12th Annual Les Turner Symposium on ALS

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

Monday, Nov. 7, 2022

8 a.m.-4 p.m. Central Time
Prentice Women's Hospital
Conference Room L (Floor 3)
250 E. Superior St., Chicago, IL 60611

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Welcome Letter

Dear friends and colleagues,

It is our pleasure to welcome you to the 12th Annual Les Turner Symposium on ALS. We are meeting in person this year and yet our symposium will also be accessible to everyone around the globe. Symposiums like this unite us and show the world our determination to work together and end vicious diseases like ALS.

There are many exciting and important developments in the field of ALS that have moved us closer to the day when we will see people living with ALS regain their strength and lead the lives they want to lead.

In the past, there were not many clinical trials in ALS, but today, clinical research is very active. Patients and doctors have many options from which they can choose a clinical trial that is the best fit for the patient. Our Les Turner ALS Center at Northwestern Medicine houses the Lois Insolia ALS Clinic, which is part of the Northeast Amyotrophic Lateral Sclerosis (NEALS) Consortium and is one of the largest ALS clinics in the country. It offers the largest group of multidisciplinary providers who put patients first, treating with compassion and expertise.

Drug discovery studies are blooming with improved preclinical assays that are translational and informative about patient conditions. For example, Dr. Richard Silverman and I discovered NU-9, the first compound that improves the health of diseased upper motor neurons in ALS. Likewise, many of our center members are developing and utilizing novel preclinical assays using novel model systems and platforms: Dr. Evangelos Kiskinis uses IPS cells, Dr. Robert Kalb utilizes worm models, and my lab uses diseased upper motor neurons.

The Les Turner ALS Center has grown to more than 80 members, working in areas of basic science research, clinical research and clinical care. The over 15 primary investigators and laboratory heads working on various areas of ALS research are all supported by NIH or other foundation grants. The team effort among members is exponentially growing, with the common goal of finding a treatment for ALS in mind.

The long-term relationship between the Les Turner ALS Foundation and the Northwestern University Feinberg School of Medicine is second to none. The partnership has led to the establishment of the Lois Insolia ALS Clinic, one of the first multidisciplinary ALS clinics in the country. In symposia like ours, we not only bring patients and clinicians together, but also scientists, so that we may share our excitement for new developments as we pave the way for a better future for all.

We again have a full day of events this year. We are thankful to our friends and colleagues who join us from Johns Hopkins Medical School, Harvard Medical School and JAX Laboratories. We will open the day with remarks of an ALS friend, followed by presentations from Les Turner ALS Center members and guests Dr. Clotilde Lagier-Tourenne and Dr. Cat Lutz. Our keynote speaker is Dr. Nicholas Maragakis of Johns Hopkins, who will be sharing recent developments in uncovering the causes of ALS. Our symposium will also feature poster presentations and a Q&A with clinicians.

We thank the Les Turner ALS Foundation as well as Mitsubishi Tanabe Pharma America, Amylyx, BrainStorm Cell Therapeutics, NuMotion, PTC Therapeutics, Biogen and Hill-Rom for their support and contributions. The cure for ALS will not drop from the sky; we will have to develop it together. As we work all our might to end ALS, we are happy to have you with us.

Sincerely,

Hande Ozdinler

Hande Ozdinler, PhD
Associate Professor, Department of Neurology, Northwestern University

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Agenda

8-9 a.m. **Registration and breakfast**

Welcome: Robert Kalb, MD, Director, Les Turner ALS Center

9 a.m. - 12 p.m. **Research presentations**

Opening remarks from Frank Granata

Metabolic Rewiring in Models of Familial ALS by Robert Kalb, MD, Director, Les Turner ALS Center at Northwestern University; Chief of the Neuromuscular Disease Division and Joan and Paul Rubschlager Professor of Neurology, Northwestern University Feinberg School of Medicine

New Insights Into Disease Mechanisms of Genetic ALS by Evangelos Kiskinis, PhD, Assistant Professor of Neurology and Neuroscience, Northwestern University Feinberg School of Medicine

Neuroimaging Insights Into the Pathogenesis of UMN-Predominant ALS Subtypes by Erik P. Pioro, MD, PhD, Medical Director of the Neuromuscular Division, Vice-Chair of Translational Neurology, and Lewis John Pollack Professor of Neurology, Northwestern University Feinberg School of Medicine

ALS, Mouse Models, and Therapeutic Strategies by Cat Lutz, PhD, MBA, Vice President for the Rare Disease Translational Center at The Jackson Laboratory

10:30 a.m. Morning Break (15 minutes)

Motors for a Cure: Why Studies of Motor Protein KIF5A Can Provide Key Insights Into the Pathogenesis of ALS by Jonathan R. Brent, MD, PhD, Assistant Professor of Neurology, Northwestern University Feinberg School of Medicine

Stathmin-2: An Emerging Therapeutic Target in TDP-43 Proteinopathies by Clotilde Lagier-Tourenne, MD, PhD, Associate Professor of Neurology, Massachusetts General Hospital and Harvard Medical School

Improving the Health of Upper Motor Neurons is Crucial for Developing Effective Treatment Strategies in ALS by Hande Ozdinler, PhD, Associate Professor of Neurology, Northwestern University Feinberg School of Medicine

12-1 p.m. **Lunch break + Research poster session + Meet-and-greet with ALS community**

1-2:15 p.m. **Keynote address**

Introduction: Evangelos Kiskinis, PhD, Assistant Professor of Neurology and Neuroscience, Northwestern University Feinberg School of Medicine

Using Human Induced Pluripotent Stem Cells as a Platform for Understanding ALS Mechanisms and Designing Therapeutics by Nicholas Maragakis, MD, Professor, Department of Neurology, Johns Hopkins University School of Medicine; Director, ALS Center for Cell Therapy and Regeneration Research; Medical Director, Johns Hopkins ALS Clinical Trials Unit; Director, Johns Hopkins Center for ALS Specialty Care at Johns Hopkins University School of Medicine

2:15 p.m. Afternoon Break (15 minutes)

2:30 - 3:40 p.m. **Clinical conversations panel**

Introduction/Moderator: Colin Franz, MD, PhD, Assistant Professor of Physical Medicine & Rehabilitation and Neurology

- Senda Ajroud-Driss, MD, Director, Lois Insolia ALS Clinic Les Turner ALS Center at Northwestern Medicine; Program Director, Neuromuscular Fellowship; Associate Professor of Neurology
- John M. Coleman III, MD, Associate Professor of Medicine (Pulmonary and Critical Care) and Neurology, Faculty, Les Turner ALS Center at Northwestern Medicine
- Lauren Webb, LCSW, Director of Support Services and Education, Les Turner ALS Foundation
- Nicholas Maragakis, MD (Keynote Speaker)

3:45-4 p.m. **Closing remarks**

Andrea Pauls Backman, MBA, Chief Executive Officer, Les Turner ALS Foundation

Unless otherwise noted, all Northwestern faculty members presenting at the symposium are affiliated with the Les Turner ALS Center at Northwestern Medicine.

Opening remarks from Frank Granata, a person living with ALS

Prior to his ALS diagnosis, **Frank Granata** was a successful finance executive whose work took him around the world. Frank was diagnosed with ALS in 2020 at the Lois Insolia ALS Clinic at the Les Turner ALS Center at Northwestern Medicine. He takes an active approach in his care and regularly uses humor, the support of his family and the Les Turner ALS Foundation's Support Services to get through the challenges of living with ALS. We are grateful to have Frank share his story about living with ALS, the impact that the disease has on his life, and his hope for research.

Closing remarks from Andrea Pauls Backman, MBA, CEO of the Les Turner ALS Foundation

Andrea Pauls Backman, MBA, has been Chief Executive Officer of the Les Turner ALS Foundation since 2015. She brings both a professional and personal passion to the ALS/MND community having cared for and lost her mother, Sally, to the disease in 2010. She came to the ALS field following a 30-year career in business management as an institutional investment manager, including as a managing director at several investment firms, and brings more than 15 years of nonprofit board management experience. She has served on the Board of Directors of the International Alliance of ALS/MND Associations since 2019 and is the board liaison to its Scientific Advisory Council. Pauls Backman holds a master's degree in business administration from DePaul University and is a certified public accountant.

Nicholas Maragakis, MD

Using Human Induced Pluripotent Stem Cells as a Platform for Understanding ALS Mechanisms and Designing Therapeutics

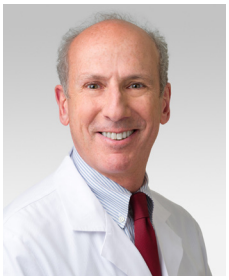
Induced pluripotent stem cells from ALS patients are increasingly used to understand the fundamental pathways by which ALS may start and progress. These cells have great versatility as they can be turned into a number of different cell subtypes of the brain and spinal cord. However, there are challenges in using these cells that remain. Nevertheless, using these cells as a platform for drug screening may give us a window into the different mechanisms by which ALS causes unique symptoms in patients and may help us refine individualized therapeutics.

