13th Annual Les Turner Symposium on ALS

Brought to you by the Les Turner ALS Center at Northwestern Medicine

Monday, Nov. 6, 2023

8 a.m.-5 p.m. Central Time
Feinberg Pavilion
Feinberg Krumlovsky Atrium
251 E. Huron St. 3rd Floor
Chicago, IL 60611

LES TURNER ALS FOUNDATION

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Welcome Letter

Dear Friends and Colleagues,

It is with great pleasure that we extend our warmest welcome to the 13th Annual Les Turner Symposium on ALS. This symposium is a day of celebration for our achievements in science, providing hope and excitement within the ALS community. Advancements in research have been very rapid. We have many reasons to celebrate.

First: The patient groups have never been more involved and active. Patients and their loved ones carry the torch with such passion and energy that it inspires everyone. The power of that energy has the potential to be the catalyst of much-needed change.

Second: The pace of discoveries has been unprecedented. The genetic causes of ALS and the underlying causes of neuronal vulnerability are being revealed one after another. We now have better preclinical platforms that generate translational information.

Third: There has been an immense collaboration among different ALS centers in the US. Our Les Turner ALS Center at Northwestern Medicine is part of the HEALEY ALS Platform Trial, which enables multiple compounds to be tested in parallel. There have been 80-100 trials worldwide, and more compounds are moving forward, one being NU-9, discovered here at home.

Fourth: More and more pharmaceutical companies share our quest to end ALS and improve the lives of patients. Some of these efforts identified new drugs for ALS and others are now in development.

As we all focus on the finish line, we form teams and collaborations. We—the scientists, clinicians, patients, and caregivers—all work hand in hand to turn the impossible into possible. Symposiums are the manifestations of such devoted efforts.

This year, we prepared a one-of-a-kind program. Dr. Frank Bennett from Ionis Pharmaceuticals will be our keynote speaker and will talk about the groundbreaking approach to mitigate ALS with anti-sense oligonucleotides. This year the FDA approved QALSODY (tofersen) as the first treatment targeting a genetic cause of ALS for people who have SOD1 mutations. Congratulations to Dr. Bennett and his team at Ionis Pharmaceuticals and your partners at Biogen. We are thrilled to have you with us!

We have Dr. Evangelos Kiskinis and Dr. Mukesh Gautam, members of our Les Turner ALS Center, as well as Dr. Darryl Bosco from University of Massachusetts, Dr. James Shorter from University of Pennsylvania, and Dr. Claire Lepichon from NIH, joining us to share their exciting research and how that helps translational efforts. We will hear from Laura Freveletti, the new CEO of the Les Turner ALS Foundation, about the achievements the foundation has made, and from Dr. Robert Kalb about what is new at the Les Turner ALS Center. I will have an information session about NU-9, and there will be an engaging discussion on the latest in ALS care and clinical research with Dr. Senda Ajroud-Driss, Tina Cascio and Janie Gobeli. This year, we have more than 30 poster abstracts from many different labs and institutions, all sharing exciting developments in the field.

None of these achievements would be possible without the Les Turner ALS Foundation and the unwavering support they show to patients, and to the improvement of science and clinical care. We are thankful to the pharmaceutical companies Mitsubishi Tanabe Pharma America, Amylyx Pharmaceuticals, Akava Therapeutics, Ionis Pharmaceuticals, Inc. and PTC Therapeutics for their support and contributions. The Mesulam Center for Cognitive Neurology and Alzheimer's Disease has been supporting the Les Turner Symposium on ALS since our inception. We thank them for sharing our vision and passion.

As we work with all our might to end ALS and other motor neuron diseases, we are happy to have you here with us today. Welcome!

P. Hande Ozdinler, PhD

P. Hande Ozdinler, PhD
Associate Professor, Department of Neurology, Northwestern University Feinberg School of Medicine

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Agenda

8:00 a.m.	**Registration and Breakfast**
8:45 a.m.	**Welcome and Opening** Hande Ozdinler, PhD, Associate Professor of Neurology, Northwestern University Feinberg School of Medicine Robert Kalb, MD, Joan and Paul Rubschlager Professor of Neurology, Chief of Neuromuscular Disease; Director, Les Turner ALS Center, Northwestern University Feinberg School of Medicine Laura Freveletti, CEO of Les Turner ALS Foundation
9:00 a.m.-12:00 p.m.	**Research Presentations**
9:00-9:25 a.m.	**Untangling the convergence of disease mechanisms in ALS using personalized iPSC technologies** by Evangelos Kiskinis, PhD, Associate Professor of Neurology and Neuroscience, Northwestern University Feinberg School of Medicine
9:35-10:05 a.m.	**How neuronal stress and brain trauma may contribute to ALS pathogenesis** by Daryl Bosco, PhD, Professor and Associate Vice Chair, Professor of Neurology, UMass Chan Medical School
10:10-10:25 a.m.	**Potential role of cardiolipin nanoparticles in improving upper motor neuron health in amyotrophic lateral sclerosis** by Mukesh Gautam, PhD, Research Assistant Professor, Ellen McConnell Blakeman Fellow, Department of Neurology, Northwestern University Feinberg School of Medicine
10:30-10:45 a.m.	**Morning Break**
10:50-11:20 a.m.	**Countering deleterious phase transitions in ALS/FTD** by James Shorter, MA, PhD, Professor, Department of Biochemistry & Biophysics, Perelman School of Medicine, University of Pennsylvania
11:25-11:55 a.m.	**From axon damage to disease: common mechanisms in neurodegeneration** by Claire Le Pichon, PhD, Investigator, National Institute of Child Health and Human Development (NICHD)
12:00-2:00 p.m.	**Lunch Break/Poster Session**
12:15 p.m.	**NU-9 Information Session (in-person only)** Hande Ozdinler, PhD, Associate Professor of Neurology, Northwestern University Feinberg School of Medicine
12:30 p.m.	**Poster Session Begins (in-person only)**
2:00 p.m.	**Opening Remarks for Afternoon Session** Laura Freveletti, CEO of Les Turner ALS Foundation
2:05 p.m.	**Keynote Address** Introduction: Robert Kalb, MD, Joan and Paul Rubschlager Professor of Neurology, Chief of Neuromuscular Disease; Director, Les Turner ALS Center, Northwestern University Feinberg School of Medicine **Antisense based therapy for rare neurological diseases by C. Frank Bennett, PhD, Executive Vice President, Chief Scientific Officer, Ionis Pharmaceuticals** Moderator: David Gate, PhD, Assistant Professor, Northwestern University Feinberg School of Medicine
3:20 p.m.	**Afternoon Break**
3:30 p.m.	**Les Turner ALS Foundation Video & Clinical Conversations** Introduction: Erik Pioro, MD, PhD, Herbert C. Wenske Professor of Neurology, Vice-Chair of Translational Neurology, Medical Director, Neuromuscular Division Senda Ajroud-Driss, MD, Professor of Neurology; Director, Lois Insolia ALS Clinic; Les Turner ALS Center; Director, MDA Clinic, Northwestern University Feinberg School of Medicine Tina Cascio, RN, BSN, Board of Directors, Les Turner ALS Foundation Janie Gobeli, ALS Research Ambassador for the Northeast ALS Consortium Moderator: Lauren Webb, LCSW, Chief Advocacy and Outreach Officer, Les Turner ALS Foundation
4:45 p.m.	**Closing Remarks** Robert Kalb, MD, Joan and Paul Rubschlager Professor of Neurology, Chief of Neuromuscular Disease; Director, Les Turner ALS Center, Northwestern University Feinberg School of Medicine

*All Northwestern faculty members presenting at the symposium are affiliated with the Les Turner ALS Center at Northwestern Medicine unless otherwise noted.

C. Frank Bennett, PhD

Antisense Based Therapy for Rare Neurological Diseases

Executive Vice President, Chief Scientific Officer, Founding Member, Ionis Pharmaceuticals
Chief Technical Officer, n-Lorem Foundation
2019 Healey Center International Prize for Innovation in ALS
2021 Lifetime Achievement Award, Oligonucleotide Therapeutic Society

Currently there are multiple genetic-based medicines being pursued for rare neurological diseases including antisense technology, gene therapy, and gene-editing technologies. Antisense oligonucleotides (ASOs) are one of the more advanced technologies. ASOs are synthetic, chemical modified nucleic acid analogs designed to bind to RNA by Watson-Crick base paring. Upon binding to the RNA, ASOs modulate the function of the targeted RNA through a variety of mechanisms. Both protein-coding as well as non-coding RNAs can be targets of ASO- based drugs, significantly broadening therapeutic targets for drug discovery compared to small molecules and protein-based therapeutics. The approval of nusinersen (Spinraza™) as a treatment for spinal muscular atrophy (SMA) and the recent approval of tofersen (Qalsody) for familial ALS validates the utility of antisense drugs for the treatment of motor neuron diseases. The application of antisense technology as potential therapy for other rare neurodegenerative diseases and neurodevelopmental disorders will be discussed.

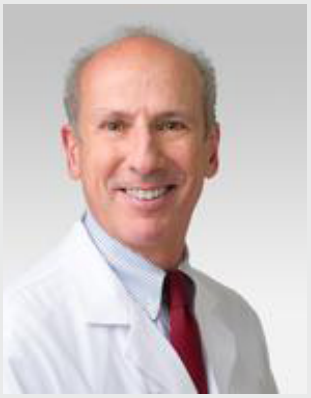
Dr. Bennett is the executive vice president and chief scientific officer at Ionis Pharmaceuticals and one of the founding members of the company. He is responsible for continuing to advance Ionis' technology and expanding the company's drug discovery platform. Dr. Bennett is also the franchise leader for gene-editing programs at Ionis. He has been involved in the development of ASO's as therapeutic agents, including research on the application of oligonucleotides for inflammatory, neurodegenerative diseases and cancer, oligonucleotide delivery, pharmacokinetics, and medicinal chemistry. Dr. Bennett led the discovery and development of nusinersen and the discovery of tofersen and tominersen.

