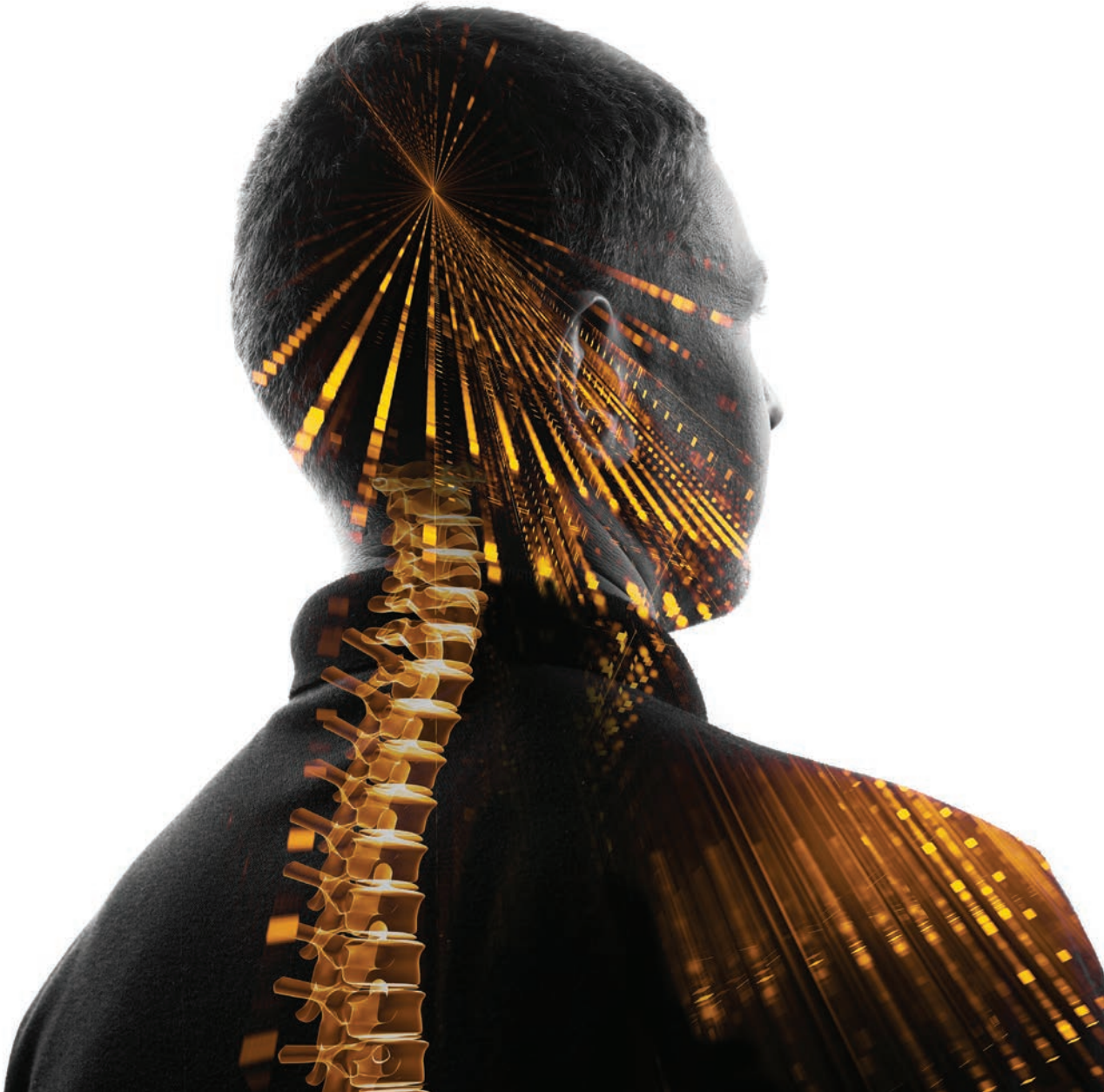
Jefferson Health

VICKIE AND JACK FARBER INSTITUTE FOR NEUROSCIENCE
OUTCOMES & RESEARCH: VOLUME 4

PRECISION MEDICINE

FOR AMYOTROPHIC LATERAL SCLEROSIS (ALS)