Nicholas Maragakis, MD, is the Medical Director of the Johns Hopkins ALS Clinical Trials Unit and Director of the ALS Center for Cell Therapy and Regeneration Research and Professor of Neurology at Johns Hopkins University. The Center for ALS Specialty Care at Johns Hopkins is a world-recognized leader in providing medical care and offering the latest in clinical trials and therapies to people living with ALS.

A common theme to Dr. Maragakis's research is the study of astrocyte biology and its role in disease pathogenesis, with a particular emphasis on how astrocytes may contribute to ALS.

His laboratory, in collaboration with others, has been interested in the development of induced pluripotent stem cells from ALS patients. His laboratory has created numerous iPS cell lines from familial and sporadic ALS patients as well as controls. His latest efforts have focused upon characterizing iPSC-derived motor neurons and astrocytes both in vitro and in vivo with an effort towards understanding disease mechanisms; in particular, modeling ALS disease heterogeneity with regard to disease progression.

Dr. Maragakis has also been heavily involved in clinical research as the principal investigator, site principal investigator, or co-investigator of numerous clinical trials in ALS, many coordinated by the Northeast ALS Consortium, for which he has served as an Executive Board member and where he is currently a member of the Scientific Advisory Board.



Robert Kalb, MD

Metabolic Rewiring in Models of Familial ALS

Mitochondrial dysfunction has been seen in nervous system tissues from ALS patients and innumerable models of ALS. Reduced generation of ATP from sick mitochondria provides the impetus for neurons to undertake countermeasures to align energy supply with demand. In this presentation, Dr. Kalb will describe how investigations of fuel utilization in cortical neurons expressing two different familial ALS-causing genes reveal alterations in the operation of glycolysis and the Krebs cycle with little observable effects on ATP production and redox state. He will also describe how manipulations of intermediary metabolism promote the survival of motor neurons in vitro, and he will posit that small molecules that affect fuel utilization by neurons may be a rational therapeutic approach for ALS patients.

Robert Kalb, MD, is Director of the Les Turner ALS Center at Northwestern University and Chief of the Neuromuscular Disease Division in the Ken & Ruth Davee Department of Neurology at the Northwestern University Feinberg School of Medicine, where he is also the Joan and Paul Rubschlager Professor of Neurology. Dr. Kalb's research uses a cell and molecular biology approach and focuses on two topics: 1.) activity-dependent development of circuits in the central nervous system and 2.) healthful compensatory responses of cells and organisms to stressful conditions. The Kalb Lab uses genetically manipulated mice, primary neuron tissue culture and *C. elegans* in its studies.

Evangelos Kiskinis, PhD

New Insights Into Disease Mechanisms of Genetic ALS

The discovery of the hexanucleotide repeat expansion in C9orf72 (C9-HRE) as the most prevalent genetic driver of ALS/FTD has stimulated intense interest in deciphering the pathophysiology associated with this mutation. The focus of this presentation will be on the interaction between dipeptide proteins generated from the C9-HRE and their toxic interaction with RNA. Dr. Kiskinis will describe the design and synthesis of a novel RNA mimetic molecule that can inhibit these C9-HRE neurodegenerative effects.

Evangelos Kiskinis, PhD, is an Assistant Professor of Neurology and Neuroscience at Northwestern University Feinberg School of Medicine and a New York Stem Cell Foundation Robertson Investigator. Dr. Kiskinis earned his 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.

Erik P. Pioro, MD, PhD

Neuroimaging Insights Into the Pathogenesis of UMN-Predominant ALS Subtypes

Multiple genetic causes of ALS point to diverse mechanisms in hereditary and nonhereditary (sporadic) forms of disease. Therefore, therapies may be most effective if personalized for differing subtypes/causes of ALS. Routine brain MRI studies have identified a unique signature in the brain of a subset of ALS patients who are younger and have more rapid disease progression. Dr. Pioro's specialized MRI studies have demonstrated distinct patterns of brain degeneration in such patients, suggestive of differing disease mechanisms. This presentation will review these findings and demonstrate how future imaging/laboratory studies may elucidate related molecular changes to guide development of future therapies.

Erik P. Pioro, MD, PhD, is Medical Director of the Neuromuscular Division and Vice Chair of Translational Neurology in the Ken & Ruth Davee Department of Neurology at Northwestern University Feinberg School of Medicine, where he is also the Lewis John Pollack Professor of Neurology. Here, he specializes in the clinical care and research of adult neurologic patients with motor neuron diseases, particularly ALS. His translational research focuses on characterizing the neuroimaging (MRI and PET) abnormalities in the brain and spinal cord of people living with ALS as well as identifying their underlying molecular correlates in patient-specific induced pluripotent stem cells and postmortem tissue. Dr. Pioro previously served as Director of the Section of ALS & Related Disorders at the Cleveland Clinic for over two decades.

Cat Lutz, PhD, MBA

ALS, Mouse Models, and Therapeutic Strategies

Advances in genome sequencing have uncovered a number of causative and contributing genes associated with ALS. Coincident with this gene discovery are advances in genetic engineering and genetic based therapeutic strategies for the disease. Mouse models play a crucial role in understanding the biology associated with the genetic mutations for ALS, provide insights into sporadic ALS disease mechanisms, and serve as patient avatars to test the efficacy of new treatments. This presentation will review some of the latest mouse models for ALS and their use in advancing therapeutics.

Cat Lutz, PhD, MBA, is the Vice President for the Rare Disease Translational Center at JAX, where she studies and develops resources for ALS and other rare neurological disorders. Dr. Lutz is a trained neuroscientist and geneticist who has worked extensively with mouse models of neurodegenerative diseases, with an emphasis on ALS. Her lab has worked to model in mice many of the genetic forms of ALS and has ensured that these preclinical mouse models are available globally to the scientific community to accelerate discovery and treatments. From 2015-2022 she established and was the Senior Director of the In Vivo Pharmacology Efficacy Testing Service at The Jackson Laboratory (JAX), where she designed preclinical ALS platforms for use in testing therapeutics for industry and academic partners.

Jonathan R. Brent, MD, PhD

Motors for a Cure: Why Studies of Motor Protein KIF5A Can Provide Key Insights Into the Pathogenesis of ALS

Mutations in distinct regions of the motor protein KIF5A lead to a broad range of neurologic diseases, suggesting that unique cellular events may be perturbed by the disruption of different functions performed by the same protein. Dr. Brent and his team found that ALS-causative mutations disrupt regulation of KIF5A activity leading to neuronal death, although the precise mechanisms are poorly understood. Using in vitro and in vivo models, Dr. Brent aims to dissect the pathways underlying KIF5A mediated neurodegeneration in ALS and related disorders. In this presentation, Dr. Brent will describe how further understanding of the pathogenicity of KIF5A mutations has the potential to advance knowledge regarding the mechanisms underlying neuronal homeostasis and selective vulnerability in ALS.

Jonathan R. Brent, MD, PhD, is an Assistant Professor of Neurology (Neuromuscular Disease) in the Ken & Ruth Davee Department of Neurology at the Northwestern University Feinberg School of Medicine. Dr. Brent completed the medical scientist training program at Columbia University, where he studied the roles of dysfunctional RNA binding proteins in ALS. He completed residency and a neuromuscular fellowship at Northwestern. Since joining Northwestern faculty in 2019, Dr. Brent has cared for patients with a broad range of neuromuscular disorders with a specific focus on ALS/MND in the Lois Insolia ALS Clinic. His research focuses on the interplay between molecular motor proteins, axonal transport, and the neuronal cytoskeleton in motor neuron diseases such as ALS. The goal of this work is to lay the foundation for the development of therapeutics targeting dysfunctional cytoskeletal proteins in ALS.

Clotilde Lagier-Tourenne, MD, PhD

Stathmin-2: An Emerging Therapeutic Target in TDP-43 Proteinopathies

Abnormal protein accumulations of the RNA binding protein TDP-43 are found in almost all instances of amyotrophic lateral sclerosis (ALS), 45% of frontotemporal dementia (FTD), and 30% of Alzheimer's disease patients. TDP-43 aggregations are associated with a striking nuclear loss of the protein. Dr. Lagier-Tourenne and her team recently demonstrated that the human RNA most affected by loss of nuclear TDP-43 is encoding the neuronal growth-associated factor called stathmin-2. Reduced levels in stathmin-2 is a hallmark in sporadic and familial ALS/FTD, and restoration of stathmin-2 expression emerges as an attractive therapeutic strategy in TDP-43 proteinopathies. Using newly generated cellular and animal models, Dr. Lagier-Tourenne and her team determined stathmin-2's essential role for neuronal regeneration and axonal maintenance and have established antisense oligonucleotides (ASOs) as a therapeutically viable approach to rescue stathmin-2 in TDP-43 proteinopathies.