Remarks

Laura Freveletti, CEO of the Les Turner ALS Foundation

Laura Freveletti is Chief Executive Officer of the Les Turner ALS Foundation. She comes to the ALS field with 30 years of experience in executive leadership, most recently as senior program officer at The Allstate Foundation. She has deep connections to the Chicago non-profit community, with experience as a senior fundraising executive at the YMCA of Metropolitan Chicago and Lyric Opera of Chicago, as well as service as board president of the Sudden Infant Death Services (SIDS) of Illinois. Laura also has led corporate marketing and community involvement at companies including Kraft Heinz, LaSalle Investment Management, and Chicago Title & Trust Company, and earned a degree in business administration from the University of Louisville. She brings a personal understanding of the impact of ALS to the position, having lost her brother-in-law to the disease.

Robert G. Kalb, MD, Joan and Paul Rubschlager Professor of Neurology, Chief of Neuromuscular Disease; Director, Les Turner ALS Center, Northwestern University Feinberg School of Medicine



In 2017, Robert Kalb became the Director of the Les Turner ALS Center at Northwestern Medicine. His laboratory studies the basic mechanisms underpinning ALS using geneticallyengineeredmice, primary neuron culture, C.elegans and yeast disease models. His work has focused on the fundamental molecular processes that go awry during disease. For example, Dr. Kalb's group discovered that derangement of energy metabolism is a key contributor to neuronal death in models of ALS. In addition, he identified two cell biological pathways (GTPase mediated vesicle trafficking and RAD23mediated protein degradation) that can be targeted for treatment of ALS. The Kalb Lab is passionately committed to bending the arc of disease and finding a cure for ALS through innovative and collaborative research.

Erik P Pioro, MD, PhD, Professor of Neurology, Northwestern University, Herbert C. Wenske Professor, Les Turner ALS Foundation, Vice Chair of Translational Neurology

In 2021, Dr. Pioro joined the Department of Neurology at Northwestern University Feinberg School of Medicine as Medical Director of the Neuromuscular Division and Vice-Chair of Translational Neurology. Dr. Pioro specializes in the diagnoses and management of adult patients with various forms of MND, including ALS. His research focuses on characterizing the MRI abnormalities in brain and spinal cord of PALS as well as identifying the underlying molecular correlates of the imaging changes in CNS tissue and induced pluripotent stem cells derived from these patients.

Remarks

Hande Ozdinler, PhD, Associate Professor of Neurology, Northwestern University Feinberg School of Medicine

Hande Ozdinler, PhD, is an associate professor of Neurology (Neuromuscular Disease) in the Ken & Ruth Davee Department of Neurology at the Northwestern University Feinberg School of Medicine. Dr. Ozdinler's research aims to understand the cellular and molecular mechanisms responsible for early vulnerability and progressive degeneration of upper motor neurons. These neuron populations are clinically relevant as their degeneration leads to diseases such as ALS, HSP and PLS. The Ozdinler Lab also works toward building effective treatment strategies, developing drug discovery platforms that incorporate upper motor neurons, and the identification of biomarkers and early detection markers.

David Gate, MD, PhD, Assistant Professor of Neurology, Northwestern University Feinberg School of Medicine

David Gate, MD, PhD, is an assistant professor of neurology in the Ken and Ruth Davee Department of Neurology at Northwestern University Feinberg School of Medicine. Dr. Gate's research is focused on the intersection of the immune system and neurodegenerative disease. His laboratory employs multi-omics strategies to interpret immune system changes related to neurodegeneration. The Gate lab is particularly interested in the interplay between T cells and neurogenerative disease antigens. Their goal is to identify novel biomarkers or immunotherapeutic targets for neurodegeneration.

Lauren Webb, LCSW, Chief Advocacy and Outreach Officer, Les Turner ALS Foundation

Lauren Webb, LCSW, is the Chief Advocacy and Outreach Officer at the Les Turner ALS Foundation. She is dedicated to forging links between policymaking and service delivery so patients and families receive not just care, but hope. With a master's degree in social work from the University of Chicago, she has worked in the neuromuscular community for over 20 years, providing direct patient care, coordinating clinical trials, and overseeing the Muscular Dystrophy Association's nationwide Care Center Network. At the Les Turner ALS Foundation, she leads efforts to provide innovative, compassionate support services with emphasis on health literacy, health equity, and patient centered care.

Evangelos Kiskinis, PhD

Associate Professor of Neurology, Northwestern University

Untangling the convergence of disease mechanisms in ALS using personalized iPSC technologies

Dr. Kiskinis will describe findings from investigating distinct genetic causes of ALS using iPSC models as well as novel mechanistic interaction between TDP43 dysfunction and neuronal excitability that is relevant for sporadic ALS disease.

Evangelos Kiskinis, PhD, is an associate professor of neurology and neuroscience at Northwestern University Feinberg School of Medicine and a New York Stem Cell Foundation Robertson Investigator. Dr. Kiskinis earned a PhD from Imperial College London and carried out postdoctoral training at Harvard University, where he pioneered the first models of ALS using personalized stem cell-based approaches. In 2015, his laboratory was established at the Les Turner ALS Center at Northwestern. The Kiskinis Lab seeks to harness the power of pluripotent stem cells to understand how neuronal function is impaired in ALS/FTD patients as well as identify points of targeted and effective therapeutic intervention for ALS/FTD. Dr. Kiskinis also serves as the scientific director of the Stem Cell Core Facility at Northwestern.

Daryl Bosco, PhD

Professor of Neurology, UMass Chan Medical School

How neuronal stress and brain trauma may contribute to ALS pathogenesis

The Bosco Lab is studying stress as an upstream trigger of neuron death in ALS. These studies are based on observations that neurons in ALS patients become overactivated because of both altered neuronal connections and metabolite imbalances within the central nervous system. Dr. Bosco will present recent research showing that ALS neurons are more susceptible to external stressors such as brain trauma.

Daryl A. Bosco, PhD, is a professor of Neurology at UMass Chan Medical School. Dr. Bosco's scientific career began with a PhD in bio-organic chemistry from Brandeis University in Massachusetts and a post-doctoral fellowship at the Scripps Research Institute in California, where she studied protein structure and misfolding in the context of neurodegeneration. Dr. Bosco was then an Instructor at Harvard Medical School in Massachusetts, where she began working on ALS. In 2008, Dr. Bosco established her independent research laboratory at UMass Chan Medical School. The Bosco Lab is investigating the mechanisms underlying ALS and related disorders such as frontotemporal dementia.

Mukesh Gautam, PhD

Research Assistant Professor of Neurology, Northwestern University

Potential Role of Cardiolipin Nanoparticles in Improving Upper Motor Neuron Health in Amyotrophic Lateral Sclerosis

Dr. Guatam will discuss nanoparticle mediated improvement of mitochondria structure as a potential treatment strategy for ALS.

Mukesh Guatam, PhD, is a research assistant professor in the Northwestern University Feinberg School of Medicine Department of Neurology. He received his MS from Barkatullah University in Bhopal, India in Bioscience with specialization in Biotechnology; and his PhD from University of Delhi, India, Department of Zoology. Dr. Gautam's research is focused on understanding underlying mechanisms that contribute to vulnerability of motor neurons in the context ALS. He is particularly interested in exosomes-mediated cellular communication, epigenetic regulation of gene expression, and identification of targets for therapy development. Dr. Gautam uses in vivo and in vitro model systems, immuno-coupled electron microscopy (iEM), correlative light electron microscopy (CLEM), extracellular vesicles, and next-generation sequencing approaches to answer his research questions.

James Shorter, MA, PhD

Professor of Biochemistry and Biophysics, University of Pennsylvania

Countering Deleterious Phase Transitions in ALS/FTD

In his talk, Dr. Shorter will provide his thoughts on how to counter deleterious phase transitions in ALS/FTD.

James Shorter, MA, PhD, is a professor of biochemistry and biophysics at the University of Pennsylvania. He received his MA in Biology from the University of Oxford and a PhD in Cell Biology from University College London. His postdoctoral training was at Yale University and the Whitehead Institute for Biomedical Research at MIT. His lab focuses on mechanisms to counter deleterious phase transitions in neurodegenerative disease.

Claire Le Pichon, PhD

Investigator, National Institute of Child Health and Human Development

From Axon Damage to Disease: Common Mechanisms in Neurodegeneration

Dr. Le Pichon will present work on axon injury signaling as a common pathway in neurodegeneration, as well as current efforts to understand fundamental differences between vulnerable and resilient populations of motor neurons in ALS.

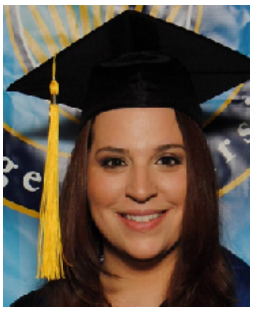
Claire Le Pichon, PhD, is an investigator in the Division of Intramural Research at the Eunice Kennedy Shriver National Institute of Child Health and Human Development. She earned her PhD in Biological Sciences from Columbia University in 2007, then joined the Translational Neuroscience group at Genentech, where she worked on several pipeline targets for neurodegenerative disease, including the axon injury signaling kinase DLK. She started her laboratory at the NIH in 2016, where she employs a multidisciplinary approach to investigate cellular mechanisms underlying neuronal dysfunction and degeneration, using mice and iPSC-derived neurons as model systems.

