequent causal mutation found in Rett syndrome. These AIMers are designed to convert the premature stop codon to tryptophan, promoting translation readthrough and protein restoration. In 293T cells, we demonstrate that MECP2 R168W protein localizes properly to the nucleus and interacts with expected co-regulatory proteins, suggesting functionality. In human and mouse neuronal lines, *MECP2* AIMers direct 30% to 90% RNA editing, respectively. With this level of in vitro editing, we restore full-length protein detectable by western and immunofluorescence assays. These preclinical investigations lay the foundation for the development of AIMers, with the potential to treat human diseases in neurology.

Directed RNA Editing for the Repair of MECP2 Mutations that Cause Rett Syndrome: Optimization of Guide Strands for Difficult-to-edit Sites

Peter A. Beal, University of California at Davis

ADARs (adenosine deaminases acting on RNA) are editing enzymes that convert adenosine (A) to inosine (I) in duplex RNA, a modification that has wide-ranging consequences on RNA function including changing the meaning of specific codons in mRNA. Site-directed RNA editing by ADARs allows for the rescue of disease-causing mutations at the RNA level. Because ADARs edit within duplex RNA, one can design guide RNAs complementary to a target sequence for the purpose of recruiting ADAR enzymes to elicit the desired edit. In this presentation I will describe how high throughput screening and rational design can inform the optimization of guide RNAs to recruit ADARs for the correction of Rett-causing mutations.

Development of Mice Modeling Missense Variants Introduced by RNA Editing to Restore MECP2 Function in Patients with Nonsense Mutations

John Sinnamon, Rett Syndrome Research Trust

The RNA editing activity of adenosine deaminases acting on RNA (ADARs) can be harnessed to repair G-to-A disease-causing mutations, however, the potential to create compensatory point mutations by RNA editing has not been explored in the context of MECP2. We have developed mouse models to test the molecular and behavioral impact of missense variants introduced by RNA editing in the context of common Rett syndrome nonsense mutations. Newly generated mouse lines containing the mutations R168X, R255X, R270X and R294X will be compared to mice containing an additional G-to-A mutation, which genetically models RNA editing of the mutant codon to a tryptophan. These studies will provide a proof of concept for RNA editing to be used as a therapy for four of the six most common Rett syndrome mutations.

Repair of Loss-of-function Mutations of MECP2 mRNA by Pentatricopeptide Repeat (PPR) Protein-mediated U-to-C Single Nucleotide Editing

Osamu Nakanishi, EditForce, Inc.

Single nucleotide editing of mRNA has a great potential to provide new strategies to treat many monogenic diseases caused by point mutations without chromatin DNA manipulation, which has another risk for undesirable mutations. Therefore, aiming for therapeutic applications, multiple efforts have been made to apply RNA deaminases, such as ADARs and APOBECs, to repair pathogenic transcripts with point mutations. However, those deaminases can catalyze only A-to-I and C-to-U conversions, so still insufficient for repair of point mutations in every direction.

Pentatricopeptide repeat (PPR) proteins are sequence-specific RNA-binding proteins known to be widely distributed in the plant kingdom and involved in RNA regulation by cleavage, splicing and nucleotide editing in mitochondria and chloroplasts. PPR proteins consist of an N-terminal domain with a tandem array of PPR motifs that specifically recognize single nucleotides and a C-terminal domain that modifies target RNAs. Based on the modular nature of the PPR proteins, we have developed single nucleotide PPR-editors consisting of an N-terminal RNA binding domain and a C-terminal enzyme domain able to catalyze not only C-to-U deamination but also U-to-C transamination.

The U-to-C transamination is especially important from therapeutic point of view because deamination of 5-methylcytosine (5mC) at CpG dinucleotides to yield T spontaneously occurs, therefore loss-of-function mutations by C-to-T mutation are frequently observed in many disease-related genes. MeCP2 is a heterochromatin-binding protein and the top eight C-to-T point mutations in the gene are known to cause about a half of Rett syndrome cases.

In this study, we design U-to-C PPR-editors aiming at direct repair of these major mutations, and test whether the PPR editors can repair the point mutations and restore MeCP2 protein expression.

Rescuing Rett Syndrome Pathology through Astrocyte Dependent MeCP2 Reactivation

Gaia Skibinski, Herophilus Inc.

Rett syndrome urgently requires disease-modifying solutions that address its genetic cause. X chromosome reactivation (XCR) is a promising therapeutic approach for X-linked disorders. We have developed a Rett patient-derived organoid model that uniquely captures the human biology of X chromosome inactivation (XCI) and the genetic background of Rett patients. Using this model, we discovered HRP-12975, a small molecule that effectively reactivates MeCP2 and restores synaptic density in Rett patient-derived organoids. In rodent models, repeated intracerebroventricular (ICV) injection of HRP-12975 demonstrates well-tolerated brain concentrations that surpass levels needed to observe reactivation in human in vitro astrocytes. Our innovative human model provides compelling evidence for the therapeutic potential of HRP-12975 in treating Rett syndrome and potentially other X-linked disorders.

Epigenome Editing of MECP2 to Rescue Rett Syndrome Neurons

Shawn Liu, Columbia University

Reactivation of the silenced wild-type *MECP2* allele on the inactive X chromosome (Xi) is a promising therapeutic strategy for treating Rett syndrome (RTT), as it attacks the root cause of this disease by restoring *MECP2* expression. We have developed epigenome editing tools consisting of a catalytically dead Cas9 (dCas9) fused with Tet1 and target gRNAs that allow for DNA demethylation in a targeted manner (Liu et al., 2016; Liu et al., 2018). We applied a multiplex epigenome editing approach to reactivate MECP2 from Xi in RTT human embryonic stem cells (hESCs) and derived neurons. Demethylation of the *MECP2* promoter by dCas9-Tet1 with target single-guide RNA reactivated MECP2 from Xi in RTT hESCs without detectable off-target effects at the transcriptional level. Neurons derived from methylation-edited RTT hESCs maintained MECP2 reactivation and reversed the smaller soma size and electrophysiological abnormalities, two hallmarks of RTT. In RTT neurons, insulation of the methylation-edited *MECP2* locus by dCpf1-CTCF (a catalytically dead Cpf1 fused with CCCTC-binding factor protein) with target CRISPR RNA enhanced MECP2 reactivation and rescued RTT-related neuronal defects, providing a proof-of-concept study for epigenome editing to treat RTT and potentially other dominant X-linked diseases. In this consortium project led by Drs. Kyle Fink, Antonio Bedalov and Shawn Liu, we are applying epigenome editing strategy with novel delivery approaches in a variety of RTT mouse models to evaluate its efficacy and safety toward developing new treatments.