Clotilde Lagier-Tourenne, MD, PhD, is an Associate Professor of Neurology at the Massachusetts General Hospital and Harvard Medical School. She is a member of the Sean M. Healey & AMG Center for ALS at Mass General and an associate member at the Broad Institute of MIT and Harvard. She trained as a medical geneticist in France and at Columbia University. Her team investigates the molecular mechanisms driving neurodegeneration in amyotrophic lateral sclerosis, frontotemporal dementia, Alzheimer's disease and Huntington's disease. She has established collaborations with academic and pharmaceutical partners to develop novel approaches to therapy, including RNA-targeting antisense oligonucleotides and immunotherapies for patients with ALS and FTD.

Hande Ozdinler, PhD

Improving the Health of Upper Motor Neurons is Crucial for Developing Effective Treatment Strategies in ALS

Movement starts in the brain. The brain component of motor neuron circuitry is responsible for the initiation and modulation of voluntary movement, which is impaired in ALS patients. ALS is characterized by the loss of both upper and lower motor neurons and progressive degeneration of the motor neuron circuitry. Developing effective and long-term treatment strategies requires that we also include the cortical component into the picture. The Ozdinler Lab is developing novel drug discovery platforms, identifying novel upper motor neuron biomarkers and gene therapy approaches to improve the health of diseased upper motor neurons, so that better and more effective treatment strategies can be developed.

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 HSP, PLS, and — with their degeneration of spinal motor neurons — ALS. 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.

Clinical Conversations Panelists

Colin Franz, MD, PhD (Moderator)

Colin Franz, MD, PhD, is a physician and scientist at the Shirley Ryan AbilityLab and Northwestern University and sees patients at the Lois Insolia ALS Clinic at the Les Turner ALS Center at Northwestern Medicine. His clinical subspecialties include neuromuscular medicine, electrodiagnostic medicine, and neuromuscular ultrasound. He is the Director of the Electrodiagnostic (clinical) and Regenerative Neurorehabilitation (research) laboratories at the Shirley Ryan AbilityLab hospital. His research is heavily inspired by the patient populations he cares for. His laboratory team takes a highly technology-oriented approach to precision neurorehabilitation. Some of his current studies include developing transient (resorbable) implanted devices to deliver therapeutics to regenerating axons and making human neurons derived from patient-derived pluripotent stem cells to determine and isolate how individual genetic factors affect neurotrauma outcomes.

Nicholas Maragakis, MD (Keynote Speaker) See bio on Page 6.

Senda Ajroud-Driss, MD

Senda Ajroud-Driss, MD, is an Associate Professor in the Ken & Ruth Davee Department of Neurology at the Northwestern University Feinberg School of Medicine, where she also serves as Director of the Neuromuscular Medicine Fellowship Program. 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 the past 16 years. Dr. Driss also leads the Les Turner ALS Center's clinical trial program.

John M. Coleman III, MD

John M. Coleman III, MD, is an Associate Professor of Medicine (Pulmonary and Critical Care) and Neurology and a member of the Les Turner ALS Center at Northwestern Medicine. He attended medical school at Loyola University Stritch School of Medicine and completed an internal medicine residency at Loyola Medical Center. He then went to the University Pittsburgh Medical Center for training in pulmonary and critical care medicine and sleep medicine. In 2013, Dr. Coleman, with his specialty in chronic respiratory failure, was recruited to Northwestern University Feinberg School of Medicine and joined the Les Turner ALS Center. Over the last 10 years, Dr. Coleman has served as one of two pulmonary physicians in the Lois Insolia ALS Clinic at the Les Turner ALS Center, providing respiratory care and support for people living with ALS. In addition, he serves as the head of the Les Turner Support Services committee and is on the board of directors of the Les Turner ALS Foundation. Dr. Coleman has published several papers and delivered international talks on proper respiratory care for people living with ALS.

Lauren Webb, LCSW

Lauren Webb, LCSW, is Director of Support Services and Education at the Les Turner ALS Foundation. Webb has worked in the neuromuscular community for over 20 years—providing direct patient care for individuals with ALS, working for a neurological specialty reference laboratory, coordinating clinical trials at Ann and Robert H. Lurie Children's Hospital of Chicago and overseeing the Muscular Dystrophy Association's nationwide Care Center Network. She has a master's degree in social work with a concentration in health administration and policy from the University of Chicago. Webb is a devoted advocate who approaches people and problems with humility, curiosity and humor.

Assessing Lower Motor Neuron Degeneration in People with Cervical Spinal Cord Injury: A Retrospective Study

Adenike Adewuyi[1], Michael Berger[2], Colin Franz[3]

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[2]*International Collaboration on Repair Discoveries (ICORD) and the Division of Physical Medicine & Rehabilitation, Department of Medicine, University of British Columbia, Vancouver, British Columbia, Canada*
[3]*The Ken & Ruth Davee Department of Neurology and the Department of Physical Medicine and Rehabilitation, Northwestern University Feinberg School of Medicine, Chicago, IL*

Motor weakness in spinal cord injury (SCI) is traditionally conceptualized as a disorder of the descending corticospinal tracts or upper motor neurons (UMN). However, there is increasing evidence that suggest that the lower motor neuron (LMN) may also be damaged at and below the spinal cord lesion. This has implications for nerve transfer surgery (NT), an exciting option to improve upper limb functions in in SCI but requires intact sub-lesional LMN health. The purpose of this study was to characterize patterns of LMN abnormality in nerve-muscle groups that are the potential recipients of NT, using a standardized electrodiagnostic examination, in individuals with subacute (injury duration >2 months, and <1year) and chronic SCI (injury duration >1 year) at injury levels C4-T1. The LMN abnormality was determined using a semi-hierarchical approach, combining the amplitude compound muscle action potential (CMAP) and abnormal spontaneous activity on needle electromyography (EMG). To date, 21 participants were included (median age, 31 years; age range 15-69 years); 16 males and 5 females with time from injury (median duration, 8 months, range, 2 months – 40 years). A high frequency of LMN abnormality was observed (90%), although there was substantial variation within and between individuals. The high frequency of LMN abnormality in recipient nerve-muscle groups has implications to candidate selection for NT surgery in subacute and chronic SCI and supports the important role of the pre-operative electrodiagnostic examination. Our results further support the inclusion of both CMAP and needle EMG parameters for characterization of LMN health.

Upper Motor Neurons are a Target for Gene Therapy and UCHL1 is Necessary and Sufficient to Improve Cellular Integrity of Diseased Upper Motor Neurons

Angela Ahrens[1], Baris Genç[1], Javier H. Jara[1], Santana S. Sanchez[1], Amiko K. B. Lagrimas[1], Öge Gözütok[1], Nuran Koçak[1], Yongling Zhu[1], P. Hande Ozdinler[1]

[1]*The Ken & Ruth Davee Department of Neurology, Northwestern University Feinberg School of Medicine, Chicago, IL*

Background: Upper motor neuron (UMN) degeneration leads to neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS), primary lateral sclerosis (PLS), and hereditary spastic paraplegia (HSP). UCHL1 (ubiquitin C-terminal hydrolase-L1) is a deubiquitinating enzyme crucial for maintaining free ubiquitin levels. Loss of UCHL1 function leads to motor function defects and progressive UMN degeneration accompanied by vacuolated apical dendrites, spine loss, and increased ER stress. Corticospinal motor neurons (CSMN, a.k.a UMNs in mice) show early, selective, and profound degeneration in Uchl1nm3419 (UCHL1–/–) mice, which lack all UCHL1 function. UMN loss occurs independent of spinal motor neuron degeneration and that UMNs are indeed effective cellular targets for gene therapy, which offers a potential solution especially for patients with predominant UMN loss.

Hypothesis: Expression of UCHL1 improves the health of UMN that are diseased due to different underlying causes.

Methods: CSMN of UCHL1-/- mice as well as hSOD1G93A and prpTDP-43A315T mice, two well characterized mouse models of ALS, representing misfolded SOD1 toxicity and TDP-43 pathology, respectively, were retrogradely transduced to express UCHL1. The impact of UCHL1 expression on UMN health was assessed at a cellular level.