Join us for an engaging discussion on the latest in ALS care and clinical research, from the perspectives of a clinician and two people living with ALS. By uniting the expertise of specialists in fields like pulmonology, nutrition, and social work, multidisciplinary care is making a difference on the quality of life and health of people living with ALS and their caregivers. The Lois Insolia ALS Clinic at the Les Turner ALS Center at Northwestern Medicine has a comprehensive multidisciplinary team and is actively involved in multi-center drug trials and other clinical research.

In this panel, you'll learn how clinicians and people living with ALS are shaping the future of multidisciplinary care, research, and community support, and you'll have an opportunity to ask questions about the latest advancements in these fields.

Senda Ajroud-Driss, MD

Senda Ajroud-Driss, MD, is a professor in the Ken & Ruth Davee Department of Neurology at the Northwestern University Feinberg School of Medicine, where she also serves as program director of the neuromuscular medicine fellowship. Dr. Driss received her medical degree from The Medical School of Tunis, Tunisia, then completed her neurology residency at the University of Illinois at Chicago and a neuromuscular fellowship at Northwestern. She is board-certified in neurology and in neuromuscular medicine and has been treating patients with ALS in the Lois Insolia ALS Clinic for nearly 20 years. Dr. Driss also leads the Les Turner ALS Center's clinical trial program.



Tina Cascio, RN, BSN

Tina Cascio, RN, BSN, joined the Les Turner ALS Foundation's Board of Directors in 2022 and serves on its Support Services Committee. Tina cared for her mom throughout her fight with ALS until her mom passed away in 2018. She is now in her own battle after having been diagnosed with ALS in 2020. Tina has been involved in community outreach and familial sponsor programs as well as the organization Her ALS Story. Tina's entire career to this point has been in the medical field and she considers herself a temporarily retired nurse. Once there is a cure for ALS, she plans on returning to care for her patients.

Janie Gobeli

Janie Gobeli serves as an advisory committee member for the HEALEY ALS Platform Trial and the QurAlis/CISCRP Global Patient Advisory Board, an ALS Research Ambassador for the Northeast ALS Consortium (NEALS), and a reviewer for the Les Turner ALS Foundation's educational materials and programs. She was diagnosed with ALS in 2021. As a former elementary education teacher and licensed cosmetologist, she is proud to work as an ALS educator, advisor, and speaker because it allows her to be a voice for all ALS warriors and follow her lifelong passion for learning and educating others.

ALS Causative Mutations in KIF5A Disrupt Autoinhibition Leading to Toxic Gain of Function

Jonathan Brent[1], Han-Xiang Deng[1], Oliver Steling-Angus[1]

[1]*Department of Neurology, Feinberg School of Medicine, Northwestern University*

The objective of this study was to elucidate the pathogenic mechanisms of ALS causative mutations in motor protein KIF5A.

KIF5A is a neuronal specific subunit of Kinesin-1, which is a microtubule motor protein that plays roles in axonal transport and cytoskeletal regulation. Mutations in distinct regions of Kinesin-1 lead to a broad range of neurologic diseases. KIF5A enables Kinesin-1 to walk along microtubules and transport various cargos such as mitochondria and RNA granules into the distal axon. ALS-causative mutations in KIF5A affect the structure of the domain of the protein that functions in cargo binding and autoregulation. However, the consequences of ALS-causative mutations on disease pathogenesis are not fully understood. Our central hypothesis was that KIF5A ALS mutations disrupt cargo binding and/or autoregulation leading to neurodegeneration.

We generated in vitro models of KIF5A ALS using transient transfection of disease causative variants into neuroblastoma cell lines. We performed immunofluorescence staining and confocal imaging to visualize the distribution of motor proteins KIF5A, KIF5B, and Dynein. We utilized Drosophila S2 cell lines to study mitochondrial motility using live-imaging.

Whereas wild type (WT) KIF5A displayed relatively homogeneous distribution, ALS mutant KIF5A developed dramatic accumulation within distal neurites. Co-expression of the ALS mutant protein with the WT caused it to accumulate as well. Mitochondria displayed similar mislocalization to distal neurites. No change was seen in the distribution of KIF5B or Dynein.

We found that ALS mutant KIF5A caused dramatic accumulation of mutant and WT protein as well as mitochondrial within distal neurite tips. These findings suggest that ALS mutation disrupts its autoinhibition causing mislocalization of its cargos through gain of function. These changes in distribution are specific to KIF5A containing motors. Our findings establish dysregulation of KIF5A activity as the underlying pathogenic mechanism for KIF5A ALS.

Targeting Mis-splicing of UNC13A and KCNQ2 in ALS Using Spherical Nucleic Acids and Antisense Oligonucleotides

Wanhao Chi[1], Jungsoo Park[2], Chad A. Mirkin[2], Johnathan K. Watts[3]

[1]*Department of Neurology, Feinberg School of Medicine, Northwestern University*
[2]*Department of Materials Science and Engineering, International Institute for Nanotechnology, Department of Chemistry, Department of Biomedical Engineering, Northwestern University*
[3]*RNA Therapeutics Institute, University of Massachussetts Medical School*

ALS is a fatal neurodegenerative disease characterized by the loss of motor neurons in the brain and spinal cord. Although genetically diverse, more than 90% of ALS patients show TDP-43 cytoplasmic aggregation and nuclear depletion. Such TDP-43 neuropathology has also been found in other neurodegenerative diseases, including frontotemporal dementia (FTD) and Alzheimer's disease (AD). TDP-43 is a DNA/RNA binding protein regulating various aspects of RNA metabolism, including splicing. Several dozen genes have been found mis-spliced in ALS/FTD patient samples, including UNC13A and KCNQ2. UNC13A and KCNQ2 encode a synaptic protein and an ion channel, which are critical for the physiological functions of motor neurons. Upon TDP-43 nuclear depletion, a cryptic exon (between exons 21 and 22) in UNC13A is spliced in, and a coding exon (exon 5) in KCNQ2 is spliced out, which ultimately leads to reduced function of both genes because of non-sense mRNA-mediated decay for UNC13A and mis-localization for KCNQ2. Here, we set out to establish a cellular system to study these mis-splicing events using human induced pluripotent stem cell (hiPSC)-derived motor neurons treated with TDP-43 siRNAs. We confirm that upon TDP-43 knockdown, UNC13A and KCNQ2 are mis-spliced. We further design gene-specific antisense oligonucleotides (ASOs) to target the mis-splicing events in these two genes and screen splice- modulating ASOs using hiPSC-derived motor neurons. Lastly, we multiplex effective ASOs from each gene using spherical nucleic acids (SNAs) and deliver multiplexed SNAs to hiPSC-derived motor neurons to restore the function of UNC13A and KCNQ2. Together, this study establishes a cellular system to study TDP-43 nuclear depletion- induced mis-splicing, identifies effective splice-modulating ASOs for UNC13A and KCNQ2, and develops multiplexed SNAs to target two mis-splicing events upon TDP-43 nuclear depletion as a proof-of-concept experiment of targeting mis-splicing in ALS and other TDP-43 dysfunction-related diseases.

Loss of Rad23a Reduces mTDP43 Aggregates in an Inducible In Vitro Model

Casey Dalton[1], Xueshui Guo[1], Karen Ling[2], Paymaan Jafar-Nejad[2], Frank Rigo[2], Robert G. Kalb[1]

[1]*Department of Neurology, Feinberg School of Medicine, Northwestern University*
[2]*Core Antisense Research, Ionis Pharmaceuticals*

TAR DNA-binding protein 43 (TDP-43) is a major pathological protein in Amyotrophic Lateral Sclerosis (ALS). At autopsy, aggregated TDP-43 inclusions are found in the cytoplasm of >95% of ALS patients. TDP-43 likely contributes to pathophysiological events by both loss-of-function (i.e., nuclear depletion) and gain-of-function (i.e., cytoplasmic aggregation) mechanisms. Many missense mutations in TDP-43 have been determined to be causative of familial ALS (fALS). These mutations increase aggregation propensity, aggravate cytoplasmic mislocalization, disrupt endogenous interactions and influence stability leading to resistance to degradation. Due to its vital endogenous functions and auto-regulatory activities, direct depletion of TDP-43 is unlikely to lead to a promising therapy. Therefore, we have explored the utility of targeting modifier genes.

RAD23 functions as a shuttle factor that binds ubiquitinated substrates and presents them to the proteasome for degradation. Rad23 exists as a single isoform in Saccharomyces cerevisiae and Caenorhabditis elegans, and as two isoforms in vertebrates (rad23a and rad23b). We have previously shown that loss of rad23 improves motor phenotypes in two C. elegans models of fALS and that antisense oligonucleotide-mediated knockdown of rad23a blunts the disease in the Tar4/Tar4 mouse model of ALS. In order to better understand the mechanism through which these protective effects are occurring, we generated doxycycline-inducible hemagglutinin-tagged Q331K human TDP-43 (mTDP-43) expressing HEK293 cells. Upon doxycycline treatment and "SarkoSpin" subcellular fractionation, we find mTDP-43 in both the sarkosyl soluble and insoluble fractions. We find that knockdown of rad23a reduces the percentage of mTDP-43 that resides in the insoluble fraction. We plan to explore how knockdown of rad23a and rad23b effect other aspects of TDP-43 pathology and whether a similar affect can be seen when expressing doxycycline inducible WT-TDP-43.