Novel tRNA Medicines, Stop Codon Disease, and Premature Termination Codons in Rett Syndrome

Theonie Anastassiadis, Alltrna

Alltrna is advancing a new class of genetic medicines for Stop Codon Disease based on the unique ability of tRNAs to readthrough premature termination codons and restore full-length functional protein production. Stop Codon Disease encompasses thousands of rare diseases, including Rett syndrome, where a premature termination codon is the genetic driver of the disease.

A premature termination codon (PTC), also called a nonsense mutation, is where the code for an amino acid has been mutated into a premature "stop" codon. This results in a truncated or shortened protein product with no or altered biological activity that causes disease. Approximately 10% of all people with a genetic disease have Stop Codon Disease, representing approximately 30 million people worldwide. Alltrna is engineering tRNA medicines that can read these PTC mutations and deliver the desired amino acid, thereby restoring the production of the full-length protein. A single tRNA medicine has the potential to restore disrupted protein production for all diseases caused by a PTC.

More than 30% of patients with Rett syndrome have a stop codon mutation in the MeCP2 gene. The MeCP2 protein needs to be tightly regulated, where too much or too little can cause disease. tRNA medicines have the potential to be differentiated from other replacement therapies and genetic medicines, where there is a risk of overexpressing the protein, in that tRNAs cannot overexpress a protein, because tRNAs do not alter cellular regulatory control.

Alltrna is presenting preclinical proof-of-concept data that illustrates the potential of the company's platform to design and deliver tRNA medicines to restore disrupted protein production for Stop Codon Disease. In patient-derived cells and an animal model of a rare human genetic disease with a PTC mutation, Alltrna's engineered tRNA oligonucleotide restores full-length protein expression. Alltrna has shown its engineered tRNA oligonucleotide has universal readthrough activity for shared genetic mutations regardless of gene or location in 25 disease models. These data demonstrate in vivo that Alltrna's engineered tRNA oligonucleotide can readthrough a PTC, independent of gene and mutation location.

Alltrna is working to advance its first drug candidates towards the clinic for a first indication in Stop Codon Disease.

Evolving Regulatory Landscape for Biologic Therapeutics in Orphan Diseases

Peter Marks, CBER, FDA

Gene therapy offers tremendous promise for the treatment of rare diseases. As we apply an evidence-based regulatory framework for these products, the FDA understands that we may need to re-evaluate and modernize our approach to their unique challenges while also ensuring that the resulting therapies are both safe and effective. In concert with this, the FDA is taking steps to facilitate more efficient gene therapy product development. This includes helping to facilitate the sharing of best manufacturing practices among academic and industrial developers, which could result in advances associated with better product quality, including consistency and yield, along with reduced costs. Additionally, the FDA will encourage the use of biomarkers as surrogate endpoints to help facilitate the accelerated approval of gene therapies for serious or life-threatening conditions. Leveraging learnings from Operation Warp Speed during the COVID-19 pandemic, FDA will be launching a pilot later this year for rare diseases. The purpose of the project will be to attempt to further accelerate the pace of development of therapeutics for very small populations with very high medical need. This pilot for rare pediatric genetic diseases will allow ongoing informal interactions during development of the product. Finally, the FDA supports work toward global regulatory convergence and, ultimately, global harmonization of regulations for these products. In fact, FDA is pursuing this goal with international partners, global regulators, and the World Health Organization. It may also explore the possibility of concurrent collaborative review of applications with global regulatory partners.

Modulating MeCP2 Levels: A Potential Therapeutic Strategy for Rett Syndrome

Huda Zoghbi, Baylor College of Medicine, Jan and Dan Duncan Neurological Institute at Texas Children's Hospital, HHMI

Rett syndrome (RTT) is a neurodevelopmental disorder caused by loss-of-function mutations in *MECP2* (Methyl CpG binding protein 2), a transcriptional regulator essential for maintenance of normal neuronal function. There is no effective treatment for RTT, and studies in mouse models have shown that increasing even mutant MeCP2 protein improves neurological symptoms. We devised a therapeutic strategy to increase mutant MeCP2 protein level by modulating *MECP2* alternative splicing. *MECP2* has two alternatively spliced isoforms: *e1* (exons 1,3,4) and *e2* (exons 1,2,3,4). Despite similar levels of transcription, *e2* is translated at a lower efficiency than *e1*. Studies on mouse models and human RTT patients suggest that E2 is dispensable for the neuronal function of MeCP2. Therefore, we hypothesize that switching *e2* to *e1* will increase MeCP2 protein level and improve symptoms in RTT models. For this, we used iPSCs from a RTT patient carrying a MeCP2 missense mutation, G118E, which causes both decreased binding to DNA as well as ~30% lowering of MeCP2 levels. When we differentiate G118E iPSCs into neurons, they display electrophysiological abnormalities and dysregulated gene expression. To test our hypothesis, we generated a CRISPR/Cas9-mediated deletion of *MECP2* exon 2 (E2KO) in the RTT iPSCs and upon differentiation into neurons, found that E2KO rescues MeCP2 protein levels. At the meeting we will share data about the effects of increasing mutant MeCP2 on both neuronal physiology and gene expression changes.

Incorporating Clinical Outcome Assessments in Rare Disease Clinical Trials

Lindsey Murray, Rare Disease Clinical Outcome Assessment Consortium, Critical Path Institute

Defining endpoints in clinical trials to treat rare diseases requires thoughtful consideration. By definition, rare diseases have limited patient populations, which limits statistical power for between group comparisons that are traditionally employed in clinical trial analyses. In addition, within and between patient heterogeneity is common, making selection of an endpoint that is applicable to the entire population challenging. Geographic distribution of the patient population and lack of established biomarkers further compound these challenges. Clinical outcome assessments (COAs) are needed to link the patient voice with clinically meaningful endpoints.