Results: When UCHL1 activity is ablated only from spinal motor neurons, CSMN remained intact. However, restoring UCHL1 specifically in CSMN of UCHL1-/- mice via directed gene delivery was sufficient to improve CSMN integrity to the healthy control levels. In addition, when UCHL1 gene was delivered selectively to CSMN that are diseased due to misfolded SOD1 toxicity and TDP-43 pathology via AAV-mediated retrograde transduction, CSMN retained their neuronal integrity and cytoarchitectural stability, and the disease causing misfolded SOD1 and mutant human TDP-43 were reduced in hSOD1G93A and prpTDP-43A315T models.

Conclusion: UCHL1 expression improved neuronal integrity and cytoarchitectural stability of CSMN diseased due to two distinct causes of neurodegeneration in ALS. UCHL1 could be a promising target for the treatment of UMN diseases.

Defining the functional role of UPF1-mediated decay in healthy and ALS-associated human motor neurons

Francesco Alessandrini[1,2], Matthew Wright[1,2], Tatsuaki Kurosaki[3], Lynne Maquat[3], Evangelos Kiskinis[1,2]

[1]Department of Neurology & Physiology, Northwestern University Feinberg School of Medicine
[2]Les Turner ALS Center at Northwestern Medicine
[3]Department of Biochemistry & Biophysics - School of Medicine and Dentistry Center for RNA Biology University of Rochester

Up-frameshift protein 1 (UPF1) is an evolutionarily conserved protein involved in several RNA surveillance pathways, playing a broad role in cellular homeostasis. UPF1 is a key player in nonsense-mediated decay (NMD), Staufen1-mediated decay (SMD) and structure-mediated decay (SRD). The precise role of UPF1-mediated decay in postmitotic neurons remains unresolved although it has been shown to enable compartmentalized gene expression, and to modulate synaptic activity and axon guidance. What also remains unclear is how these pathways are modulated in neurodegenerative diseases with impaired mRNA homeostasis such as Amyotrophic Lateral Sclerosis (ALS). We and others have shown that UPF1 overexpression rescues neurotoxicity in several genetic ALS models, however this mechanism remains controversial.

Here, we used spinal motor neurons differentiated from iPSCs to comprehensively define the role of UPF1-mediated decay in this most vulnerable cell type afflicted in ALS patients. We performed gene expression and mRNA stability analysis from RNA-Sequencing in neurons treated with a siRNA for UPF1, in combination with RIP-Seq for the active form of UFP1 (pUPF1). These overlapping datasets highlighted a stringent set of transcripts that are targeted for degradation. To better understand the physiological role of these pathways in post-mitotic neurons we analyzed the features of target transcripts and find that only a small proportion (~20%) harbor PTCs. We find that ~50% of UPF1 targets have high GC content and highly structured 3'UTR. Many UPF1 targets show also a previously described binding site for UPF1 in the 3'UTR, as well as longer 3'UTRs and shorter 5'UTRs. Further, UPF1 targets in motor neurons are strongly enriched for function within specific pathways, suggesting that UPF1 plays a regulatory role. We showed that protein-ubiquitination activity and autophagy pathways are effectively modulated by UPF1 inhibition.

Utilizing the stringently defined class of UPF1 targets we next asked how the pathway is modulated in response to ALS-associated mRNA perturbations. Analysis of RNA-Seq datasets from mutant C9orf72 ALS patient neurons, neurons treated with TDP-43 siRNA and postmortem ALS patient samples suggests that TDP-43 dysfunction subtly impairs the efficiency of NMD-dependent degradation. Our results suggest that TDP-43 dysfunction may burden mRNA surveillance pathways. More broadly, our study provides a comprehensive description of UPF1 activity in post-mitotic cells and offer novel insights into the role of UPF1 in ALS-associated degeneration.

Targeting mis-splicing in ALS using antisense oligonucleotides

Wanhao Chi[1], Jonathan K. Watts[2], Evangelos Kiskinis[1,3,4]

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[3]*Simpson Querrey Institute, Northwestern University, Chicago, IL*
[4]*Department of Neuroscience, Northwestern University Feinberg School of Medicine, Chicago, IL*

TDP-43 cytoplasmic aggregation and nuclear depletion occur in more than 90% of ALS and ~50% of frontotemporal dementia (FTD) patients. TDP-43 regulates RNA metabolism, and because of its trans-localization and loss-of-function, at least several dozen genes are mis-spliced in ALS/FTD patient samples, including *UNC13A* and *KCNQ2*. *UNC13A* and *KCNQ2* encode a synaptic protein and an ion channel respectively, that play fundamental roles in 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 mislocalization for *KCNQ2*. Here we set out to establish a cellular system to study these mis-splicing events using human induced pluripotent stem cell (iPSC)-derived motor neurons treated with TDP-43 siRNAs. We confirm that the cryptic exon inclusion in *UNC13A* and the coding exon exclusion in *KCNQ2* depend on TDP-43 nuclear depletion. We further design gene-specific antisense oligonucleotides (ASOs) to target the mis-splicing events in these two genes and screen splice-modulating ASOs using human iPSC-derived motor neurons. Lastly, we multiplex ASOs from each gene using spherical nucleic acids (SNAs) and deliver multiplexed SNAs to iPSC-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.

My ALS Decision Tool™: An Interactive, Online Tool to Aid in Informed Decision Making

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[1]*Les Turner ALS Foundation*

My ALS Decision Tool™ explains ALS treatment options in easy-to-understand language, breaking down the benefits and risks. The interactive tool also includes questions to help people living with ALS reflect on their needs and values. Based on each person's answers, the tool suggests resources and next steps.

My ALS Decision Tool™ was launched in late 2021 and is a web-based tool to help people living with ALS make informed health care decisions. It is the first of its kind of resource in the US designed to help people living with ALS make complex medical decisions for essential aspects of their ALS care. My ALS Decision Tool™ currently has information about breathing and nutrition support devices.

My ALS Decision Tool™ was co-produced with people living with ALS, caregivers, a health literacy technology company, international researchers, and health professionals.

Hypothesis: The goal of the ALS decision support tool is to provide clear, relevant and actionable information to help people living with ALS make informed decisions and facilitate conversations with health care providers about how to manage their care and treatment. Shared decision-making tools are an essential component of patient-centered care.

Methods: Using user testing, website analytics and firsthand feedback from people living with ALS, we were able to analyze the success of the tools.

Results: Since the launch, these tools have been viewed over 4,000 times. Feedback from patients and families has been very favorable. Due to the success and positive feedback received, new genetic testing and clinical research modules are currently under development.

Conclusion: My ALS Decision Tool™ was created to contribute to improved 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. People living with ALS often face big health care decisions. The My ALS Decision Tool™ is freely available to any ALS healthcare or advocacy organization to share with people living with ALS.

lesturnerals.org/resources

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). Heat shock proteins (HSPs) act as a key factor in the regulation of protein homeostasis, and perturbations in this process may play a significant role in ALS. However, limited emphasis has been placed on investigating the direct relationship between HSP functionality and disease pathogenesis. Interestingly, a recent whole-exome sequencing study identified mutations in the gene DNAJC7, which encodes for an HSP40 protein, in several ALS patients. While most of these variants are stop-gain, many are missense and located in functional protein domains, suggesting the disease mechanism is driven by haploinsufficiency. DNAJC7 acts as a co-chaperone for HSP70 to help impede inappropriate polypeptide folding. However, little is known about the specific function of DNAJC7 in the CNS and motor neurons (MNs) specifically, which is the cell type that predominantly degenerates in ALS patients. What also remains unknown is how DNAJC7 dysfunction leads to disease pathogenesis. To address these questions, we employed a combination of CRISPR/Cas9 gene editing, induced pluripotent stem cell (iPSC) technology, mass spectrometry (MS)-based quantitative proteomics, and transcriptomic analysis. In this study we have generated an allelic series of iPSC lines harboring pathogenic mutations of DNAJC7. We unbiasedly identified the endogenous binding partners of DNAJC7 in human motor neurons using co-immunoprecipitation MS. The DNAJC7 interactome included several RNA binding proteins (RBPs), a family of proteins implicated in ALS. Using biochemical fractionation, we found that loss of DNAJC7 affects RBP solubility. To assess the functional consequence of this altered solubility, we performed RNA Seq and found broad differential expression and characterized mis-splicing in mutant DNAJC7 expressing human MNs. This suggests that DNAJC7 plays a role in the functionality in RNA regulatory elements. Taken together, these results implicate DNAJC7 in multiple potential pathogenic modalities.