Cell Type-Specific Alterations of the Excitability of Motor Cortical Pyramidal Neurons in a Model of Juvenile Onset ALS

Soumil Dey[1], Mukesh Gautam[2], Hande Ozdinler[2], Marco Martina[1]

[1]*Department of Neuroscience, Feinberg School of Medicine, Northwestern University*
[2]*Department of Neurology, Feinberg School of Medicine, Northwestern University*

Amyotrophic lateral sclerosis (ALS) severely impacts both upper and lower motoneurons. Several recent papers show that, in ALS, dysfunction of upper motor neurons is a major feature of the disease and that cortical neuronal hyperexcitability is a main contributor to this dysfunction, although the mechanisms of hyperexcitability remain unclear. Mutations in the Alsin gene are a causative factor for juvenile-onset amyotrophic lateral sclerosis and hereditary spastic paraplegia. In the alsin knock-out (AlsinKO) mouse model, cortico-spinal motor neurons (CSMN) show apical dendrite degeneration and deformation in mitochondria and Golgi apparatus, but nothing is known about their electrophysiological phenotype. In the present study, we examined the electrophysiological features of layer 5 CSMNs in AlsinKO mice at a pre-symptomatic time point (4-6 weeks-old mice). To this end, we took advantage of a CSMN reporter line (UCHL1-UeGFP) in the AlsinKO background where GFP expression in the motor cortex is restricted to CSMNs, enabling visualization of these neurons in acute cortical slices. Surprisingly, patch clamp recordings showed that CSMNs from the AlsinKO mice showed significantly less intrinsic excitability compared to wild-type counterpart, as their I/O curve was shifted to the right by ~200 pA. Accordingly, the action potential threshold was considerably more positive in AlsinKO mice. Interestingly, non-CSMN pyramidal neurons of the AlsinKO mice also showed a right shift of the input-output curve compared to wild-type counterpart, but this shift was considerably smaller (~120 pA). The significance of our results is three-fold. First, they show that long before the onset of behavioral symptoms, excitability of M1 pyramidal neurons is severely affected, suggesting a potential target for pharmacological modulation. Second, they show an unexpected decrease in intrinsic excitability, possibly as a consequence of homeostatic adaptations. Third, they show intriguing cell-selectivity in the effects of the alsin mutation, suggesting that these effects are regulated by the selective expression of specific ion in each neuronal cell type.

An Interactive, Online, Informed Decision-Making Tool for Genetic Counseling & Genetic Testing in ALS

Anne Marie Doyle[1], Lauren Webb[1], Laynie Dratch[2], Lisa Kinsley[3], Jennifer Roggenbuck[4]

[1]*Les Turner ALS Foundation*
[2]*University of Pennsylvania*
[3]*Northwestern University Feinberg School of Medicine*
[4]*The Ohio State University Wexner Medical Center*

Genetic counseling and testing are now critical components of Amyotrophic Lateral Sclerosis (ALS) care, with the first FDA approved genetic therapy and the growing number of gene-targeted trials in the clinical pipeline. New evidence-based, consensus guidelines for genetic counseling and testing in ALS assert that everyone with ALS should be offered genetic testing. This aligns with The Morris ALS Principles, which state that people with ALS should be treated as informed partners in their care, and everyone with ALS should be given options for genetic counseling and testing. However, navigating the many implications of genetic testing can be daunting for patients and families, especially those with limited health literacy. Furthermore, genetic counseling is a limited resource, thus, alternative service delivery models or supplemental education methods must be explored.

The Les Turner ALS Foundation drew upon international resources and research to develop a decision tool that uses plain language to introduce key concepts related to genes, genetic testing, and ALS research. The genetic module for My ALS Decision Tool™ explains how genetic testing works, guides users through the benefits and downsides of genetic testing, explores the emotional challenges that may come with genetic testing, and describes what people living with ALS can learn from genetic testing, and why this information matters.

The goal of the tool is to educate and empower people living with ALS and their care partners so that they feel confident in their decision-making about genetic testing. We feel the use of this tool may improve health outcomes and both reduce inequities in health care and caregiver burden throughout the ALS journey by empowering people with ALS to make early and informed decisions about their care. This tool fills a gap in existing resources with convenient, understandable educational support. We plan to assess the tool's usability through web analytics and user feedback, and its utility through pre and post-use outcome measures such as empowerment.

Find the new My ALS Decision ToolTM on Genetic Testing, along with modules on breathing and nutrition and other educational resources, at lesturnerals.org

High Density Multi-Electrode Array Recordings Help Investigate the Upper Motor Neuron Health and Connectivity in ALS

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Cortical hyperexcitation is one of the underlying causes of upper motor neuron (UMN) vulnerability in amyotrophic lateral sclerosis (ALS), and early cortical connectivity defects contribute to disease pathology. However, the exact electrophysiological patterns of diseased neurons are not fully understood. Here, we use a high-density multi-electrode array to investigate neuronal firing patterns, network bursts, and connectivity within the context of both health and disease. We utilize well-characterized ALS mouse models that are developed based on mutations detected in ALS patients, and that display progressive UMN loss. Crossing hSOD1G93A, hPFN1G118V and TDP-43A315T mouse models, with UCHL1-eGFP mice helped generate ALS disease models in which UMNs are genetically labeled with eGFP, so that multi-array electrophysiological recordings can be performed with cellular precision and resolution, which was not possible before.

Presently, either mixed cortical cultures are established, or acute brain slices are prepared on the high-density multi-electrode arrays (HD-MEAs), which have 4096 electrodes arranged in a 64x64 grid on a complementary Metal–Oxide Semiconductor chip. Active Pixel Sensor technology enables 3-5 min recordings controlled by the BrainWave 5 software (3-Brain) at a sampling rate of 10kHz. Raw voltage traces are analyzed to detect and sort spike events, performed with the Precise Timing Spike Detection algorithm at a differential threshold set to 10 times the standard deviation followed by spike sorting via principal component analysis. Network burst detection is then performed using either a rate-threshold or recruitment threshold to identify large scale synchronization and synaptic connectivity. Further network analysis is carried out in MATLAB to identify patterns of functional connectivity. These metrics reveal key differences in the firing rates, signal propagation, and network dynamics of upper motor neurons at both cellular and systems level.

Our ongoing studies investigate the electrophysiological properties of cortical neurons and how they may be altered in different animal models of UMN disease. After understanding the alterations that occur during disease, and how such alterations relate to disease onset and progression, the data generated from high-density microelectrode arrays can be used as a marker in the drug discovery process. This will help identify compounds that contribute to functional improvement and reveal novel treatment strategies for UMN disease.

Defining the Mechanisms by Which Mutations in DNAJC7 Increase Susceptibility to ALS/FTD

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The accumulation of insoluble and misfolded proteins is commonly associated with degeneration of neurons in amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) patients. Heat shock proteins (HSPs) play a central role in the regulation of protein homeostasis by facilitating effective folding, trafficking, and degradation of both nascent and aged polypeptides. While it has become increasingly clear that perturbations in in the proteostasis network play a significant role in ALS/FTD, limited emphasis has been placed on investigating the direct causal relationship between the functionality of HSPs and disease pathogenesis. Heterozygous, loss-of-function mutations in the DNAJC7 gene, which encodes for the HSP40 protein DNAJC7 have recently been identified as a cause for rare forms of ALS. The DNAJC7 protein acts as a co-chaperone for HSP70 chaperones, thereby facilitating HSP70-polypeptide interactions and appropriate polypeptide folding. However, little is known about the specific function of DNAJC7 in the central nervous system and motor neurons specifically, the cell type that predominantly degenerates in ALS patients. Our primary hypothesis is that DNAJC7 haploinsufficiency leads to the accumulation of misfolded HSP70 client proteins resulting in the disruption of biological processes critical to the function and survival of vulnerable MNs. Here, we used mutant DNAJC7 cellular models, patient induced pluripotent stem cell (iPSC)-derived spinal motor neurons (MNS) and CRISPR/Cas9 gene-editing, in combination with mass spectrometry (MS)-based quantitative proteomics and RNA-Sequencing to elucidate how ALS/FTD-associated mutations in DNAJC7 contribute towards neuronal dysfunction and degeneration.

Developing A Semi-high Throughput Platform to Advance Drug Discovery Efforts for Upper Motor Neuron Diseases

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Even though ALS is defined as degeneration of both upper and lower motor neurons, currently no preclinical drug discovery platforms use upper motor neuron (UMN) health as a readout. We developed UeGFP mice, a reporter line for UMNs. Crossing UeGFP to well-characterized ALS mouse models that recapitulate different disease pathologies in patients, helped develop UMN model systems that degenerate due to different underlying causes. Therefore, we develop a cell-based and mechanism-focused drug discovery platform that utilizes the health of diseased UMNS as a readout. Since translation is at a cellular level, our goal is to identify compounds that would be effective in patients.

Dissociated cortical cells isolated from postnatal-day 3 (P3) of control (UeGFP) and diseased (hSOD1-UeGFP, PFN-UeGFP, TDP-UeGFP) pups are plated on glass bottom 96-well plates with a density of 16,000 cells/well and cultured either in the presence of serum free medium or with compounds of interest for 3 days. They are fixed and subjected to immunocytochemistry to better visualize UMNs, astrocytes, and microglia. High-throughput imaging, cellular quantification and assessment of cellular processes are used to determine changes in UMN health upon treatment.

UMNs are distinguished from other cells/neurons by their eGFP expression. UMNs become diseased due to different underlying causes in the SOD1, TDP and the PFN models. Yet, even at P3, they display a common cellular pathology: shorter axons and reduced branching and arborization. Importantly, they respond to compound treatment by extending their axon and by improving branching and arborization. High-throughput imaging and analyses help determine total cell numbers, the total neurite length, total branch points, per well and per condition. We assess the impact of previously identified FDA drugs on the health of UMNs that are diseased due to different underlying causes.