A MESSAGE FROM THE PRESIDENT/CEO, VICKIE AND JACK FARBER INSTITUTE FOR NEUROSCIENCE

Dear Colleagues,

Thomas Jefferson University Hospital's Departments of Neurology, Neurosurgery and Neuroscience at the Vickie and Jack Farber Institute for Neuroscience – Jefferson Health are constantly engaged in research that will help discover innovative treatments for various neurologic conditions, thus improving patient care.

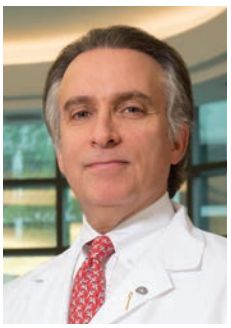
I am pleased to be sending you the fourth volume of our series that describes our research and illustrates the superior patient care that our departments and physicians are engaged in at Jefferson. In previous brochures we have described research in spinal cord injury, cognitive functioning in temporal lobe epilepsy, and outlined ongoing clinical trials and treatment for both aneurysms and stroke.

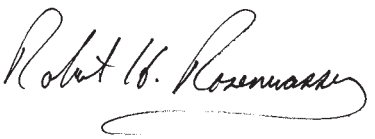
In this current volume, we highlight the work Jefferson Health is doing in the area of amyotrophic lateral sclerosis (ALS), a particularly devastating condition. Piera Pasinelli, PhD, Davide Trotti, PhD, Aaron Haeusler, PhD, and Hristelina Ilieva, MD, PhD, from the Jefferson Weinberg ALS Center, examine a group of proteins in the blood, urine and cerebrospinal fluid (CSF) in people with ALS and employ patient-derived motor neurons by using induced pluripotent stem cells (iPSCs) to identify accurate ALS biomarkers for disease diagnosis and progression, and to identify the molecular signature of different groups of patients. Such biomarkers represent the template for the identification of the pathogenic mechanisms and therapeutic targets specific for different forms of ALS, and for understanding how patients respond to therapy. Such biomarkers, pathways, and targets are critical for advancing clinical trials of ALS treatments, thus extending life and bringing us closer to a cure for ALS.

At Jefferson Health, we value our clinicians and researchers who contribute to the well-being of patients with neurological impairment and disability. We continue to use stem cell treatments and other approaches for patients with various neurologic conditions. All our treatment modalities are based on substantive research.

I invite you to explore our research for patients with ALS in the following pages. I hope you will find it informative to read about the ways we are working to enhance the quality of life for these, and other, patients.

Sincerely,





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INTER-DISCIPLINARY SYMPTOMATIC TREATMENT AND RESEARCH: A CLINICAL & RESEARCH INTEGRATED PROGRAM FOR ALS

The Jefferson Health Weinberg ALS Center

Every year, approximately 5,000 Americans hear the words: "You have ALS." It's a devastating diagnosis with still no known cure or treatment to halt the disease. Riluzole and Radicava, the two FDA-approved drugs for ALS only work marginally, slowing down disease progression by 3 to 6 months, depending on the patient. The only hope for ALS patients and their families is in the precision medicine research to identify better therapies and in personalized clinical care to alleviate the patients' symptoms, improve their quality of life, and reduce their financial and emotional burdens.

With this drive, Jefferson Health operates a multi-disciplinary, patient-centric, clinical and research integrated program (CRISP) where the complex needs of ALS and ALS/FTD patients and their caregivers are met. (See Figure 1)

The Clinical and Research Integrated Strategic Program (CRISP) model approaches organization of health care by disease rather than by medical specialty.

This integrated approach breaks down the traditional silos of the academic model and places the needs of the patient at the center of care.