SBT-272 improved mitochondria structure and function and preserved Upper Motor Neurons with TDP-43 Pathology

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Background: Mitochondrial dysfunction is a common underlying pathophysiology for the upper motor neurons (UMNs), which is shared both by ALS patients with TDP-43 pathology and by well-characterized mouse models of ALS with TDP-43. SBT-272 is a CNS penetrant, small molecule that targets the mitochondrial phospholipid cardiolipin and it is an investigational drug in phase I human trial. Functionally it restores mitochondrial inner membrane cristae from damage and therefore improves mitochondrial function. We have shown that SBT-272 treatment increased axonal outgrowth and branching of diseased UMN, cultured from early postnatal stage from prp-hTDP-43A315T-UeGFP mice. Here, we investigate whether in vitro results translate into improvements in vivo, by chronic treatment of prp-hTDP-43A315T-UeGFP mice with SBT-272.

Methods: A) Motor cortex from prp-hTDP-43A315T-UeGFP mice were cultured and treated with SBT-272, edaravone and AMX-0035 to study UMN axonal outgrowth and branching B) Mitochondrial membrane potential was measured using TMRE flow cytometry with and without SBT-272 treatment. C) prp-hTDP-43A315T-UeGFP and UeGFP litter mates were dosed with SBT-272 (1.0 and 5.0 mg/kg/day) via intraperitoneal injection, from P60 until P120. D) Immunohistology was performed on perfused brains to determine loss of GFP+ UMN, count of activated microglia and active astrogliosis in the motor cortex.

Results: SBT-272 treatment improved mitochondria membrane potential and axonal outgrowth of TDP-43+/eGFP+ UMN in vitro. SBT-272 was superior to edaravone and AMX-0035 on axonal growth outcome. In vivo administration of SBT-272 led to UMN retention (Vehicle: 29.58 ± 1.32; 1.0 mg/kg/day: 51.40 ± 0.93; 5.0 mg/kg/day: 49.93 ± 1.76, n = 4-5 mice per group; $P < 0.0001$), and significantly reduced activated microglia (Iba1+: vehicle: 37.08 ± 3.73; 1 mg/kg/day SBT-272: 9.13 ± 0.57; 5 mg/kg/day SBT-272: 13.27 ± 2.03; $P < 0.0001$) and astrogliosis (GFAP+: Vehicle: 59.75 ± 7.31; 1 mg/kg/day SBT-272: 20.07 ± 1.85; 5 mg/kg/day SBT-272: 24 ± 4.1, n = 5; $P < 0.0001$). We report good correspondence between in vitro and in vivo potencies of SBT2-72 in the well-characterized TDP-43 model of ALS.

Conclusion: Chronic administration of SBT-272 is neuroprotective to UMNs with TDP-43 pathology and support the development of SBT-272 for treatment for UMN diseases, such as ALS.

The aging brain microenvironment drives development of disease-associated microglia

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Microglia are long-lived, self-renewing, tissue-resident macrophages of the central nervous system. We hypothesized that aging is associated with a decline in the homeostatic functions of microglia, including clearance of protein aggregates and cellular debris, synaptic maintenance, and secretion of trophic factors. To test this hypothesis, we performed bulk and single-cell RNA-sequencing (scRNA-seq) on FACS-purified microglia isolated from brains of young (4-9mo) and old (18-24mo) mice with and without intratracheal instillation of infuenza A virus (IAV) and 30-day recovery. We identified reproducible transcriptomic remodeling during aging that is largely unique to microglia, characterized by two distinct cell states: one characterized by interferon-responsive gene expression, and another characterized by upregulation of metabolic genes involved in glycolysis and beta-oxidation, and markers of disease-associated microglia (DAM), including *Apoe*, *Spp1*, *Lpl*, and *Clec7a* in concert with downregulation of key mediators of homeostatic microglial function, including *Mertk*, *Cx3cr1*, *Tmem119*, and *P2ry12*. Notably, signatures associated with IAV were not detected in any cell type after recovery. In parallel, we also performed scRNA-seq on live microglia from patients who died from COVID-19 or non-inflammatory conditions. We observed a concerted upregulation of key NFKB targets including *CDKN1A* (P21), as well as reductions in markers of cell division including *MKI67* in microglia from COVID-19 patients, suggesting that persistent NFKB activation may lead to cell-cycle arrest that may be unique to severe COVID-19. To determine whether age-related changes in microglia are cell autonomous or are driven by the aging brain microenvironment, we depleted microglia in young and old mice using the M-CSF-R inhibitor PLX3397 and performed transcriptomic profiling on FACS-purified repopulating microglia. After repopulation, microglia from old animals retained the transcriptomic signature of aging, including the expression of DAM associated genes. Our findings suggest changes in the brain microenvironment lead to persistent inflammation in microglia which is not further antagonized by IAV infection. In severe COVID-19, however, microglia exhibit persistent NFKB-responsive gene expression that may ultimately lead to CNS dysfunction.

Knockdown of *rad23a* confers benefits in a mouse model of TDP43 proteinopathy

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In several neurodegenerative diseases, the TAR DNA binding protein of 43 kDa molecular weight (TDP43) mis-localizes to the cytoplasm and adopts a non-native conformation leading to aggregation. It is likely that a combination of loss- and gain-of-function events underlies its noxious effects on neuronal health and survival. Neuronal expression of a mutant version of TDP43 in the nervous system of *Caenorhabditis elegans* yields a marked uncoordinated (unc) locomotor phenotype and in a screen for modifiers, we found that loss of *rad23* suppressed the unc phenotype. In addition, in a rat spinal cord *in vitro* model we found expression of mutant TDP43 killed motor neurons and this was suppressed by RNAi mediated knockdown of the two mammalian orthologs *rad23a* and *rad23b*. We have now extended these observations to the mouse model in which wild type human TDP43 is expressed under the control of the Thy-1 promotor (the Tar4/4 mouse). Intracerebroventricular injection of antisense oligonucleotides (ASO) targeting *rad23a*, but not a scrambled sequence, into the newborn mice leads to a dramatic, specific and sustained knockdown of its target. Reducing the abundance of RAD23A increases the average life span of TAR4/TAR4 from 22 ± 3 days to 31 ± 3(*) days ($p < 0.001$ in Gehan-Breslow-Wilcoxon statistical test). This beneficial effect was seen in both males and females. Knockdown of *rad23a* reduced the abundance of cytosolic TDP43 as well as sarkosyl-insoluble TDP43. The significant increase in total ubiquitin levels in Tar4/4 mice was blunted by knockdown of *rad23a*. This work indicates that reducing the abundance of RAD23A may be useful in neurodegenerative diseases with mislocalized and misfolded TDP43.

Identification of Predicted Key Interaction Domains of TDP-43 Reveals Involvement in Downstream Cellular Events

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Background: TDP-43 is a DNA/RNA binding protein that is implicated in key cellular events, and 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.

Methods: 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 of the two protein structures and analyzes different potential conformations for proteins 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 pairings are 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.

Results: 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.

Discussion: 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 spots 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.

High density multi-electrode array recordings in-vitro help investigate upper motor neuron health and connectivity in ALS

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Background: Cortical hyperexcitation is suggested as a potential contributor to upper motor neuron (UMN) vulnerability in ALS. In addition, defects in cortical connectivity may contribute to disease pathology. We aim to characterize the electrophysiological profile of neuronal networks in healthy motor cortex and the motor cortex of disease models of ALS, such as hSOD1G93A, hPFN1G118V and TDP-43A315T, which recapitulate different aspects and pathologies of the disease.

Hypothesis: Cortical connectivity defects will inform about ALS pathology and will form a baseline to test UMN response to therapeutic drug screenings.

Methods: Transgenic UMN reporter lines were generated to study UMN response at a cellular resolution. We utilize high density multi-electrode array (HD-MEA) technology to record action potentials and local field potentials of primary cortical neuronal cultures prepared from UCHL1-eGFP, hSOD1G93A-UeGFP, hPFN1G118V-UeGFP and TDP-43A315T-UeGFP mice. The HD-MEA, based on active pixel sensor technology employing a complementary metal–oxide semiconductor chip, records extracellular voltage variations simultaneously across 4096 microelectrodes, elucidating the electrophysiological differences.

Results: We find that basal spontaneous activity, defined by single-spiking events, begin to occur as early as 14 days in-vitro (DIV) and bursting events synchronize by 21 DIV. Currently, we are testing changes in cortical connectivity and activity of UMNs diseased due to misfolded SOD1 toxicity, profilin mutations and TDP-43 aggregation.

Discussion: The HD-MEA system, with its optimized micro-electronics allowing high spatial-temporal resolution, offers a valuable tool to understand the dynamic properties of neuronal networks and lays the foundation for future functional studies with respect to drug discovery.