The US Food and Drug Administration (FDA) describes 4 types of COAs: patient-reported outcome measures (PROs), observer-reported outcome measures (ObsROs), clinician-reported outcome measures (ClinROs), and performance outcome measures (PerfOs). PROs rely on self-report on symptoms that cannot be measures objectively (e.g., pain severity, shortness of breath). ObsROs rely on a report of observable signs, events, or behaviours related to a patient's health condition by someone other than that patient or a health professional, in contrast to a ClinRO where these signs, events, or behaviours are reported by a healthcare professional. PerfOs include measurements of a clearly defined task.

FDA has produced 4 guidance documents which describe the best practices for developing and/or modifying a COA for a specific context of use (e.g., use in Rett syndrome clinical trials). This session will provide an overview of best practices for identifying what concepts should be measured with a COA for a specific disease, steps for developing or modifying a selected COA, and considerations for implementation in clinical trial programs.

Digital Biomarkers for Rett Syndrome: Sleep, Activity and Circadian Rhythms

Gari Clifford, Emory University, Georgia Institute of Technology

We developed a Rett syndrome severity marker based upon the interactions between movement and sinoatrial activation of the heart, plus circadian rhythm measures. A total of 396 features of heart rate variability, rest activity and cosinor rhythmometry metrics, multiscale entropy and multiscale network representation metrics were extracted from synchronous ECG and acceleration signals recorded by wearable devices. LASSO regularized logistic regression classifier was used to classify the severity of Rett syndrome. Using 42 Individuals we demonstrated a mean accuracy of 76% by a leave one out cross validation approach. We subsequently applied this to another unseen cohort of 20 Rett syndrome patients and achieved equivalent performance (an accuracy of 75%). We then examined features of this index and the component features to identify physiological changes after intervention of ketamine treatment. No statistically different change was seen between ketamine and placebo. In this talk I'll discuss these issues and point the way forward for the use of these metrics in on and off-body sensing for Rett syndrome sufferers.

AI-powered Radio Signals Paving the Way for Objective Biomarkers in Rett Syndrome

Dina Katabi, MIT

Current outcome measures for Rett syndrome, such as RBSQ and CGI-I, are subjective and do not adequately capture nuanced differences in disease severity and progression. With the increasing number of drugs targeting Rett syndrome, there is a pressing need for more objective and quantifiable biomarkers.

In this talk, we introduce a novel approach that can quantify symptoms of Rett syndrome, including loss of motor function, breath holding, hyperventilation, and sleep disturbances. Central to our method is Emerald, a contactless radio device enhanced with artificial intelligence (AI) technology. Operating unobtrusively in the background, much like a WiFi router, Emerald employs AI to analyze radio signals in a room. This allows it to infer a patient's breathing, sleep, and motion patterns without the necessity for wearable sensors. We will present results from an observational clinical study with Rett syndrome patients, illustrating how this technology offers objective and robust markers for the disease.

Pilot Study of the MC-10 Bio-stamp and Emerald Device in the Rett Population

David Lieberman, Boston Children's Hospital

As part of an effort to quantify physiologically relevant biomarkers related to disrupted autonomic function in Rett syndrome, we utilized two devices in the patient's home for up to 4 weeks in a cohort of pediatric and adult aged Rett patients. We examined use of MC10 Biostamp, an FDA-cleared wearable patch that employs a wireless remote monitoring platform intended for the continuous collection of ECG data utilized to derive ECG derived measures of apnea and hyperventilation. We also utilized the Emerald Device, a touchless biosensor which uses a highly sensitive radio-based sensor that detects radio waves that reflect off of the body and can thereby measure components of ambulation, respiration, and sleep. Fifteen patients used the MC10 patch with analytics, with many reporting problems with adhesion of the patch as well as skin Irritation and discomfort. Fourteen patients utilized the Emerald device and found it very easy to use and very straightforward to deal with. On the other hand, daily questionnaires recording patient's anxiety, sleep pattern, and seizure frequency were reported as tedious for the caregivers to complete. A second cohort of Rett patients will be examined utilizing the Emerald device, standard polysomnography, and novel wearable devices.

Electrophysiological Signals as a Potential Biomarker for Rett and Related Disorders

Eric Marsh, Children's Hospital of Philadelphia

We have been evaluating the utility of EEG and evoked potentials as a biomarker of cortical function in Rett syndrome (RTT). As a number of disease-modifying therapeutics are currently under development, there is a pressing need for biomarkers to objectively and precisely assess the effectiveness of these treatments. We have performed resting state EEG, visual (VEP) and auditory (AEP) evoked potentials in over 75 individuals with RTT, aged 2 to 37 years, and control participants across five sites as part of the Rett Syndrome and Related Disorders Natural History Study.
We have analyzed the data for associations of primary measures with clinical severity. We have found group level differences in RTT subjects compared to control participants. We have also found that slowing of the EEG or reduction of amplitude was associated with RTT-related severity. Yearly recordings yielded similar findings and evidence of test-retest reliability of EPs at the individual level. The current data indicate the promise of evoked potentials as an objective measure of disease severity in individuals with RTT and support the need for future research to validate these measures for clinical use.

The Hunt for Endophenotypes in Rett Syndrome

John Foxe, University of Rochester Medical Center

This presentation will discuss outcomes from a series of recent high-density electrophysiological findings by our research group that point to highly anomalous cortical processing of auditory inputs in females with Rett syndrome, and a potential relationship between these measures and metrics of disease severity. We have shown that **1)** early auditory sensory processing of simple frequency deviations are delayed and weakened in Rett, **2)** that early auditory sensory processing of duration deviations are only registered at rapid stimulation rates in Rett and fail at slower rates, and that these processes are also delayed and weakened, even when present, **3)** that the canonical series of auditory evoked potential (AEP) components to simple tone and phonemic inputs is severely disrupted in Rett, and these data suggest that measures of these temporal processing stages (e.g. the P2 component) may have utility as high reliability classifiers of Rett children relative to neurotypical controls. In turn, the extent of deficit in the P2 is associated with disease severity in this group, suggesting that this measure may serve as an objective measure of disease severity/ progression. We hold, however, that it is unlikely that any one neurophysiological measure will have perfect utility as a neuromarker of Rett and that a much likelier scenario is that a multivariate endophenotype approach will yield considerably higher utility in this domain. We propose to develop such a multivariate classifier using a set of easily deployable, clinically tractable assays that can be performed within a reasonable clinical visit. Based on considerable preliminary work, we have identified 8 experimental paradigms with high likelihood to yield actionable measures.