Being able to distinguish UMNs in the tissue culture allow developing a cell-based drug discovery platform, focusing on the responses of UMNs with a cellular precision. Having UMNs that are diseased with different underlying causes and pathologies, help develop a mechanism-focused approach, so that compounds or combination of compounds that are most effective for a given cause can be determined.

RAD23 Enhances the Degradation of Proteins That Cause Familial Amyotrophic Lateral Sclerosis (ALS)

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Accumulation of aggregated disease-proteins in neurons is a cardinal feature of neurodegenerative disease such as ALS and is direct evidence for abnormal protein homeostasis. Enhanced degradation of disease proteins is a promising strategy for re-establishment of proteostasis and neuronal health. RAD23 was originally identified in a screen for proteins in yeast required for resistance to the toxicity of ultraviolet radiation and subsequently discovered to be a shuttle factor that presents ubiquitinated clients to the proteasome for degradation. Mammals have two orthologs of RAD23, rad23a and rad23b, that have similar overall structure. They have both redundant as well as unique cell biological activities. To determine if either protein influences a familial ALS causing protein, we expressed G85R superoxide dismutase (SOD) in primary rat cortical neuron cultures (lacking astrocytes) with or without RAD23A. We find that the overall abundance of mutant SOD (mSOD) is significantly reduced by co-expression of RAD23A. We hypothesized that RAD23A reduces the abundance of mSOD by accelerating its degradation. To test this idea, we co-incubated mSOD + RAD23A with the proteasome inhibitor MG132 or the autophagy inhibitor bafilomycin A1. Each inhibitor partially reverses the effect of RAD23A on mSOD abundance. These results suggest that RAD23A promotes degradation of mSOD by both the proteasome and autophagy. Going forward we will determine whether RAD23B has a similar effect on mSOD abundance and whether the effects of RAD23 are also seen with another fALS causing protein, TDP43. The identification of factors that reduce the abundance of toxic proteins in ALS may have therapeutic implications.

Ambient Glutamate and Motoneuronal Excitability: the Role of System xc- in ALS Pathogenesis

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Glutamate excitotoxicity is canonically viewed as an important mediator in the pathogenesis of Amyotrophic Lateral Sclerosis (ALS). In both human and animal studies, neural networks exhibit elevated extracellular glutamate concentrations coupled with hyperexcitability which ultimately begets denervation. Not surprisingly, the contribution of synaptic glutamate has been heavily scrutinized in ALS etiology; the role of ambient (tonic), extrasynaptic glutamate, however, has yet to be examined in this regard. In the central nervous system (CNS) ambient glutamate is supplied by the cystine/glutamate antiporter, system xc-, which imports cystine from the extracellular compartment and exports glutamate in exchange. Importantly, system xc-, with specific protein subunit xCT, is markedly upregulated in both animal ALS models and human patients. Remarkably, transgenic mice lacking a functional system xc- (xCT-/- mice) exhibit extended lifespan as well as 60-80% lower ambient glutamate concentrations when compared to wild-type (WT) controls. In contrast, SOD1G93A mice, a transgenic model for ALS pathology, display truncated lifespan, enhanced system xc- activity, and elevated ambient glutamate concentrations. In this study, we explored the role of ambient, extrasynaptic glutamate in ALS by assessing the antiporter's contribution to the deleterious hyperexcitability of motoneurons. Thus, we employed a novel in vitro spinal cord preparation to electrophysiologically measure motoneuronal excitability in xCT-/-, WT, and SOD1G93A mice. Additionally, cerebral spinal fluid (CSF) was sampled from each genotype, and glutamate concentrations were analyzed using mass spectrometry. Our results revealed decreased motoneuronal excitability in xCT-/- mice, as evidenced by enhanced short-term depression (STD), when compared to WT and SOD1G93A counterparts. Contrarily, SOD1G93A mice exhibited attenuated STD as compared to xCT-/- and WT mice, thus revealing increased motoneuronal excitability. Furthermore, the decreased excitability in xCT-/- mice was concomitant to reduced CSF glutamate levels, whereas the increased excitability in SOD1G93A mice was attendant to elevated glutamate levels. These preliminary data suggest that ALS-induced system xc- upregulation, and the obligate release of ambient glutamate, drive the motoneuronal hyperexcitability which leads to degeneration. Future experiments will i) confirm whether pharmacological antagonism of system xc- can mitigate motoneuronal hyperexcitability and ii) probe the mechanism by which ambient glutamate alters cellular properties in the disease. Collectively, our data portend a novel therapeutic intervention which may ameliorate the noxious effects of glutamate excitotoxicity in ALS thereby attenuating or precluding motoneuronal dysfunction.

TDP-43 Protein Interactome Informs about Perturbed Canonical Pathways and May Help Develop Personalized Medicine Approaches for Patients with TDP-43 Pathology

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TDP-43 is a DNA/RNA binding protein that is implicated in key cellular events. It has numerous domains with distinct functions. TDP-43 pathology is one of the most broadly observed proteinopathies in amyotrophic lateral sclerosis (ALS) and is defined by protein aggregations, including phosphorylated TDP-43. It is important to identify the correlation between protein-protein interactome activity with its key functions and how these protein interactions contribute to disease pathology.

To identify the location of the interaction sites of these proteins, High Ambiguity Driven protein–protein Docking (HADDOCK) software was utilized. This program takes input from the two protein structures and analyzes different potential conformations for protein interaction. The output is the lowest possible energy interaction dimer. This newly formed dimer complex is then analyzed to determine the locations of interaction between TDP-43 and binding partners. After repetitive analysis with TDP-43 and all of its known binding partners, the amino acid residues that interact in each protein pairing is assessed. The interactome data is utilized to visualize which of the binding partners interact within the established domains of TDP-43. The resulting proteins predicted to be interacting within each domain underwent computational analysis via Ingenuity Pathways Analysis (IPA) to highlight the associated canonical pathways, upstream and downstream regulators, and significantly affected cellular pathways.

The recurrent interaction residues of TDP-43 with binding partners represent the key areas within the amino acid sequence that will have the greatest impact on functionality if inactivated by mutations or otherwise. Additionally, the predicted binding partners within each of the established domains help predict druggable domains as well as the functionality and downstream effects of each of the domains within TDP-43.

Our analysis represents a novel computational approach to protein-protein interaction analysis, which provides data on the downstream cellular events associated with the binding partners of TDP-43 and its key functionally active domains. The specific domain "mapping" also provides a potential avenue for the identification of key locations for drug development. In addition, these studies will help understand why mutations in different regions lead to specific pathologies, and how more personalized treatment strategies can be developed.

Modeling Physiologic Metabolism in iPSC-Derived Motor Neurons

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Amyotrophic Lateral Sclerosis (ALS) is a debilitating neurodegenerative disease which selectively targets upper and lower motor neurons (MNs) of the brain and spinal cord. Mitochondria play a central role in MN biology by facilitating cellular energy generation though the catabolic production of ATP, and the anabolic generation of metabolites and macromolecules. Mitochondrial dysfunction and oxidative stress are commonly associated with the selective degeneration of ALS MNs. However limited emphasis has been placed on accurately modeling human MN metabolism in vitro, and thus investigating the causal relationship between mitochondrial metabolism and disease pathogenesis. Traditional cell culture media utilized to maintain neurons is specifically formulated to reduce metabolic stress, and is not physiologic. Little is known about how a physiologic metabolic milieu affects MN mitochondrial metabolism, and how mitochondria in this context contribute to disease. Here, we aim to address this fundamental limitation by utilizing Human Plasma-Like Media (HPLM), which closely approximates the extracellular metabolite composition of human plasma & cerebrospinal fluid. In preliminary experiments, we find HPLM can support iPSC-derived MNs and the maintenance of MN cell identity. Using mass spectrometry analysis, we show HPLM remodels the intracellular metabolome of healthy MNs and increases the presence of mitochondrial metabolites. Additionally, ALS patient-derived MNs exhibit an increased sensitivity to oxidative cell death in HPLM. The capacity of HPLM to alter MN metabolism renders it a promising platform for further investigation into novel metabolic mechanisms of MN vulnerability in ALS.

Neurofilament as a Marker of Motor Neuron Death in an In-Vitro Stretch Injury Model

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Neurofilament is a useful clinical biomarker of neurotrauma and neurodegenerative diseases since it reflects cytoskeleton pathology. Recent work in the field of amyotrophic lateral sclerosis (ALS) demonstrates that systemic neurofilament levels rise with disease progression and motor neuron loss. After spinal cord injury, spinal motor neuron loss can occur but is highly variable from one patient to the next. We hypothesize that the clinical biomarker, neurofilament, may have utility for early detection of spinal motor neuron loss after traumatic injury. We applied traumatic injuries to human stem cell-derived motor neurons to test this hypothesis using an in vitro stretch injury model. We manipulated the extent of biaxial strain and genotype of the motor neurons to determine if neurofilament levels could detect different degrees of motor neuron degeneration after injury.

We hypothesize that this mutation will reduce the level of neuroprotection through a decrease in the release of BDNF. We will quantify this by measuring the release of neurofilament heavy-chain in an in-vitro stretch injury model.

Two independent sets of isogenic human induced pluripotent stem cells (hiPSC) lines were generated using CRISPR-Cas9 consisting of the val and met genotype of the val66met SNP. These were differentiated into motor neurons following an established differentiation protocol. Flexible 96-well plates were used to perform in-vitro stretch injury. Cell viability was evaluated using lactate dehydrogenase assay (Promega) post-injury. We quantified neurofilament heavy-chain via ELISA assay (Novus Biologicals). Images were taken via Leica LASX Inverted Fluorescent Microscope, and analyzed using NIS Elements software.