Phase 3b, Multicenter, Randomized, Double-Blind, Parallel-Group Study to Evaluate Efficacy and Safety of Oral Edaravone Administered Over 48 Weeks in Patients with Amyotrophic Lateral Sclerosis (MT-1186-A02)

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Introduction: An intravenous (IV) formulation of edaravone (Radicava®/Radicut®) was shown to slow the rate of physical functional decline in amyotrophic lateral sclerosis (ALS). This ongoing, multicenter, phase 3b, double-blind, parallel group, randomized study is currently evaluating 2 dosing regimens of Radicava ORS® (edaravone) oral suspension, which was recently approved by the US Food and Drug Administration for use in patients with ALS.

Objective: To evaluate and compare the long-term safety, efficacy, and tolerability of 2 dosing regimens of oral edaravone suspension (MT-1186) for a period of 48 weeks in patients with ALS.

Methods: Study MT-1186-A02, which began in December 2020 and is estimated to complete in December 2023, will equally randomize patients into 2 treatment groups. Group 1 will have oral edaravone (105-mg dose) administered once daily for 28 days for 12 cycles, and group 2 will have oral edaravone administered for 14 days, followed by placebo for 14 days in Cycle 1. Subsequently, in group 2, oral edaravone will be administered for 10 days followed by placebo for 18 days in Cycles 2-12. Study MT-1186-A02 is anticipated to include approximately 380 adult patients diagnosed with definite or probable ALS, baseline forced vital capacity ≥70%, and baseline disease duration ≤2 years. The primary objective is to evaluate the efficacy of each dosing regimen based on the changes from baseline to week 48 in the revised ALS Functional Rating Scale score. Secondary objectives will evaluate the safety and tolerability of each dosing regimen. Exploratory objectives include investigation of changes in nerve conduction tests and biomarkers.

Results: Ongoing.

Summary/Conclusion: This study will provide important information on the safety, efficacy, and tolerability of 2 dosing regimens for oral edaravone suspension in patients with ALS.

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Significant immunomodulatory effects observed with NurOwn (MSC-NTF cells) treatment reveal a reciprocal relationship in changes in CSF inflammatory and anti-inflammatory biomarkers in a phase 3 trial

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Introduction: Neuroinflammatory mechanisms play an important role in ALS disease progression through direct and indirect effects on motoneurons (Brown et. al. 2017). MSC-NTF cells are known to reduce T cell activation, induce T and B regulatory cells and secrete anti-inflammatory cytokines and micro-RNA molecules (Kern et. al. 2020), in addition to delivering neuroprotective factors. miR-146a, secreted by MSC-NTF cells, is a key regulator of the astrocyte-mediated inflammatory response (Iyer et. al. 2012). Previously we demonstrated reductions in inflammatory CSF biomarkers in a phase 2 clinical trial in ALS participants (NCT02017912) following a single intrathecal dose of autologous MSC-NTF cells (100 - 125 x 106 cells; Berry et. al. 2019). We sought to confirm these biomarker observations and to explore if a reciprocal relationship with CSF anti-inflammatory biomarkers existed with inflammatory markers following repeated administration of autologous MSC-NTF cells in a phase 3 ALS clinical trial (NCT03280056).

Methods: CSF was collected at 7 time points, at baseline (before treatment) and through week 20. CSF biomarker methods and statistical analyses were previously described (Cudkowicz et. al. 2022).

Results: We observed a reciprocal relationship with MSC-NTF cells treatment between inflammatory and anti-inflammatory biomarkers, with significant longitudinal decreases in inflammatory (MCP-1, SDF-1a) and increases in anti-inflammatory (miR-146a) biomarkers observed at 20 weeks, while values in the placebo treatment group remained unchanged. These results confirm the immunomodulatory effects observed in the ALS phase 2 randomized clinical trial.

Conclusion: MSC-NTF cell therapy results in reciprocal longitudinal changes in CSF inflammatory and anti-inflammatory biomarkers through 20 weeks. These observed modifications to the ALS inflammatory microenvironment may contribute to slowing the rate of ALS disease progression.

References:
1. Brown et al, NEJM 2017
2. Kern et al, Amyotrophic Lateral Sclerosis Frontotemporal Dementia, 2020
3. Iyer et al, PLOSOne 2012
4. Berry et al, Neurology 2019
5. Cudkowicz et al, Muscle & Nerve, 2022

Analysis of Ataxin2 Targets as Mediators of Amyotrophic Lateral Sclerosis

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To better understand the molecular neuropathology of ALS, we have been analyzing the mechanisms by which the ALS risk gene and RNA-binding protein Ataxin2 functions in neural function and degeneration. Stress granules play a central role in ALS pathogenesis. Ataxin2 is a component of these stress granules that harbor translationally arrested mRNAs and translation factors. Using Ataxin2 RNA immunoprecipitation and Ataxin2 RNAi perturbation in combination with RNA-sequencing, we have identified hundreds of potential biochemical and functional RNA targets of Ataxin2. Among these functional targets is the transcription factor CrebA which we in turn demonstrated is an in vivo effector of Ataxin2 function in mediating Huntingtin-mediated neurodegeneration. We hypothesize that a subset of these Ataxin2 targets is critical to mediating Ataxin2 function in ALS pathogenesis. Our first objective is to screen these candidates in novel high throughput in vivo behavioral screens to identify those capable of suppressing circadian and sleep phenotypes in TDP-43-based models of ALS in Drosophila. We have developed a high throughput behavioral platform in Drosophila that enables in vivo screening of genetic modifiers of TDP43 effects on circadian rhythm function. Interestingly, disrupted circadian rhythms of the hormone cortisol as well as disturbed sleep have been observed in those with ALS, consistent with disrupted clock function. This platform, in contrast to fly eye morphology screens, will also enable the detection of disease modifiers at steps preceding cell death when therapeutic intervention would ideally be timed. Using this approach, we have identified candidate mediators of TDP43 effects on circadian behavior. Our ultimate goals are to reveal novel mechanisms by which Ataxin2 functions and to provide a number of solid, evidence-based candidates for pharmacological intervention to develop ALS therapeutics.

Stabilizing Microtubules Rescues Disrupted Nucleocytoplasmic Transport in Loss-of-Function Models of the ALS Gene NEK1

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The overwhelming majority of ALS is sporadic in nature, while a small (<12%) but highly informative fraction of patients suffers from familial forms of disease. Recently, a number of whole-exome sequencing studies have identified a significant enrichment of loss-of-function (LOF) variants in a novel identified gene NEK1, which encodes the NIMA-related kinase 1 (NEK1) protein, in ALS patients. Following numerous validation studies, these LOF heterozygous variants have since been estimated to confer susceptibility for up to 3% of all (sporadic/familial) ALS patients and can thus be considered the third most common genetic cause of ALS. NEK1 is one of 11 NIMA-related kinases (NEKs) that functions in cell cycle regulation, meiosis, and DNA damage in dividing cells. However, the physiological role of NEK1 in MNs, as well as the functional impact of ALS-linked mutations, has yet to be fully elucidated.

Mass spectrometry-based proteomics analyses identified novel NEK1 interactors and NEK1-dependent protein expression changes in human iPSC-MNs that converged on proteins enriched for function in the microtubule (MT) cytoskeleton and nucleocytoplasmic (N/C) transport. Two key NEK1 interactors involved in these pathways, a-tubulin (TUBA1B) and importin beta-1 (KPNB1), contain predicted NEK1 phosphorylation motifs and were shown to be phosphorylated by NEK1 via in vitro kinase assays. Using two independent iPSC-MN models of NEK1 haploinsufficiency, we additionally found strong evidence for functional impairments in MT homeostasis and nuclear import following NEK1 loss-of-function. These effects were also observed in vivo, following reduction of the Drosophila NEK1 homologue niki, and produced age-dependent motor and survival phenotypes. Importantly, treatment with the MT-stabilizing drug paclitaxel restored NEK1-dependent deficits in both pathways.

ALS associated TDP-43 pathology disrupts KCNQ2 function

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder that affects motor neurons of the brain and spinal cord. Nuclear depletion and cytoplasmic aggregation of TAR DNA Binding Protein 43 kDa (TDP-43) in patient post-mortem tissue is a neuropathological a hallmark of ALS. TDP-43 is an essential DNA/RNA binding protein predominantly localized to the nucleus where it is responsible for crucial RNA-processing and transport. Mislocalization of TDP-43 to the cytoplasm has recently been linked to the mis-splicing of several genes that appear to be human and neuron-specific targets. We describe here that nuclear loss of TDP-43 leads to mis-splicing of the voltage-gated potassium channel subunit KCNQ2 (Kv7.2). Kv7.2 is expressed widely in neurons of the CNS where it is a key regulator of neuronal excitability, demonstrated by its associated with severe developmental epilepsy. With TDP-43 in the cytoplasm, a significant proportion of Kv7.2 transcripts are mis-spliced leading to a removal of exon 5. The functional repercussion of TDP-43 driven KCNQ2 mis-splicing, as well as the relevance to ALS pathophysiology remains unclear. Using several model system including heterologous expression of KV7 plasmids, stem cell derived-neurons, and postmortem ALS tissue, we find that the deletion of exon 5 results in a non-functional Kv7 channel, cytosolic protein aggregation and altered neuronal excitability.