Figure 1. The Jefferson Health Weinberg ALS Center: a model for CRISP.
Source: Weinberg ALS Center

SCIENCE BEHIND ALS: CURRENT AREAS OF STUDY

As much as ALS is clinically a complex disease, thus requiring inter-disciplinary care spanning multiple clinical, social and psychological areas, it is also complex and heterogeneous at the molecular level. In order to tackle this complexity, the Weinberg ALS Center operates four research laboratories, and a clinical-research program, under the guidance of Piera Pasinelli, PhD; Davide Trotti, PhD; Aaron Haeusler, PhD; and Hristelina Ilieva, MD, PhD. Each laboratory has unique expertise tailored to studying a specific aspect of the disease, together covering the breath of ALS pathogenesis. (See Table 1). Because each patient (or group of patients) is unique, the Weinberg Center's approach to research starts in the clinic where biofluids and/or cells are obtained from patients. These are taken to the laboratory, where they are used to identify biomarkers and to explore pathogenic processes. Ultimately, each process is a potential therapeutic target that hopefully can be translated back to individual patients in the clinic.

Table 1. Summary of Current Research Projects

Area of Research	Specific Projects
Disease transmission	• Investigating the spread of disease pathology between neurons • Elucidating the contribution of astrocytes to disease toxicity
Modelling	• Creating of a genetic mutant mouse model of C9orf72 ALS • Creating of cellular models of ALS, including iPSC lines and neuronal lines
DNA/RNA dysfunction due to C9orf72 repeat expansion	• Evaluating DNA damage caused by repeat expansions • Investigating the secondary DNA and RNA structures formed by repeat expansion and the effect of methylation • Characterizing the interaction between repeat expansions and methylation enzymes • Studying protein sequestration by pathological RNA structures
Dipeptides formed by repeat expansions	• Determining the molecular requirements for pathology • Understanding the role of dipeptides in disease onset and progression • Elucidating the neurotoxic effects of arginine rich dipeptides on protein synthesis machinery • Evaluating the effects of dipeptides on synaptic transmission, calcium dynamics and synaptic vesicle proteins
Biomarker	• Identifying molecules within patient biofluids to serve as biomarkers of disease onset and progression
Pharmacoresistance	• Investigating and improving drug delivery across the blood-brain and spinal cord barriers

Whilst research within the Weinberg ALS Center encompasses many areas, some recent work has focused on C9orf72 repeat expansions – the most recently discovered, and prevalent, gene mutation associated with ALS – and examination of pharmacoresistance in ALS.

Articles based on these two areas of research are described below.

Ongoing Research into C9orf72 Repeat Expansions[1]

The discovery of C9orf72 repeat expansions by two independent outside groups[2,3] has provided novel insights into the pathogenesis of ALS (and frontotemporal dementia) and highlights the importance of non-coding repeat expansions and RNA toxicity in neurodegenerative diseases. Jefferson Health is engaged in building on this recent discovery with their own research, which is described here.

A hexanucleotide repeat expansion (HRE) of a noncoding GGGGCC repeat within the chromosome 9 open reading frame 72 (C9orf72) gene is now seen as a major cause of ALS. Approximately 90% of ALS cases are sporadic and the other 10% have a family history of the disease. The C9orf72 HRE represents the most common genetic cause of both familial and sporadic ALS.

Dr. Haeusler and his team have looked at normal human C9orf72 alleles, which have 2 to 25 intronic GGGGCC repeats, with the majority having fewer than 8 repeats and more than half having only 2 repeats. Expanded repeats associated with ALS, however, are thought to have variable lengths, ranging from 10 to thousands of HREs, but correlations between the repeat lengths and clinical onset or disease progression have yet to be established.

Researchers at Jefferson Health have been examining how the HRE forms DNA and RNA G-quadruplexes with distinct structures that promote RNANDNA hybrids (R-loops). Their research suggests that the structural polymorphism causes a repeat-length-dependent accumulation of transcripts aborted in the HRE region. These transcribed repeats bind to ribonucleoproteins in a conformation-dependent manner. Specifically, nucleolin, an essential nucleolar protein, preferentially binds the HRE G-quadruplex, resulting in cellular stress.