Using Digital Health Technologies to Advance Clinical Drug Development

Kate Lyden, VivoSense Inc

Digital health technologies (DHTs) provide an opportunity to dramatically advance the way health care is delivered and clinical drug development trials are conducted. DHTs, such as wearable, implantable, and ambient sensors, can be deployed remotely, passively, and continuously to assess how patients feel and function in real world environments. These types of data provide many potential benefits to develop novel, patient-centric, measures that are more sensitive to treatment benefits, speed up trial timelines, reduce trial costs, increase recruitment reach and retention, and ultimately bring more drugs to market. This talk will focus on the potential, progress, and challenges of using DHTs in clinical drug development. Specific examples of how DHTs are being used in Rett syndrome drug development will be examined and discussed.

Using Patient-centric EMR Data for Robust Rare Disease Research and Drug Development

Nasha Fitter, Ciitizen

Natural history information is critical for drug development and for clinicians and caregivers to understand patient symptoms and disease progression. Unfortunately, most rare diseases lack high-quality and complete datasets due to patient/caregiver fatigue and movement between health systems. Ciitizen is a patient-consented, real world evidence platform created by rare disease families that use a patient's own HIPAA right of access to collect the entirety of their medical records. The platform then utilizes AI and machine learning, along with human review to abstract and code thousands of terms from unstructured medical records. These data elements are used to create rich natural history studies, show burden of disease and unmet need, and justify primary and secondary endpoints. Recently Praxis Precision Medicines utilized Ciitizen's dataset for *SCN2A-DEE* as their single source of RWE data in their IND submission with the FDA, accelerating their clinical trial by four years. The goal of the Ciitizen platform is to provide rich, longitudinal data for rare disease cohorts that can be easily accessed by patients, caregivers, advocacy groups, clinicians, academia and industry.

Combining Untapped Resources to Expedite Targeted Therapeutics (CURETT) Initiative: A Vision

Cary Fu, Vanderbilt University Medical Center
Bernhard Suter, Texas Children's Hospital, Baylor College of Medicine

A major focus of RSRT has been to identify and fill gaps in the research landscape to enable therapeutic development. Two underutilized resources are 1) existing medical records and 2) parent experiences. To make these data accessible we've launched the CURETT initiative.

Through the CARE study we have partnered with Ciitizen to extract salient clinical information from medical records, allowing years of medical history to build comprehensive patient journeys. Almost 200 US patients are enrolled. While limitations are still being identified such as variable data quality across the study, preliminary analyses indicate important advantages including availability of pre-diagnosis data and enrichment of adults who are typically under-represented in natural history studies of neurodevelopmental disorders.

We have also established the Rett Syndrome Global Registry, an online study where parents share their experience caring for a loved one with Rett. Built to clinical trial standards the SHARE study will initially launch in 9 languages with nearly 200 enrolled patients and will collect comprehensive parent experiences on care strategies, symptom histories, and other areas.

Though the CARE and SHARE studies are valuable on their own, their power increases when families participate in both, allowing us to tie medical records to symptom burden at home. Ciitizen data has previously been used to support an IND application in early-onset SCN2A developmental and epileptic encephalopathy, and the Rett Syndrome Global Registry adheres to FDA guidance on using real world data in regulatory decision making. We aim to leverage the combined strengths of these resources to develop a robust historical control group that will help expedite genetic medicine trials to help us CURETT.

POSTER ABSTRACTS

DNA Base Editing of MeCP2 C-terminal Deletions

Guy J., Alexander-Howden B., von Bock und Polach T., Selfridge J., Bird A.*

Rett syndrome (RTT) is a severe neurological disorder caused by mutations in MeCP2. The majority of RTT mutations affect one of the two known functional domains: the methyl-DNA binding domain (MBD) or the NCoR-interaction domain (NID). Inactivating either domain affects the ability of MeCP2 to recruit the NCoR corepressor complex to methylated chromatin.

A number of RTT mutations do not appear to affect these functional domains but instead lead to a drastic reduction in the level of MeCP2 protein. These include small frameshifting deletions in the C-terminus of MeCP2, which account for about 10% of RTT cases.

Studies in mice have shown that the resulting truncated MeCP2 protein is fully functional, but present at only 10% of wild-type levels in the brain, resulting in RTT-like phenotypes.

C-terminal deletions (CTDs) can also be found in individuals who do not display severe neurological symptoms. Detailed examination of these "neutral variants" and RTT CTDs reveals a pattern: pathogenicity is determined by the frameshifted reading frame. In particular, RTT CTDs share the common C-terminal sequence Pro-Pro-Stop.
We propose that the particular geometry of prolines hinders translational termination, triggering a process similar to nonsense-mediated decay whereby both protein and mRNA are degraded.

Mutating a single nucleotide in the stop codon of a mouse CTD allele causes translation through to the following stop, avoiding these C-terminal prolines. This restores the truncated protein to WT levels and prevents the appearance of RTT-like phenotypes.

An adenine base editor (ABE) was used to introduce the same change in transfected cells with high efficiency. In order to deliver the editing machinery *in vivo* it is necessary to split the ABE into two parts due to the limited capacity of AAV vectors. This was achieved using split intein sequences, allowing splicing of the two protein segments into functional ABE when co-expressed. These will now be tested further in CTD mice.

Organ-enriched microRNAs Detectable in Blood Plasma as Peripheral Epigenetic Biomarkers of Rett Syndrome

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Background

Minimally invasive diagnostic tools are needed to better characterize, predict, and monitor disease progression and treatment response in Rett syndrome (RTT) patients. At DiamiR, we developed an approach based on targeted selection and quantitative analysis of microRNAs enriched in specific brain regions and other organs/tissues known to be affected by MeCP2 mutations/deletion, and detectable in blood plasma. microRNAs are short, non-coding regulatory molecules whose levels change in disease. The main objectives of this study was (i) to confirm our earlier microRNA profiling data generated with plasma samples from RTT patients and age-matched healthy study participants; (ii) to evaluate additional pre-selected microRNAs; and (iii) to assess longitudinal changes in levels of microRNA biomarkers.