We have related the degree of in-vitro stretch injury to motor neuron viability by quantifying an increase in lactate dehydrogenase and neurofilament release in supernatant media over four different injury displacements. Imaging analysis consisted of neurite length and number of Islet ½ positive motor neuron nuclei per field, where we observed a decrease with greater displacements.

Previous studies have demonstrated impaired BDNF release in the met genotype. As neurofilament heavy chain secretion is used as a clinical marker for injury in conditions such as ALS and other neurodegenerative diseases, we hope to delineate it as a marker of motor neuron death in in-vitro studies. The release of lactate dehydrogenase, a cellular feature of damage and toxicity, validates our injury models.

NU-9, a Novel Small-Molecule Inhibitor of Amyloid Beta Oligomers, Rescues Reactive Astrogliosis in Pre-symptomatic 5xFAD Mice

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It is well established that Amyloid beta oligomers (AβOs) are the most neurotoxic form of amyloid beta (Aβ), yet failed clinical trials of fibril-selective Aβ drugs have led some to doubt Aβ as an Alzheimer's disease (AD) target. Failure in treatments that neutralize non-toxic fibril Aβ, however, should not undermine the potential of treatments aimed at neutralizing toxic AβOs. Indeed, an analysis of recent Aβ-targeting drugs in clinical trials showed that a patient's improvement in cognition strongly correlated with their drug's selectivity for AβOs. Our lab recently discovered that the novel small molecule compound NU-9, originally identified for its ability to inhibit SOD1 aggregation in ALS mice, also prevents the formation of AβOs from Aβ monomer in vitro. In order to test the compound in vivo, we administered NU-9 orally to 5xFAD mice for 60 days and observed neuropathological changes. As increasing evidence has implicated AβOs in the induction of gliosis and inflammation, now considered primary pathogenic events in AD, we were particularly interested in NU-9's effect on these phenomena. Using super resolution microscopy, we observed one AβO proteoform, probed by the conformationally selective clinical trial drug ACU193, to bind in abundance along reactive astrocyte processes. As this AβO proteoform was reduced by NU-9 in vitro, we were especially intrigued at NU-9's potential impact on reactive astrocyte levels. Remarkably, 5xFAD mice given both 20 mg/kg and 100 mg/kg treatment of NU-9 had a 3-fold decrease in reactive astrocyte levels from controls, suggesting that the AβO proteoform bound along reactive astrocyte processes may also induce their activation. Taken together, we present evidence of a reactive astrogliosis-inducing AβO proteoform and introduce NU-9 as a novel small molecule inhibitor of AβOs with great therapeutic potential for the treatment and prevention of AD.

Loss of Function of the ALS-Associated NEK1 Kinase Disrupts Microtubule Homeostasis and Nuclear Import

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Loss-of-function variants in NIMA-related kinase 1 (NEK1) constitute a major genetic cause of amyotrophic lateral sclerosis (ALS), accounting for 2 to 3% of all cases. However, how NEK1 mutations cause motor neuron (MN) dysfunction is unknown. Using mass spectrometry analyses for NEK1 interactors and NEK1-dependent expression changes, we find functional enrichment for proteins involved in the microtubule cytoskeleton and nucleocytoplasmic transport. We show that α-tubulin and importin-β1, two key proteins involved in these processes, are phosphorylated by NEK1 in vitro. NEK1 is essential for motor control and survival in Drosophila models in vivo, while using several induced pluripotent stem cell (iPSC)–MN models, including NEK1 knockdown, kinase inhibition, and a patient mutation, we find evidence for disruptions in microtubule homeostasis and nuclear import. Notably, stabilizing microtubules with two distinct classes of drugs restored NEK1-dependent deficits in both pathways. The capacity of NEK1 to modulate these processes that are critically involved in ALS pathophysiology renders this kinase a formidable therapeutic candidate.

Traumatic Injury Causes Selective Degeneration and TDP-43 Mislocalization in iPSC-derived C9orf72-associated ALS/FTD Motor Neurons

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A hexanucleotide repeat expansion (HRE) in C9orf72 is the most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). However, the penetrance of the repeat expansion is incomplete, suggesting an interplay between genetics and environmental stressors. Neurotrauma as a result of traumatic brain or spinal cord injury has been shown to increase the risk of ALS/FTD in epidemiological studies. Here, we combine patient-specific induced pluripotent stem cells (iPSCs) with a custom-built device to deliver biofidelic stretch trauma to C9orf72 patient and isogenic control motor neurons (MNs) in vitro. We find that mutant MNs exhibit selective degeneration after a single incident of severe trauma, which can be partially rescued by pretreatment with a C9orf72 antisense oligonucleotide (ASO). A single incident of mild trauma does not cause degeneration but leads to cytoplasmic accumulation of TDP-43 in C9orf72 MNs. This mislocalization, which only occurs briefly in isogenic controls, is restored in mutant MNs after 6 days. Lastly, repeated mild trauma ablates the ability of patient MNs to recover. These findings highlight alterations in TDP-43 dynamics in C9orf72 patient MNs following traumatic injury and demonstrate that neurotrauma compounds neuropathology in C9orf72 ALS/FTD. More broadly, our work establishes an in vitro platform that can be used to interrogate the mechanistic interactions between ALS/FTD and neurotrauma.

The Role of Aurora B Kinase in the Development of Neurodegenerative Phenotypes in ALS

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that affects the upper and lower motor neurons. There is evidence that nuclear dysfunction in motor neurons occurs in ALS, leading to a decrease in cellular health. However, it is unknown what causes this nuclear dysfunction to develop and how it contributes to disease pathogenesis. While the cause of ALS is unknown, the most common genetic risk factor for ALS is an expansion of a hexanucleotide repeat in the chromosome 9 open reading frame 72 (C9orf72) gene. This repeat expansion leads to the expression and accumulation of 5 different dipeptide repeat proteins; however, it is unknown how these dipeptide repeats contribute to disease pathogenesis. Our data shows that two of the C9orf72 dipeptide repeats, poly-RP and poly-RG, are cytotoxic when expressed in neuronal cells, producing nuclear pathological phenotypes. To explore a potential mechanism underlying the development of these pathologies, we focused on a signaling pathway that is critical for regulating nuclear processes. Aurora B kinase is a serine/threonine kinase that localizes to chromosomes and has been reported to play an important role in both mitosis and cytokinesis. Based on its predominantly nuclear localization and functions, our hypothesis is that an impairment in Aurora B kinase activation contributes to the development of nuclear pathologies associated with the poly-RP and poly-RG dipeptides. To address this hypothesis, we transfected rat primary cortical neurons with expression plasmids containing the dipeptides. We found that there are alterations in Aurora B kinase levels in cells expressing the poly-RP and poly-RG dipeptides, specifically a decrease in cells expressing the poly-RP dipeptide and an increase in cells expressing the poly-RG dipeptide. Our findings indicate that alterations in Aurora B kinase may be involved in the development of pathological phenotypes in C9orf72-associated ALS, perhaps establishing an overall mechanism of neurodegeneration.

p38-Alpha Contribution to the Pathogenesis of Amyotrophic Lateral Sclerosis

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Axonal degeneration represents an early and critical pre-symptomatic event in both sporadic and familial forms of Amyotrophic Lateral Sclerosis (ALS), but the underlying mechanisms remain unknown. The development of ALS models, most notably transgenic SOD1-G93A mice, revealed effects of mutant, misfolded superoxide dismutase 1 (SOD1) that long precede axonal pathology, including aberrant activation of p38 mitogen-activated protein kinases (MAPK) and inhibition of fast axonal transport. Despite this knowledge, the contribution of specific p38 kinase isoforms to MN axonopathy has not yet been evaluated.

In this work, we assessed the contribution of p38, the most abundant p38 MAPK isoform expressed in neurons, to major neuropathological features of mSOD-G93A mice including locomotor deficits, axonal pathology, and aberrant glial cell activation. Towards this, we performed genetic experiments involving YFP-SOD1-G93A and p38 αAF/WT knock-in mice, where a mutant MAPK14 allele encodes a non-activatable version of p38α. These studies uncovered a delay in locomotor deficits and reduced degeneration of spinal cord axons in mice featuring mSOD-G93A expression and attenuated of p38 signaling. Interestingly, glial activation was unaffected in these mice, suggesting the beneficial effect of p38 attenuation may selectively impact neurons. Collectively, our findings reveal a significant contribution of p38 to mutant SOD1-induced axonal pathology, suggesting this kinase might represent a relevant therapeutic target to treat ALS.

Investigating the Impact ALS and Neonatal Epilepsy Causative KIF5A Mutations on Intracellular Trafficking and Cytoskeletal Structure

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KIF5A, a member of the kinesin superfamily, is a neuron-specific motor protein with mutations associated with several neurodegenerative and neurodevelopmental diseases. Of interest, are Amyotrophic Lateral Sclerosis (ALS), a progressive motor neuron disease, and Neonatal Intractable Myoclonus (NEIMY), a severe form of neonatal epilepsy. Despite their diverse clinical phenotypes, both mutations occur in a similar location of the C-Terminal domain, the area associated with cargo binding and the protein's autoinhibition. We developed in vitro models of ALS and NEIMY KIF5A variants to investigate their impact on the cytoskeleton and cargo transport. Our results suggest that both mutations disrupt the protein's autoregulation, causing protein accumulation in the tip of the axon. The phenotype of the NEIMY variant is far more severe, causing the formation of large aggregates towards the axon tip and complete depletion of the protein from the soma. These aggregates appear to sequester Kinesin Light Chain-1 (KLC1), indicating the full KIF5A complex is affected, but obstruct KIF5A cargo such as lysosomes and mitochondria. The NEIMY aggregates disrupt the cytoskeletal structure by creating a physical blockage in the axon. This causes a gap in the cytoskeleton and hinders cargo transport to the distal axon. Our findings indicate the primary mechanisms of the both diseases being gain of function, but truncating the protein at the NEIMY mutation site causes only partial suppression of the phenotype, implicating loss of function as a secondary mechanism of action. Overall, our findings further reveal the underlying mechanisms of the clinically divergent syndromes ALS and NEIMY.