Traumatic injury exacerbates neuropathology in C9orf72 ALS/FTD motor neurons

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There are many documented mutations that have been implicated in amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Research and epidemiological studies have demonstrated that some of these mutations are not completely penetrant. This incomplete penetrance suggests that environmental factors can contribute to an ALS/FTD diagnosis. Neurotrauma is an established epidemiological risk factor in neurodegenerative disease, but a specific clinical link between trauma and ALS/FTD remains controversial. One common pathology between ALS/FTD and traumatic injury cases is the nuclear depletion and cytosolic accumulation of TAR DNA-binding protein 43 (TDP-43). However, it is unclear if the mechanisms causing mislocalization in ALS/FTD or traumatic injury overlap. Furthermore, it is unclear if ALS/FTD-associated mutations intrinsically increase vulnerability to neurotrauma. The limitations of epidemiological studies and inability to prove cause and effect facilitates the need to develop biofidelic preclinical models that recapitulate the human condition. In this work, we aim to investigate neurotrauma within the scope of C9orf72, the largest genetic cause of ALS/FTD, using a highly reproducible system designed to model stretch injury in motor neurons derived from human induced pluripotent stem cells (iPSCs). We find that mutant C9orf72 motor neurons exhibit increased susceptibility to a single episode of severe trauma that presents immediately following injury. A single traumatic event induces extended TDP-43 mislocalization in ALS motor neurons, whereas only a brief mislocalization is observed in isogenic controls. This results in increased mis-splicing of STMN2 and UNC13A, downstream targets of TDP-43, in mutant C9orf72 motor neurons. Notably, multiple injuries induced sustained mislocalization of TDP-43 beyond the recovery time of a single stretch injury. Trauma exacerbates specific neuropathology in mutant C9orf72 motor neurons, evident as an increased presence of toxic RNA foci. Increased production of dipeptide repeat proteins was not observed. Lastly, pre-treatment with antisense oligonucleotides (ASOs) targeting the C9orf72 repeat expansion prevented the trauma-induced increase in RNA foci. Taken together, our results characterize the role trauma plays in enhancing neurodegenerative disease pathology through extended mislocalization of TDP-43 and increased production of RNA foci in mutant C9orf72 motor neurons.

2-Year-Old and 3-Year-Old Italian ALS Patients with Novel ALS2 Mutations: Identification of Key Metabolites in Their Serum and Plasma

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Pathogenic variants in ALS2 have been detected mostly in juvenile cases of amyotrophic lateral sclerosis (ALS), affecting mainly children and teenagers. Patients with ALS2 mutations demonstrate early onset cortical involvement in ALS. Currently, there are no effective treatment options. There is an immense need to reveal the underlying causes of the disease and to identify potential biomarkers. To shed light onto the metabolomic events that are perturbed with respect to ALS2 mutations, we investigated the metabolites present in the serum and plasma of a three-year-old female patient (A0) harboring pathogenic variants in ALS2, together with her relatives, healthy male and female controls, as well as another two-year-old patient DH, who had mutations at different locations and domains of ALS2. Serum and plasma samples were analyzed with a quantitative metabolomic approach to reveal the identity of metabolites present in serum and plasma. This study not only shed light onto the perturbed cellular pathways, but also began to reveal the presence of a distinct set of key metabolites that are selectively present or absent with respect to ALS2 mutations, laying the foundation for utilizing metabolites as potential biomarkers for a subset of ALS.

Neuroimaging insights into the pathogenesis of UMN-predominant ALS subtypes

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Amyotrophic lateral sclerosis (ALS) is an incurable and progressively fatal neurodegenerative disease that manifests with varying proportions of upper motor neuron (UMN) and lower motor neuron (LMN) dysfunction, as well as occasional cognitive/behavioral impairment. Such clinical phenotypes are distinguishable by clinical, electrodiagnostic and neuroimaging studies.

Conventional magnetic resonance imaging (cMRI) of brain using T2- and proton density (PD)-weighted sequences reveals hyperintense signal along the corticospinal tract (CST) in a proportion of ALS patients who present with UMN-predominant signs (e.g. spasticity, pathologic reflexes). These ALS-CST+ patients tend to be younger and have significantly faster disease progression than other patients with UMN-predominant ALS who show no CST hyperintensity (ALS-CST–). We hypothesize that such ALS-CST+ patients comprise a clinical subtype distinct from other ALS subtypes, including ALS-CST– patients and those with classic ALS (cALS) in whom UMN and LMN dysfunction is more equally present.

Advanced MR techniques such as diffusion tensor imaging, diffusion tensor tractography, and novel analytic approaches such as fractal dimension (FD) analysis, graph theory network analysis, and 3D FD analysis using machine learning all reveal significant differences in brain abnormalities between ALS-CST+ and the aforementioned ALS subtypes. This indicates differences in regional brain degeneration and suggests potential differences in disease pathogenesis.

Future MRI studies of ALS patient CNS will explore additional abnormalities distinguishing disease subtypes. Generation of motor neurons and neuroglia from induced pluripotent stem cells from ALS-CST+ and ALS-CST– patients will be analyzed for potential molecular signatures (e.g. RNA, protein) distinguishing them.

Linking genes to neurorehabilitation practice: Development of human stem cell model to isolate gene-neurotrauma interactions

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Background: Neurotrauma is the most common injury leading to death and long-term disability. Injury complexity has made it hard to understand the high variability in outcomes, even when similar injuries occur. Genetic variances, which might have implications in the ways the central nervous system responds to trauma and motor rehabilitation, are poorly understood.1 The use of human isogenic stem cell lines, where a genetic variant is isolated against uniform background, allows us dissect the role of genetic variants in neurorehabilitation models. We study the val66met single nucleotide polymorphism (SNP) in the brain derived neurotrophic factor (BDNF) gene, which has been associated with altered neurorehabilitation outcomes.2,3 We hypothesize that motoneurons generated from human induced pluripotent stem cells (hiPSC) carrying the met genotype with respect to this SNP will develop normally, but have impaired activity-dependent release of BDNF and higher vulnerability to injury.

Methods: Two independent sets of isogenic hiPSC lines were generated using CRISPR-Cas9 consisting of the val and met genotype of the val66met SNP. These were differentiated into motoneurons following an established differentiation protocol.4 96 well plates with multi-electrode arrays (MEA) were used for electrophysiological characterization using Maestro Pro (Axion BioSystems). Flexible plates were used to perform in-vitro stretch injury.5 BDNF protein collected from media supernatant was measured using BDNF ELISA (R&D Systems) 48 hours post-plating. Cell viability was evaluated using lactate dehydrogenase assay (Promega) 48 hours post-injury.

Results: The results show initial firing at day 5 after plating, and bursting activity at day 10. Firing rate increased from day 5 to 25 in all lines, with no statistical significant differences in firing rates between all lines per day. Val genotype showed 40% higher BDNF release compared to met genotype. Met genotype showed increased cell death after injury (25% Lagrangian strain).

Conclusion: Our results demonstrate impaired BDNF release phenotype in met genotype, supporting previous animal studies.3 Due to increased vulnerability to injury, the met genotype could have a role in acute neuronal survival after injury. Cell genotype did not alter motoneuron differentiation, electrophysiological development or firing patterns. Future research involves transitioning to 3D in-vitro models to increase biofidelity of injury and cellular diversity, to better define mechanisms contributing to high outcome variability and develop personalized rehabilitation approaches.

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

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Background: 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.

Method: 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.

Results: 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.

Conclusion: 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.

Cell biology of dipeptide repeat protein cell-cell transmission

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C9orf72 ALS is the most common genetic form of ALS, resulting from an intronic GGGGCC hexanucleotide repeat expansion in the *C9orf72* gene. One proposed patho-mechanism 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. 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 method and bath applied HA (hemagglutinin tagged)-$(PR)_{20}$ to both HeLa cells and primary rat mixed spinal cord cultures. HA-$(PR)_{20}$ is rapidly internalized and localizes to the nucleolus as soon as 30 minutes in both cell types. We applied several endocytosis inhibitors to demonstrate that poly-PR internalization is accomplished through endocytic processes. Poly-PR endocytosis was potently inhibited by temperature shifts, clathrin-mediated inhibitors, dynamin inhibitors, and macropinocytosis inhibitors. We plan to use a proximity labeling technique 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 in internalization of toxic DPRs and targeting them could prevent transmission between cells and slow disease progression.