Results of this study demonstrate that the distinct C9orf72 HRE structural polymorphism at the DNA/RNA level initiates molecular cascades leading to ALS pathologies. This study of C9orf72 provides the basis for a mechanistic model for repeat-associated neurodegenerative diseases.

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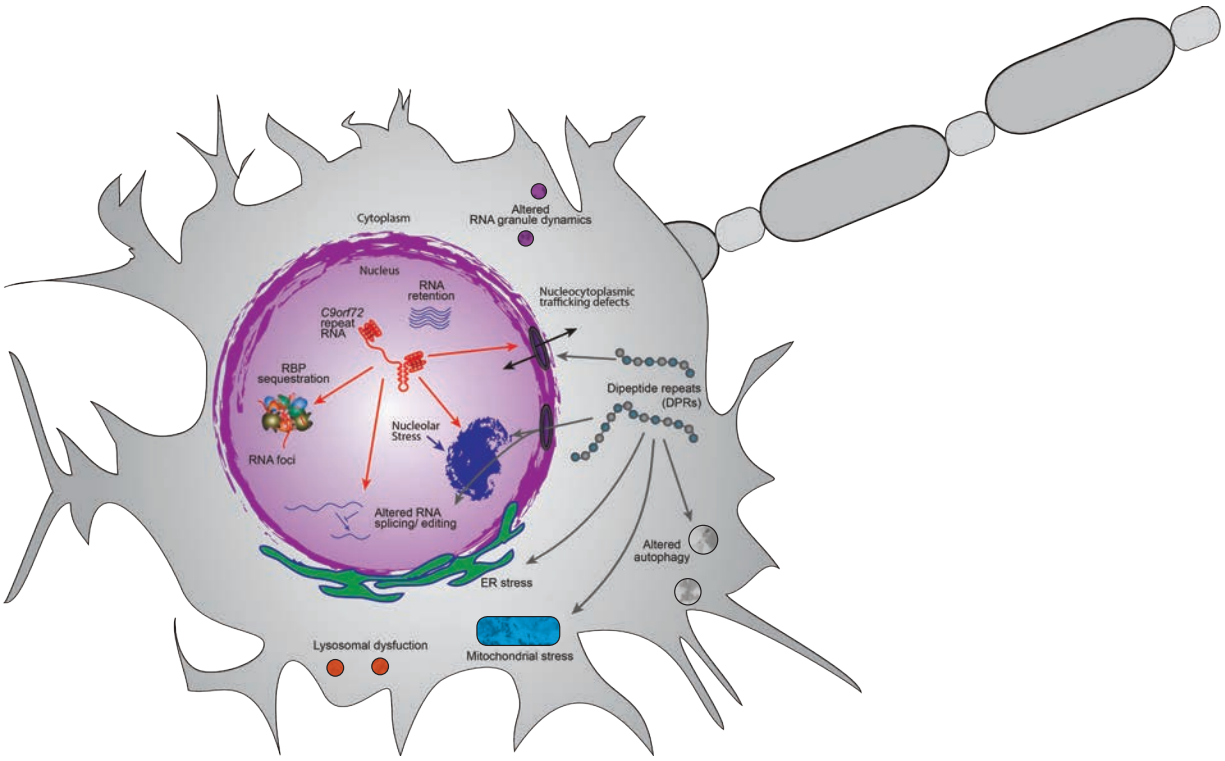

Figure 2. Pathogenic pathways to neurodegeneration and potential therapeutic targets in C9orf72-linked ALS.
Source: Aaron Haeusler, PhD

Nucleotide Repeat Expansions and C9orf72 in ALS[1]

Nucleotide repeat expansions (NREs) have been associated with over 30 neurological and neuromuscular disorders with non-AUG-dependent translation of NREs emerging as a potential common pathogenic feature of many of the diseases. NREs are prevalent mutations in ALS as well.[2] Repeat-associated non-AUG (RAN) translation of these repeat regions produces mono- or di-peptides that contribute to the pathogenesis ALS. The mechanisms and drivers of RAN translation are not yet well understood. Our results provide a new mechanistic understanding for the role of RAN translation in driving disease pathogenesis and elucidate new therapeutic approaches to attenuate NRE-linked neurodegeneration, which can be tested in the clinic immediately.