Materials & Methods

Concentrations of 45 pre-selected microRNAs were analyzed in EDTA plasma samples of 163 study participants, including 81 RTT patients and 82 age-matched controls. RNA from plasma samples was isolated using the MagMAX mirVana kit (ThermoFisher). microRNA concentrations were measured by qPCR using custom plates (Qiagen) pre-plated with lyophilized miRCURY LNA-based primers for a 45-microRNA panel. Differentiation of RTT, RTT sub-groups, and control by microRNA pairs and their combinations (classifiers) was evaluated using a proprietary software program developed at DiamiR.

Results

microRNA pairs/classifiers were shown to effectively differentiate between RTT patients and control of three age groups (best classifier AUC=0.94 for <5-yr-olds, AUC=0.91 for 6-15-yr-olds, AUC=0.77 for >16-yr-olds). Several microRNAs were also shown to present efficient biomarkers of secondary pathology: walking ability (AUC=0.81), hyperventilation/breathing problems (AUC=0.75), and epilepsy (AUC=0.89). Further, liver-enriched microRNAs demonstrated promise in detecting liver abnormalities. Longitudinal data showed consistent changes in levels of microRNAs in RTT patients, although this sample sub-set was small (n=9). Further, the data supporting analytical validation of the platform technology to be used for a Lab-Developed Test (LDT) for RTT will be presented.

Conclusions

These data support the development of assays based on the analysis of cell-free brain/other organ-enriched microRNAs detectable in blood plasma to facilitate better understanding of RTT-associated pathophysiological processes and development of therapeutics for RTT.

Development of Pentatricopeptide Repeat (PPR) Protein-mediated RNA Regulation Technologies for Therapeutic Applications

Yusuke Yagi, Mizuho Ichinose, Masaru Ohta, Osamu Nakanishi and Bernard Gutmann EditForce, Inc., Fukuoka, Japan*

Pentatricopeptide repeat (PPR) proteins are sequence-specific RNA-binding proteins known to be widely distributed in the plant kingdom and involved in RNA regulation by cleavage, splicing and nucleotide editing in mitochondria and chloroplasts. PPR proteins consist of an N-terminal domain with a tandem array of PPR motifs that specifically recognize single nucleotides and a C-terminal domain that modifies target RNAs. We are currently developing new gRNA-free RNA editing technologies including one-base editing for controlling RNA functions aiming for therapeutic applications. In this poster presentation, we would like to introduce the nature of the PPR motifs for nucleotide recognition and applications of the PPR proteins including functional inhibition of repeat-expansion transcript, upregulation of translation and U-to-C/C-to-U conversions for one-base repair.

Mouse Models for Evaluating MECP2 Reactivation and Rescue of RTT Via XIST Inactivation and Targeted DNA Methylation Editing

Kyle Fink,[1] Shawn Liu,[2] and Antonio Bedalov[3]

1. UC Davis, 2. University of Columbia, 3. Fred Hutchinson Cancer Center

The majority of Rett syndrome (RTT) patients are females heterozygous for *MECP2* mutation, in which random X chromosome inactivation (XCI) during development leaves ~50% of neurons without functional MeCP2 protein, thereby creating cell-autonomous neuronal dysfunction. Corresponding mutations in hemizygous males lead to severe neonatal encephalopathy and early death. Mice carrying null alleles of *Mecp2* closely mimic symptoms seen in patients, including irregular breathing, stereotypical limb movements and shortened lifespan, and are thus faithful models of RTT. Male *Mecp2*-null mice start exhibiting symptoms as early as 30-60 days of age, with only half of the animals surviving beyond 75 days whereas the heterozygous females have a near-normal lifespan with neurologic deficits that are delayed (~6 months) and highly variable in severity. A major breakthrough in RTT research was the demonstration that RTT-like symptoms in adult mice can be reversed by genetic or viral restoration of MeCP2 protein. Thus, reactivation of the silenced wild type (WT) allele of *MECP2* from the inactive X chromosome (Xi) presents an exciting therapeutic opportunity that attacks the root cause of this disease by restoring MeCP2 function, which is the aim of the consortium led by Drs Fink, Lui and Bedalov.

Our preliminary data demonstrate our strategy's feasibility. First, we found that conditional deletion of the long non-coding RNA XIST, which is required for the XCI, from the Xi in mouse brain reactivates *Mecp2* in up to 5% of neurons, which was sufficient to promote survival of RTT mice (Adrianse et al *Epigenet Chromatin* 2018). This also suggested that XIST-independent mechanisms maintain *Mecp2* repression on the Xi.

Because we have previously shown that XIST deletion in the mouse brain causes near-complete loss of H3K27me3 and H2AK-Ub1 histone repressive marks on the Xi, but only minimal loss of DNA methylation, we hypothesized that **DNA methylation is critical to maintain *Mecp2* repression**. Accordingly, our *in vitro* data using human RTT hESCs and differentiated neurons demonstrate that targeted demethylation of the *MECP2* promoter on Xi by dCas9-Tet1 fusion protein directed to the *MECP2* locus via sgRNA, a DNA methylation editing tool pioneered by Dr. Liu, robustly reactivates *MECP2* from the Xi without detectable off-target effect at the transcriptional level and rescues small soma size and electrophysiological deficits, two hallmarks of RTT neurons (Qian et al *Sci Transl Med*, 2023).