Moving Forward After Loss: Support for Those that Have Lost Their Partners to ALS

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The Les Turner ALS Foundation has provided educational and social support groups for people living with Amyotrophic Lateral Sclerosis (ALS) as well as their spouses, caregivers, families, and friends for many years. What had not been in place was a formal, therapeutic bereavement group that provided support to those who lost their loved ones to ALS. Due to the required social distancing during the COVID-19 pandemic, people had to grieve while in isolation, resulting in further feelings of emotional distance from family and friends, a lack of understanding and/or acceptance by others as being a person in mourning, and the development of complicated, unresolved grief. The Foundation realized there was an opportunity to expand our services in a meaningful way for a population of grievers who were isolated.

In August 2020, the Foundation started Moving Forward After Loss, a partner bereavement group, as this relationship is most directly impacted by loss. This 90-minute, virtual, six-week series was led by a member of our Support Services Team. The group followed a therapeutic curriculum focusing on topics like, "What is Grief? What is mourning?" On average five to eight participants attended weekly; an optimal size for creating an intimate and safe experience. The intention of the group was to provide people with education about grief along with coping techniques.

Upon completion of the series, participants filled out a survey to describe their level of satisfaction with the group. A majority of respondents stated they were more aware of their feelings and experiences of grief, had tools to move through their grief, and would recommend this group to others. All respondents said that the group met their expectation. Forty-five people who have lost a partner to ALS have been supported from August 2020-August 2023.

The bereavement process is not linear. Nevertheless, the unanimous satisfaction with the group and the general trends towards accepting loss demonstrate the significant role in supporting people during their grief process. By providing education and validation in a supportive setting, we have provided the space to help people to move forward in their grief while still honoring the ones that they have lost.

Cell Biology of Dipeptide Repeat Protein Cell-Cell Transmission

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C9orf72 ALS is the most common monogenic form of ALS, resulting from a GGGGCC hexanucleotide repeat expansion in the C9orf72 gene. One proposed mechanism of disease is a toxic gain-of-function from dipeptide repeat proteins (DPRs) which are products of repeat-associated non-AUG translation. DPRs have been shown in vitro to participate in a neurodegenerative disease spreading mechanism termed cell-cell transmission, yet the mechanism of how DPRs can spread remains incompletely understood. We utilized a culture technique, with donor and receiver neurons, to demonstrate cell-cell transmission of one of the toxic arginine-rich DPRs, poly-PR, in primary rat hippocampal neurons. To understand how poly-PR is internalized into cells, we used a simplified in vitro method in which we bath applied 2μM synthetic hemagglutinin, HA, tagged PR of twenty repeats, HA-PR20, to both HeLa cells and primary rat mixed spinal cord cultures. HA-PR20 was rapidly internalized and localized to the nucleolus as soon as 30 minutes in both cell types. We applied several endocytosis inhibitors to demonstrate that poly-PR internalization is an active process accomplished through endocytosis. Poly-PR endocytosis was potently inhibited by temperature shifts, clathrin-mediated inhibitors, dynamin inhibitors, and macropinocytosis inhibitors. To gain further insight into the mechanism of poly-PR uptake, we will use a proximity labeling method followed by mass spectrometry to identify proteins within 1 nm of poly-PR at the cell surface. We will use RNA interference techniques to knock down proteins of interest and determine their importance in poly-PR uptake. We hypothesize that these identified proteins could be important for the internalization of toxic DPRs and targeting them could prevent intercellular transmission and slow disease progression.

A Novel Mouse Model to Reveal the Role and the Impact of Neuroimmune Modulation in ALS with TDP-43 Pathology

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Motor neuron loss is associated with neuroinflammation and accumulation of protein aggregation in amyotrophic lateral sclerosis (ALS). TDP-43 pathology is one of the most common forms of protein aggregation and the contribution of neuroinflammation is well-documented. However, we lack cell-based model systems that would bring cellular clarity and precision to our understanding for the mode of interaction, site of initiation and the underlying molecular basis of neuronal degeneration. To investigate the contribution of neuroimmune modulation in ALS pathogenesis, we generated a novel mouse model, prpTDP-43A315T-MCP1-CCR2, by crossing prpTDP-43A315Tmice with MCP1-CCR2 reporter line, in which cells expressing MCP1 (monocyte chemoattractant protein-1) and CCR2 (CC chemokine receptor 2) are genetically labeled by monomeric red fluorescent protein-1 and enhanced green fluorescent protein, respectively. Therefore, cells that are responsible for the initiation of innate immunity are fluorescent in one of the best-characterized mouse models of TDP-43 pathology, and can be traced, isolated and assessed with cellular precision. To investigate the interplay between the peripheral and the central nervous system, we studied both the peripheral immune system organs, including spleen, thymus and inguinal lymph nodes, as well as the brain and the spinal cord of prpTDP-43A315T-MCP1-CCR2 mice at early symptomatic (P60) and late symptomatic (P100) stages. MCP1-CCR2 mice are used as healthy controls. Our initial investigations suggest an overall increase in the levels of CCR2+ cells around the blood vessels and meninges, suggesting a leakage from the BBB in the presence of TDP43 pathology, and robust infiltration of neuroimmune cells to the brain parenchyma early in the disease. Infiltrating cells were in close vicinity with Iba1+ microglial cells and GFAP+ astrocytes, which are present at high levels in layer V of the motor cortex of prpTDP-43A315T-MCP1-CCR2 mice. Our observations suggest a utility for this novel mouse model, as it allows visualization and cellular assessment of neuroimmune axis within the context of TDP-43 pathology in both the CNS and the periphery. Since translation is at the cellular level, MCP1+ and CCR2+ cells in this novel TDP-43 model will shed light onto the cellular mechanism responsible for the initiation and progression of TDP-43 pathology and will help develop therapeutic approaches related to neuroimmune modulation in ALS.

Development of In Vitro Human Stem Cell Model to Study Motor Neuron Degeneration after Mechanical Injury

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Spinal cord injury (SCI) is a major cause of disability with uncertain prognosis and limited treatment options. Many SCI treatments are dependent on intact lower motor neurons (MNs) such as diaphragm pacing, nerve transfers, and functional electrical stimulation to name a few. However, clinical studies have shown that the extent of MN loss after human spinal cord injury is highly variable, and often affects multiple spinal segments above and below the center of the lesion and is detectable in about 80% of patients by clinical electrophysiology. About half of affected SCI patients have severe MN loss. This variability complicates the search for optimal treatments and may be influenced by factors such as injury location, severity, age, and genetics. In a traumatic SCI event, mechanical forces dislocate or fracture the vertebrae, leading to compression of the spinal cord due to misalignment of the vertebral canal.

To simulate this event in vitro, we cultured spinal cord organoids from HB9-GFP expressing (fluorescent motor neuron reporter) human embryonic stem cells following an established protocol. Then, we used a custom-built machine driven by an electromagnetic voice coil to compress these organoids to 65% of their initial height and release them in a period of 30 milliseconds. We collected culture media two hours after injury to quantify injury phenotype using to measure cell death biomarkers (lactate dehydrogenase and neurofilament light chain).

Results: Our cell death biomarker assays demonstrated significantly elevated levels of lactate dehydrogenase and neurofilament light chain levels after 65% compression injury to human spinal organoids compared to sham controls. Still underway, we are quantifying the extent of motor neuron degeneration by quantification of HB9-GFP and other MN markers.

This model provides an opportunity to investigate the influence of patient genotype on SCI motor neuron death, with implications for predicting neurotrauma patient prognosis and treatment options for better quality of life. Future work will involve using this paradigm to study genetic factors that modify neurotrauma outcomes as well as screen drug candidates for neuroprotection.

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Optimizing Wireless Dual-Site Electrical Stimulation for Enhanced Axon Regeneration and Functional Recovery in Peripheral Nerve Injuries

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Therapeutic electrical stimulation (TES) of peripheral nerves has been shown to enhance axon regeneration in clinical and preclinical studies. Recent TES protocols have shown that stimulation delivered proximal or distal to the nerve repair site has separate and distinct benefits on regeneration and functional recovery. Here we test the hypothesis that combining TES at these two sites will have an additive benefit. Prior work from our group demonstrates that six, consecutive daily bouts (1-hour) of TES is superior to the current clinical standard of a single 1-hour treatment so we required an implantable nerve stimulator. To accomplish this, we developed novel wireless, battery-free, and fully implantable peripheral nerve interface with dual nerve cuffs for multisite TES.

To enhance peripheral nerve regeneration with optimized TES protocol enabled by a novel dual-site wireless, battery-free resorbable implant made from advanced materials.

We used an electrical stimulation protocol (1 hour, 20 Hz) previously shown to enhance peripheral axon regeneration, and we applied it to a rodent sciatic nerve model. Stimulation was delivered both distally (on the sciatic nerve) and proximally (on the tibial nerve) from the point of transection in the dual stimulation model. A proximal-only, distal-only, and sham device model were also used to determine the effectiveness of the combined treatment.