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Proteome-Wide Degradation Dynamics in ALS SOD1 Patient Neurons Reveal Disrupted VCP Homeostasis

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Mutations in SOD1 cause ALS through gain-of-function effects driven by protein misfolding. The mechanisms by which mutant SOD1 protein impairs proteostasis in patient motor neurons (MNs) are unclear. Here, we used an induced pluripotent stem cell (iPSC) isogenic model coupled to stable isotope labeling with amino acids in cell culture (SILAC) and mass spectrometry-based proteomics, to monitor proteome-wide degradation dynamics. We found that several proteins involved with polypeptide folding and cytoskeletal pathways degrade more slowly in patient-derived MNs. Amongst the “older” proteins is the ALS-causal VCP, which plays a key role in proteasome-dependent degradation and autophagy. The interactome of VCP is altered in mutant SOD1 MNs in vitro, while overexpression of VCP rescues mutant SOD1 toxicity in patient neurons in vitro and C. elegans model in vivo. Our results suggest that VCP contributes to SOD1-dependent degeneration, linking these two known ALS causal genes and highlighting the importance of selective protein degradation impairment in ALS pathophysiology.

Peripheral Nerve Interfacing to Deliver Therapeutic Electrical Stimulation with a Wireless, Battery-Free System

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Introduction: Therapeutic electrical stimulation of peripheral nerves has been shown to enhance axon regeneration in clinical and preclinical studies. Our recent work has shown that stimulation delivered proximal and distal to nerve repair site have distinct benefits on regeneration and functional recovery, so here we test the hypothesis that combining these two sites of therapeutic stimulation will have additive benefit. Our approach is to deliver therapeutic electrical stimulation with a wireless, battery-free and fully implantable peripheral nerve interface, a new class of devices can offer capabilities that match or exceed those of their wired or battery-powered precursors.

Objective: To employ novel bioresorbable materials for a type of thin, flexible and wireless implant that provides precisely controlled therapeutic electrical stimulation of the injured nerve in order to both augment axon regeneration and reduce denervation related muscle atrophy.

Methods: 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 control model were also utilized to determine the effectiveness of the combined treatment.

Conclusion: In devices with optimized, wireless designs, our novel polymers enable safe, short-term operation for enhancing functional recovery in rat model of nerve injury. Long term experimental measurements are still underway to determine extent of therapeutic benefits.

Comprehensive electrical stimulation therapy to improve phrenic nerve regeneration and recovery of diaphragm muscle function on rats

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Phrenic nerve injury leads to diaphragmatic paralysis, causing shortness of breath, recurrent pneumonia, anxiety, insomnia, morning headache, excessive daytime somnolence, orthopnea, and fatigue. There is lack of epidemiological studies to show the prevalence of phrenic nerve injury due to underrecognizing the injury, at our center an unpublished retrospective analysis showed idiopathic phrenic neuritis to be the most common single cause of phrenic nerve injury. Natural recovery not only takes up to 2 years but still leaves two thirds of patients with unsatisfactory diaphragmatic function. Here, we propose an integrated therapeutic electrical stimulation approach to both the phrenic nerve and affected diaphragm with novel, wirelessly powered battery free devices to achieve a better functional recovery. These devices reduce the risk of infection inherent with transcutaneous temporary leads, avoid the use of externally powered hardware that can be dislodged when caring for a patient, and do not require a secondary removal surgery. The phrenic nerve stimulator uses advanced materials that bioresorb harmlessly to benign products and the elastomeric polymer cuff design interfaces seamlessly with the proximal nerve stump. With the right stimulation parameters, there is an improvement on the speed and efficiency of axon regeneration and reinnervation. The diaphragm stimulator utilizes highly stretchable, bioresorbable wire electrodes that can be attached directly to the muscle surface and ensure an ideal electrical interface. With direct, paced, stimulation, we can decrease the muscle degeneration during the denervated period and maintain respiratory function. Together, we demonstrated a comprehensive electrical stimulation therapy to enable better recovery from phrenic nerve injury on a rat model.

Characterizing the Target ALS RNA-Seq data set through differential expression, cellular deconvolution, and missplicing analysis

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Amyotrophic lateral sclerosis (ALS) is the third most common neurodegenerative disease, with approximately 5,000 new patients diagnosed each year in the US alone. Despite a decades-long global effort, few effective treatment options exist, and several large-scale consortiums have been assembled to facilitate research into the underlying disease mechanisms.

Here, we obtained RNA-Seq data from cerebellum, cortex, and spinal tissues from the Target ALS genomic repository. For each tissue, we performed differential expression analyses between healthy subjects and C9orf72, SOD1, and sporadic ALS patients. As expected, cerebellum and occipital cortex tissues showed few to no differentially expressed (DE) genes between healthy subjects and ALS subtypes [FDR<0.05]. On the contrary, in motor cortex and spinal tissues – which are affected in ALS – we found between 55 (in sporadic motor cortex) and 1027 (in SOD1 lumbar spine) DE genes compared to healthy, with spinal subsets showing the strongest changes in all ALS subtypes. Gene set enrichment analyses of spinal tissues indicated strong overexpression of genes related to microglia and underexpression of genes related to oligodendrocytes, suggesting changes in cell type composition may be driving differential expression.

To test this hypothesis, we performed cellular deconvolution of the tissue samples using CIBERSORTx and identified drastic cell type differences in ALS cervical and lumbar spine. In all ALS subtypes, we observed significant increases in the proportion of astrocytes, microglia, and oligodendrocyte progenitor cells compared to healthy samples. Decreases were seen in the proportion of oligodendrocytes (significant in all ALS subtypes) and excitatory neurons (significant in C9orf72 and sporadic subtypes). Notably, oligodendrocytes in SOD1 samples were significantly decreased in relation to other ALS subtypes as well as healthy.

Finally, we investigated TDP-43 dysfunction by searching for TDP-43-related missplicing events in each sample. We find evidence of increased TDP-43-related missplicing events in motor cortex and spinal tissue samples of some C9orf72 and sporadic patients. As expected, our data do not show any evidence of TDP-43-related dysfunction in SOD1 ALS.

Together, this work serves as an exploration and characterization of the Target ALS RNA-Seq resources, a large-scale data set derived from CNS tissues of ALS patients and healthy subjects. Our goal is to provide insight to other researchers in order to effectively use these data for downstream analyses.

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 cases. 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.

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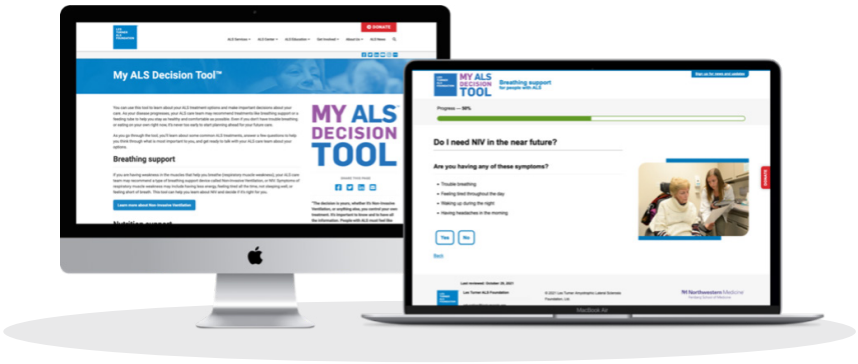

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About The Les Turner ALS Foundation

The story of the Les Turner ALS Foundation begins with one man's ALS diagnosis in 1976. Les Turner, a Chicago-area businessman, husband and father, was frustrated by the lack of local resources available to manage the devastating effects of the disease. His family and friends shared his determination to ensure that other people with the disease would have the support, resources and hope for a cure that were unavailable to him.

Today, the Les Turner ALS Foundation is the oldest independent ALS group in the country. For 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.

The Foundation began funding ALS research annually in 1979, followed by the opening of the multidisciplinary Lois Insolia ALS Clinic in 1986. Since 1979, the Foundation has donated more than $32 million directly to Northwestern University in addition to millions more indirectly and has been a steadfast partner in the establishment, direction and growth of the ALS program. Established in 2014, the Les Turner ALS Center at Northwestern Medicine brings together the worlds of ALS research and clinical care and is led by the most well-respected and successful clinicians and researchers in the field.

In 2022, the Foundation made a commitment of $1.1 million to the Les Turner ALS Center at Northwestern Medicine, funding research toward more effective treatment and cures for ALS as well as world-class multidisciplinary patient care at the Lois Insolia ALS Clinic. Led by Robert G. Kalb, MD, the Center has launched a new ALS Research Pilot Grant Program that supports promising early-stage ALS research projects by a broad group of scientists.

Together, we will create a world free of ALS. To learn more and donate to support our work, please visit **lesturnerals.org**.

Notes