We hypothesize that reactivation of *MECP2* from the Xi will rescue RTT-associated phenotypes in mice. The consortium has developed three new transgenic mouse models (1,2,3) and two novel methods of delivery of epigenetic editors (4,5) for *MECP2* reactivation *in vivo* including: **1) Xi-linked Mecp2-NanoLuciferase-tdTomato dual reporter mice**

for high sensitivity detection and quantification of Mecp2 reactivation; **2) MeCP2-null heterozygous female model of severe RTT** with exclusive inactivation of the X-chromosome harboring the WT *Mecp2* for measuring reactivation-induced rescue, which circumvents evaluating delayed and variable phenotypes in *Mecp2* heterozygous with random XCI; **3) MeCP2-null heterozygous female model of severe RTT** harboring **the WT human *MECP2* on the Xi** for the *in vivo* testing of human sgRNAs; **4) Cre recombinase-dependent dCas9-Tet1** transgenic line for efficient and tissue-specific DNA methylation editing *in vivo*; and **5) intein-mediated split TET1-dCAS9** for AAV9 delivery. This combination of transgenic models to measure reactivation efficacy (1) and rescue (2,3) with those that enable efficient *in vivo* editing *via* genetic means (4) and AAV9-mediated delivery (5) comprise a state-of-the-art tool kit to evaluate the *in vivo* feasibility of a *MECP2* reactivation strategy for treatment of RTT.

Employing Prime Editing Systems to Precisely Correct Rett Syndrome Mutations

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**contributed equally to this work*

Rett syndrome is caused by loss-of-function mutations in the regulatory gene *MECP2* in neurons. Because the expression of *MECP2* must be finely tuned for proper neurological function, genome editing approaches that directly restore gene function through the correction of mutations are an important therapeutic approach for Rett syndrome. Prime editing systems enable precise sequence changes within a genome in post-mitotic cells, such as neurons, without introducing double strand breaks, which reduces the likelihood of creating unwanted modifications to the genome. We are focused on improving the efficiency of prime editing for the correction of T158M (c.473C>T), which is one of the most common point mutations in Rett patients. The local sequence neighboring this mutation is potentially challenging for correction using base editing systems because base editing at nearby bystander positions would produce nonsynonymous sequence changes. We have realized improvements in the efficiency of MECP2 T158M correction through optimization of the prime editor protein and pegRNA. These efforts have focused on assessing editing outcomes in the context of either ribonucleoprotein (RNP) or mRNA delivery of the prime editing reagents, as these modalities can be translated to transient delivery systems for *in vivo* MECP2 correction in the CNS. In addition, we have developed an efficient method to identify potential off-target sites genome-wide for prime editing systems (PE-tag), and we have applied this method to our prime editing components designed to correct the T158M mutation. Together our improved prime editing reagents and off-target analysis method provide a foundation for increasing the efficiency and safety of prime editing systems for the precise correction of T158M and other common Rett mutations, as we transition to animal models as a step forward along the path toward achieving MECP2 gene correction in patients.

Engineering Nme2Cas9 Adenine Base Editors for Bystander-free Correction of Four MECP2 Mutations that Cause Rett Syndrome

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Cas9 base editors enable precise, RNA-guided nucleotide changes without introducing double-strand breaks. Adenine base editors (ABEs) can catalyze precise T:A-to-C:G conversions, including corrections of C:G-to-T:A mutations that cause disease. Four such C:G-to-T:A mutations in MECP2 exon 4 – c.502C>T (R168X), c.763C>T (R255X), c.808C>T (R270X), and c.916C>T (R306C) – are collectively responsible for ~25% of Rett syndrome cases. ABEs require positioning of the target adenine within a precise distance of the Cas9 protein's PAM sequence (e.g., NGG for wildtype SpyCas9). Potential ABE-based approaches to therapeutic genome editing can be thwarted by the absence of a suitable PAM as well as the presence of one or more bystander adenines within the editing window. Delivery challenges also complicate therapeutic applications of ABEs, especially in organ systems such as the central nervous system (CNS), where MECP2 editing would be required. Currently, AAV vectors are among the most promising delivery vectors for CNS applications. However, most ABEs are based on SpyCas9 or its PAM-minimized derivatives, which are too large to fit into a single AAV vector along with the necessary guide RNA.

We identified and characterized Nme2Cas9, which is compact, resistant to off-target editing, and active in human cells. Importantly, it requires an N4CC PAM for targeting, enabling Nme2-ABEs to target sites near sequences that are devoid of purines on the edited strand. We and others recently established Nme2Cas9-based ABEs as among the first to be deliverable by a single AAV vector, including in the CNS. However, editing efficiencies were modest with some guides, editing windows were broad, and tissue-specific promoter choices were highly constrained due to the size of the protein effector and the cargo limits of AAV vectors.

We have now used a variety of Cas9 engineering approaches with the aim of increasing the editing activity, targeting scope, and AAV compatibility of Nme2-BEs. We first used deaminase domain insertion to position the deaminase proximal to the predicted displaced DNA strand, based upon Nme2Cas9 crystal structures. The domain-inlaid Nme2Cas9 variants demonstrated shifted editing windows dependent on the site of insertion, as well as increased activity relative to that of the N-terminally fused variant. We further expanded the editing scope of the domain-inlaid Nme2-ABE by creating a derivative that only requires a N4CN PAM, nearly doubling the number of guides and sites available for Nme2-ABEs. We achieved this latter improvement in targeting scope by transplanting into Nme2Cas9 the PAM-interacting domain of a distinct but poorly active Cas9 homolog (SmuCas9) that we had previously discovered. We also minimized the lengths of the linkers flanking the embedded TadA8e deaminase domain, partially relieving single-AAV space constraints. Finally, we engineered site-specific mutations that increase Nme2-ABE editing efficiency in human cells.

Using these improved Nme2-ABE variants, we have identified guides that direct the correction of all four of the pathogenic *MECP2*-causing mutations noted above, without significant bystander editing. These results provide a potential route toward the precise therapeutic editing of up to ~25% of Rett syndrome cases. We will present our preclinical developmental efforts to maximize editing efficiencies and validate these guide/effector configurations for therapeutic applications.

Delivery and Optimization of Epigenome Editors to Reactivate MECP2

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Rett syndrome (RTT) is a severe neurodevelopmental disorder characterized by a period of normal infancy followed by regression in developmental milestones, resulting in developmental delays with absence of speech, ataxia, loss of purposeful hand movements, and seizures. RTT is caused by mutations in *MECP2* on the X chromosome and shows an X-linked dominant inheritance pattern, exclusively in females. Because of X chromosome inactivation (XCI), females with RTT are mosaic of cells expressing the mutant and wild-type alleles of *MECP2*. In line with the RSRT X-reactivation consortium, our research platform involves creating gene-modifying proteins that can recognize and bind to specific gene sequences in the *MECP2* gene.