At an early regenerative time point (two weeks), the dual model showed an additive effect of the combined treatment through delayed muscle atrophy. At six weeks, both dual and proximal outperformed the sham model when measuring muscle re-innervation and atrophy, suggesting effectiveness of the device and the dual treatment.

Our data suggests there is additive benefit to the combination of proximal and distal TES into a dual-delivery protocol for early post-injury outcomes. However, at later time points, TES delivered to the proximal nerve and dual site have comparable effects. In addition, we demonstrate a novel wireless implant system made from resorbable materials that enabled multiple days of dual site TES.

Newly Diagnosed ALS Support Series: Providing Education, Support, & Community

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Support and educational needs of people newly diagnosed with Amyotrophic Lateral Sclerosis (ALS) can differ greatly due to several factors including but not limited to delayed diagnosis, health literacy skills, geographical proximity to an ALS Center, eligibility for clinical trial, and access to FDA-approved treatments. In addition, receiving a diagnosis of ALS is associated with feelings of distress due to changes that can impact someone physically, emotionally, and financially alarmingly fast due to the two-to-five-year life expectancy. This often leads to delayed decision-making which can have an impact on quality of life. The months following a diagnosis reveal a critical area of support.

At the Foundation, we began an internal audit of our current practices for supporting those newly diagnosed. We analyzed our educational materials and explored first-hand experiences of those living with ALS and caregivers during those crucial first six months. This revealed an opportunity to update our print and web related materials, while fusing our support group model with our online educational curriculum resulting in the Les Turner ALS Foundation's Newly Diagnosed ALS Support Series; a first-of-its kind program.

The eight-part series covers topics identified, by those interviewed, as imperative, for example, "Advanced Directives." Each session is led by a Support Services Coordinator, occurs online, and lasts one-hour. Eight people on average have attended each session; people newly diagnosed with ALS and their partners. Feedback occurs after each session via online survey. To date, a majority have expressed satisfaction in the sessions, an increased confidence, and a plan to implement something learned. One participant elaborated, "We have learned something interesting at each session of the series. Not only does the series answer questions, but also makes you think of new questions specific to our own situation. It's also been nice to be involved with people who are in the same boat as we are."

The months immediately following an ALS diagnosis are life-altering and for some can be isolating. There is a need to support those newly diagnosed and their families. By offering a unique hybrid educational support series we have found a way to allow people the time and space to adjust to the diagnosis, while still providing education in a timely manner to foster informed decision-making, in a supportive environment.

Addressing ALS/MND Caregiver Needs Through Support and Education

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The physical and emotional toll of Amyotrophic Lateral Sclerosis (ALS) not only impacts the person with the disease but also their support system, including paid and unpaid caregivers also referred to as informal/family caregivers. Approximately 80% of home care services are provided by informal family caregivers (ALS Association, 2020) and are not paid. Due to the concentrated focus and amount of time dedicated to caring for the person living with ALS, a caregiver's needs are often suppressed, resulting in stress and a potential decline in their mental and physical well-being. This is particularly true if the caregiver does not have adequate support, information, resources, and guidance, which negatively affects their ability to provide care to the person living with ALS.

Through analysis of our materials, an area of development was identified to expand our service reach to caregivers. We first began with a literature review and audit of our resource materials, reviewed the Alliance of ALS/MND Associations' Fundamental Rights for Caregivers of People Living with ALS (Rev. April 2021) Lesturnerals.org/caregiver-rights, and conducted a needs assessment to understand specific caregiver needs (support group taskforce, & individual interviews). Following this work, we developed a caregiver education and support program that addresses specific caregiver needs, implemented the support groups and launched educational materials, and determined program outcomes through interviews and satisfaction surveys.

Initial outcomes through interviews and satisfaction surveys have been positive. Participation in support groups has grown over time. Interviews have revealed feedback like, "Our group is a blessing for each participant. They support one another, care for one another, and most importantly, they are able to express their stress, frustration and fears freely without worry about judgment or hurting their loved ones."

Caregivers often report that caregiving has provided benefits, such as, finding a sense of purpose, feeling a sense of accomplishment, knowing you are making a difference, building deepening connections with loved ones, and increasing confidence to handle challenging situations. However, caregivers often experience physical and emotional distress, which can lead to a decreased quality of life. By providing targeted programming to caregivers through support groups and educational materials, the Foundation has identified new resources to help decrease caregiver burden. Ongoing analysis of groups and materials will continue. To learn more visit lesturnerals.org/resources

Internet-Supervised Home-Based Spirometry through Telemedicine in Amyotrophic Lateral Sclerosis

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Pilot Implementation study of At-Home Telespirometry (AHT) measurement of Erect-seated forced vital capacity (eFVC) suggested positive results in precision (Bland-Altman), repeatability, feasibility of longitudinal measurement, increased assessment of supine forced vital capacity (sFVC) in home situation and differential rate of loss of eFVC as function of initial eFVC at baseline [Young 2023, Narapureddy 2023]. Slope measurements of eFVC change differ by stratification of initial eFVC at baseline in parallel with increased rate of eFVC loss and increased rate of number of eFVC 3 % predicted monthly drops in cohort with eFVC < 60 % predicted at baseline [Young 2023, Brooks 2023].

A total of 100 ALS subjects from two ALSA-certified ALS Clinics in the US (SUNY Upstate, Atrium Health) will enter a clinical study to measure erect-seated and supine slow vital capacity [eSVC, sSVC] from home using a portable spirometer (MIR, Italy), ZEPHYRx mobile Remote Monitoring application and ZEPHYRx Provider Dashboard over 6 months. Clinical and statistical analysis will follow the same procedure as the previous pilot FVC study. Cohorts stratified according to <60%, 60-80% and > 80% predicted eSVC at baseline will be followed longitudinally to define eSVC and sSVC changes between cannonical quarterly (q3month) in-clinic visits to assess whether clinically significant changes in eSVC and sSVC identify respiratory complications requiring initiation of ventilator support before the next in-clinic visit.

This prospective study [NCT05106569] has nearly completed recruitment of 100 ALS subjects. Baseline characteristics of enrolled ALS subjects will be similar to ALS subjects completing the pilot FVC study [Young 2023]. An interim analysis of the first 50 subjects to complete the study will be presented.

Stratification of eSVC obtained from baseline in-clinic measurement is predictive of ALS disease trajectory [Chio 2022; [BRB1] [EY2] Ackrivo 2019, Elamin 2019, Pirola 2019]. This ongoing clinical study, using AHT to measure eSVC and sSVC between canonical in-clinic visits at home, aims to identify clinically significant, between clinic visit eSVC and sSVC changes, that may require earlier respiratory management than would occur from quarterly in-clinic visits.

Peripheral Immune Dysregulation in Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease in which immune dysregulation is a common feature. Recent studies show that genetic risk factors for ALS are associated with peripheral immune dysfunction. However, it is unknown if the immune system is differentially impacted in genetic vs. sporadic ALS. Here, we employed single cell RNA sequencing of over 600,000 peripheral blood mononuclear cells (PBMCs) from 22 ALS patients and 18 age-matched healthy controls. Among ALS subjects were four carriers of C9orf72 repeat expansions, which account for about 40% of familial ALS cases2. We also assessed nine subjects with fast progressing and nine with slow progressing sporadic ALS. We then utilized a novel CRISPR-mediated library preparation method to enrich for immune transcripts. We identified 31 immune cell types through multimodal reference mapping. We highlight several transcriptionally dysregulated cell types in ALS vs. healthy controls, including cytotoxic CD4+ T cells and a subset of intermediate B cells. Notably, our data show disparate changes to the peripheral immune system in genetic vs. sporadic ALS. Specifically, we identified a high number of dysregulated genes in monocytes derived from patients carrying C9orf72 repeat expansions compared to healthy controls. Altogether, our findings reveal a dysregulated peripheral immune transcriptome that might play a role in the onset or progression of sporadic and genetic ALS.

LES TURNER ALS FOUNDATION

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About The Les Turner ALS Foundation

The Les Turner ALS Foundation was founded in 1977 by the family and friends of Les Turner, who was diagnosed with ALS at the age of 36. Les's wife Ina and their three young boys turned to Les's brother-in-law and best friend, Harvey Gaffen, for help. At a time when information and research on ALS was almost non-existent, they set out to raise funds to provide vital research and resources to people living with ALS and their families.

In 1979, the Foundation established one of the world's first laboratories devoted to ALS research at Northwestern Medicine, followed by the opening of the Lois Insolia ALS Clinic in 1986, which provides world-class multidisciplinary care for people living with ALS. Since then, the Foundation has directly funded over $32 million in ALS research and clinical care, as well as millions more in indirect funding.

Today, the Les Turner ALS Foundation is the Midwest's leading ALS organization. For more than 45 years, it has been our mission to provide the most comprehensive care and support to people living with ALS and their families so they can confidently navigate the disease and have access to the most promising therapies. We treat each person like family, supporting them every step of the way, and provide their loved ones with answers and encouragement.

Under the leadership of Robert G. Kalb, MD, Joan and Paul Rubschlager Professor of Neurology, Chief of Neuromuscular Disease; Director, Les Turner ALS Center, Northwestern University Feinberg School of Medicine, the Les Turner ALS Center comprises more than 70 members working across Northwestern's Chicago and Evanston campuses, uniting expertise across scientific disciplines to generate new insights and significant advances in the fight against ALS.

Funding for ALS research at the Les Turner ALS Center is made possible through the support of our generous donors. Please consider joining them by making a donation or enrolling in our monthly giving program, which will enable long-term investments in ALS care and research — and provide hope to people living with ALS and their families everywhere.

Together, we will create a world free of ALS. To learn more, visit **lesturnerals.org.**