The consortium lead by Drs. Liu, Bedalov and Fink have developed epigenome editing tools consisting of a catalytically dead Cas9 (dCas9) fused with Tet1 and target gRNAs that allow for DNA demethylation in a targeted manner (Liu et al., 2016; Liu et al., 2018). We applied a multiplex epigenome editing approach to reactivate MECP2 from Xi in RTT human embryonic stem cells (hESCs) and derived neurons. It is important to note that epigenetic editor fused DBDs are particularly large due to the complexity and potential need for combinatorial approaches (Halmai et al., 2020). The inclusion of additional effector domains such as the catalytic domain of the human DNA demethylase TET1 (2.2 kb) fused to an alternative DBD strategy such as catalytically inactive SpdCas9 (3.7 kb) would rapidly reach and exceed the capacity of 4.9 kb in adeno-associated viral vectors (AAV). Our group has addressed this concern by developing an intein-mediated trans-splicing *Spd*Cas9 that efficiently packages in two AAV capsids as a gold standard for gene therapy delivery. Here we show the optimization of evolved capsids at delivering the dual AAV into the mouse brain, novel delivery platforms, as well as evaluation of smaller promoters to increase efficient packaging and delivery.

Base Editing Restores MECP2 Protein in R255X Patient Derived Neurons

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Rett syndrome is a rare X-linked neurodevelopmental disorder occurring in 1:10,000 female births. Rett symptoms manifest at 6-18 months of age and are followed by rapid developmental regression and progressive language/motor deterioration. Rett is caused by mutations in Methyl-CpG Binding Protein 2 (MECP2), a ubiquitously expressed transcriptional regulator. MECP2 is abundantly expressed in CNS neurons and essential for neurodevelopment, including synapse and dendritic formation. The most prevalent Rett mutations are caused by C to T transition mutations, which are all amenable to base editing. Therefore, in this study, we engineered an HEK293T-AAVS1-mCherry-MECP2 cell line for high-throughput screening of guide/base editor combinations for the 7 most prevalent mutations. We identified editing strategies for multiple MECP2 mutations, and here we show that base editing can effectively correct R255X, the third most common Rett mutation. We found that in vitro base editing using LNPs can effectively restore MECP2 protein in R255X patient-derived NGN2 neurons in a time-dependent manner. Finally, base editing ameliorated Rett neuronal phenotypes, including nuclear size and gene expression levels.

Design of the REVEAL Pediatric Study of TSHA-102 Gene Therapy for Rett Syndrome

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The US FDA has recently cleared an Investigational New Drug application, allowing Taysha Gene Therapies to initiate the REVEAL Pediatric study, the first trial of TSHA-102 gene therapy for girls with Rett syndrome (RS). Efficacy and safety of TSHA-102 were tested first in animal models and are under clinical evaluation in the REVEAL Adult study (NCT05606614). Compared to adult treatment, gene therapy in early childhood may have greater potential to transform the lives of RS patients, e.g., delaying loss of ambulation or other irreversible outcomes.

The REVEAL Pediatric study will be a Phase 1/2, multicenter, dose-finding study to evaluate the safety and efficacy of TSHA-102 in 3 – 8-year-old (yo) girls with RS. Part A of the study is an open-label, single-arm escalation study intended to define a maximum tolerated or maximum administered dose (MTD or MAD) of TSHA-102. Immunoprophylaxis will begin at Day -7 and will be tapered from Week 17 to Week 25 (oral prednisone) and starting from Week 38 (sirolimus).

In Cohort 1 (n=3), subjects 5 – 8 yo will receive a single intrathecal dose of $5x10^{14}$ viral genomes (vg), with Independent Data Monitoring Committee (IDMC) safety evaluation scheduled 42 days after the first and third patient treatments, to identify any dose-limiting toxicities (DLTs). Finding of 1 DLT will trigger expansion of Cohort 1 to 6 subjects; should two DLTs occur in Cohort 1, the initial dose will have surpassed the MTD. Otherwise, Cohort 2 (n=3) will be treated with $1x10^{15}$ vg, and safety evaluation will occur as before. Should 0/3 (or 1/6) Cohort 2 patients experience DLTs, the MAD of $1x10^{15}$ vg will be applied in Part B.

All Part A efficacy outcomes will be evaluated with descriptive statistics, relative to each subject's baseline data. Outcomes will include objective metrics, e.g., electroencephalography for seizure assessment, auditory and visual evoked potentials, mismatch negativity, and whole-body polysomnography, plus physical, functional, and cognitive scoring by study personnel and caregivers. Caregiver-assessed outcomes will capture aspects of RS identified as specifically burdensome in this disease: communication, hand movement control, breathing, and mobility difficulties.

Part B will enroll 6 subjects (Cohort 3; 5 – 8 yo), randomized to an immediate and a delayed treatment cohort (ITC and DTC); following IDMC review, 6 additional subjects (Cohort 4; 3 – 5 yo) will be randomized. Treatment will be with TSHA-102 at the MAD or MTD determined previously. Efficacy and safety evaluations will be conducted at prespecified intervals before and ≥2 years after treatment. For outcomes with potential evaluator bias, patient videos will be assessed by independent experts blinded to subjects' treatment status.

Early Safety and Efficacy Observations Following the First Use of TSHA-102 Gene Therapy in a Patient with Rett Syndrome

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The REVEAL Adult study (NCT05606614), a first-in-human trial of TSHA-102 gene therapy, is actively enrolling women ≥18 years old with Rett syndrome (RS). The first enrolled participant received TSHA-102 treatment with immunoprophylaxis at the Centre Hospitalier Universitaire Sainte-Justine (Montréal, Canada). As specified in the study protocol, her case was reviewed by the Independent Data Monitoring Committee (IDMC) on Day 42 post-treatment.

Participant 1 is a 20-yo woman diagnosed with RS at age 3. She presented with global delay, followed by slow regression of skills. She could sit at 6 months but never crawled or walked independently or developed a pincer grasp. She learned a few words from 11 months onwards, which she eventually lost. Following speech and hand-use regression, stereotypic hand movement developed after age 2. She has been unable to stand without support since age 6 and stopped reaching or grasping around age 7. Seizures, developing at age 3, proved refractory to multiple medications. They are generally well-controlled with clobazam and phenytoin, despite occasional breakthrough seizures during viral infections or when her blood phenytoin declines to <100 µmol/L. Ongoing medical challenges include persistent constipation, recurrent pneumonia, scoliosis, osteopenia, and a benign thyroid nodule identified in January 2023.

Following sirolimus and prednisolone initiation at Day -7, TSHA-102 was injected intrathecally on Day 0. The participant experienced a clinically nonsignificant blood pressure decline, resolving within 8 hours. Oximetry following treatment showed stable tissue perfusion. Treatment-related adverse events included: an episode of mild (Grade 1) pyrexia on Day +1 (deemed TSHA-102-related), managed with acetaminophen and resolving in <24 hours; and mild irritability starting on Day +5 (prednisolone-related). Post-treatment hematology and other labs were mostly unremarkable; liver enzyme excursions seen in Week 2 had all resolved by Week 4. There were no serious adverse events, irrespective of causality.

Starting Week 2 after TSHA-102 treatment, caregiver reports and key efficacy metrics showed clinical improvement. Week 4 clinical global impression change scores rated by physicians (CGIi) and caregivers (PGIi) were rated at 2 ("Much improved") and 3 ("A little better"), respectively. RS Behavior Questionnaire (RSBQ) total score showed a 23-point improvement over baseline; of the 8 RSBQ subscales, 6 showed substantial improvement, including "Hand behaviors" (declining from 12 to 5 points). The "Night-time behaviors" score declined from 3 to 0 points, consistent with caregiver reports that the patient was sleeping peacefully all night, for the first time in their memory. Conversely, there were no significant changes observed on the Revised Motor Behavior Assessment (R-MBA) or any subscales. However, the participant started being able to sit unassisted for a few minutes by Day 35. Finally, whereas two seizures were recorded in the 10-day run-up to therapy, none occurred between Days 0 and +35, despite low blood phenytoin.

Because Participant 1 experienced no dose-limiting toxicities, and considering the rapid clinical benefit she appears to be experiencing, the IDMC approved continuation of the REVEAL Adult study, with Participants 2 and 3 to receive the same dose of TSHA-102 as Participant 1, later in this calendar year.

A Novel X-reactivation Gene Therapy is Highly Efficacious and Shows Superior Biodistribution in Large Animals

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X chromosome inactivation is a developmentally regulated process in females, wherein one X chromosome per cell is inactivated through a complex stepwise process involving the Xist RNA. This process allows for dosage regulation in females for X-linked gene expression. As a consequence, female tissue affected by X-linked genetic disorders, such as Rett syndrome, will be a mixture of cells expressing the mutated version of the gene or the healthy copy depending on which X chromosome is active in any given cell. This mosaicism complicates gene replacement strategies significantly as the healthy cells are at risk of being overdosed. Notably, all cells that express the loss of function mutation contain a healthy copy on the silenced chromosome which, upon re-expression, could ameliorate the disease phenotype. Thus, therapies that reactivate the X chromosome may be an effective strategy for Rett. Here, we show that targeting sequestration of miR106a using a sponge (miR106sp) leads to the partial reactivation of the X chromosome and subsequent increase in MeCP2 expression. Furthermore, viral vector-based delivery of miR106sp showed efficacy in both Rett patient derived neurons, with a broad spectrum of MeCP2 mutations, as well as in a severe female Rett mouse model. To ensure optimal therapeutic benefit effective targeting of the central nervous system is critical for success in the treatment of neural developmental disorders including Rett syndrome. Alcyone Therapeutics has developed a novel precision delivery platform, Falcon,™ allowing to optimize drug delivery to the brain. We demonstrated superior biodistribution of AAV9 in terms of uniformity, deeper brain structure penetration, and reduction of off-target effects in nonhuman primates. In summary, our x-reactivation genetic therapy is highly promising for Rett syndrome, and other X-linked disorders, and will be delivered with a novel technology allowing improved delivery and reduced off targets.

Behavioral and Neurophysiological Phenotypes of Female Mecp2 Heterozygous Mice that May Aid Clinical Translation of Novel Therapeutic Approaches

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Biogen

Loss of ability to communicate, deficits in gross and fine motor function, and seizures are among the clinical features cited as most impactful in terms of disease burden for parents and caregivers of Rett patients. Since Rett syndrome is an X-linked disorder, it is ideal to explore phenotypes in models that recapitulate the cellular mosaicism of this disorder. We performed longitudinal characterization of several potentially translatable phenotypes in a cohort of female, *Mecp2* heterozygous mice. Our data indicate that these mice present with motor coordination deficits, abnormal auditory startle responses, and increases in spike wave discharge events even at the earliest timepoint tested (~8 weeks of age). In addition, we find age-dependent deficits in auditory-evoked potentials beginning ~10-12 weeks of age. Together, these data contribute to our understanding of behavioral and neurophysiological phenotypes in a mouse model that mirrors key aspects of clinical Rett syndrome and may be valuable for evaluation of new candidate therapies.

Developing Lipid Nanoparticle Formulations for mRNA Delivery in the Brain

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Advances in CRISPR technologies have made genome editing a promising therapeutic strategy for genetically defined neurological diseases. However, delivering CRISPR tools to the brain remains a challenge. Adeno-associated virus (AAV)-mediated delivery is limited in cargo packaging capacity and is vulnerable to off-target effects due to continuous expression of the CRISPR/Cas9 construct. A non-viral approach based on lipid nanoparticles (LNPs) would circumvent these limitations. Here, we demonstrate that LNPs can deliver mRNAs to the brain after injection into the cerebrospinal fluid (CSF) or brain tissue. We show that a branched ionizable lipid with bioreducible arms outperformed the industry-standard MC3 ionizable lipid in delivering mRNA to the brain. Using the mTmG and nTnG reporter mouse models which show a change in fluorescence after Cas9 editing or Cre-mediated recombination, we show that the LNPs traveled throughout the CSF and delivered mRNA to different compartments of the ventricular system. Cre recombination was most intense in the ependymal cells lining the ventricles with some deeper editing observed for a subset of formulations. Intraparenchymal injection showed editing throughout the striatum and along white matter tracts. We continue to work on improving delivery to neurons throughout the brain by engineering lipid and protein composition and formulation parameters.

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