This study conducted by Drs. Pasinelli, Trotti and Haeusler analyzed whether different cellular stressors promote RAN translation of dipeptide repeats (DPRs) that are associated with the G4C2 hexanucleotide expansions in C9orf72.

Pathological features resulting from non-AUG-dependent translation have been well- catalogued in patients carrying an NRE mutation. However, the cellular and cell type-specific mechanisms that increase this translation are still not well understood. Jefferson Health's study examined the mechanisms in disease-relevant cells and neurons that lead to the accumulation of toxic DPRs in cells.

Using the C9orf72 NRE-linked neurodegenerative paradigm, Jefferson Health's research demonstrates in neurons that excitotoxic stress and increased neuronal excitation drive the production of polypeptides through RAN translation. This result alone has important pathogenic implications in cell models relevant to neurodegenerative disease, since C9orf72-NRE patient-derived iPS motor neurons show age-dependent hyperexcitability or hypoexcitability.

Additionally, new mechanistic insights into the relationship between RAN translation and activation of the integrated stress response (ISR) were uncovered, highlighting a potential therapeutic target. FDA-approved compounds that target components of the ISR have been previously administered in clinical trials for other neurological and neuromuscular disorders.

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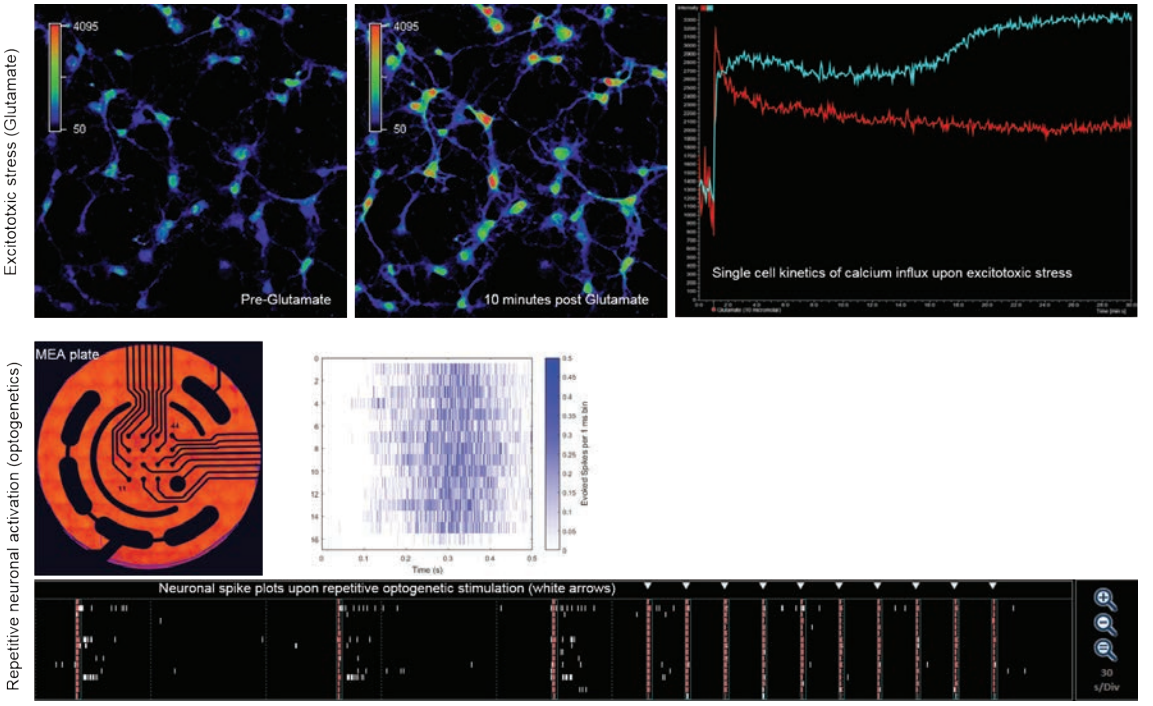
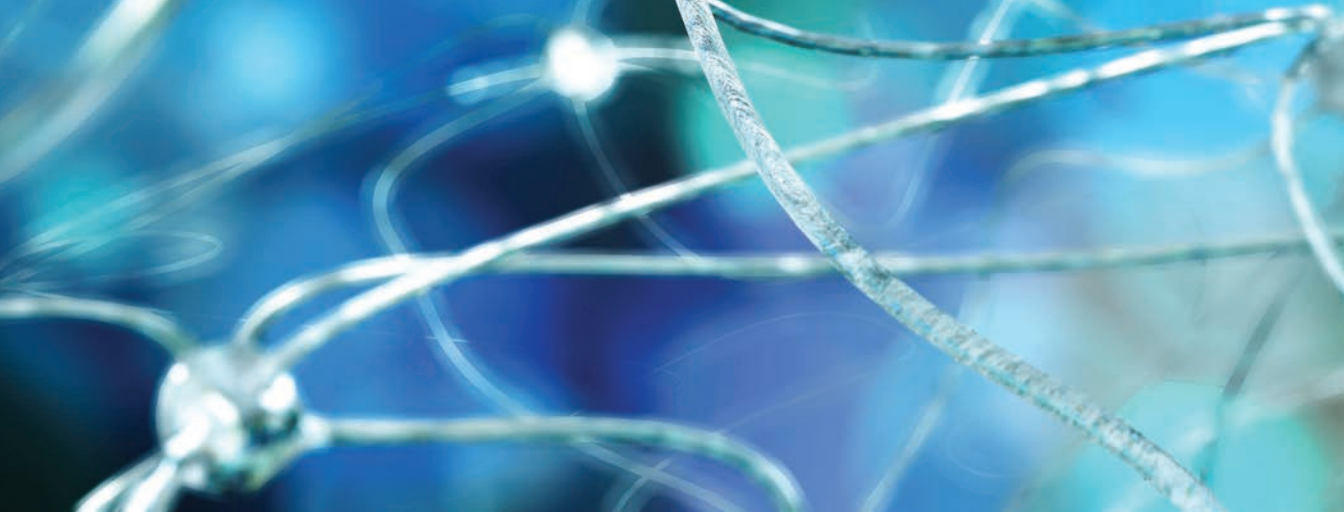


Figure 3. Overview of Excitotoxic stress and repetitive neuronal activation paradigms to model C9orf72 repeat associated non AUG (RAN) translation in cortical neurons reported in Westergrad et al., 2019. (Top) Excessive levels of extracellular glutamate is causative of excitotoxic stress resulting in robust changes in intracellular calcium which is frequently observed in ALS patients. (Bottom) Repetitive neuronal activity through expression and activation of light activated ion channels (channelorhodopsins) using Axion Lumos microelectrode array (MEA) in neurons increases spiking activity which closely mimics hyper excitability in a subset of ALS patients.
Source: Karthik Krishnamurthy, PhD, Post-doctoral Fellow, Jefferson Weinberg ALS Center (unpublished data)



Blood-Brain Barrier Driven Pharmacoresistance in ALS[1]

Researchers at Jefferson Health have found that the complexity of ALS etiology and the pathologic changes in the blood-brain barrier and blood-spinal cord barrier (BBB-BSCB) contribute significantly to limiting the development of a successful pharmacotherapy. A new understanding of ALS-linked genes and genetic mutations is providing a pathway to elucidating pharmacoresistance in ALS.

The BBB is essential for proper neuronal function, homeostasis, and protection of the CNS microenvironment from blood-borne pathogens and neurotoxins and is an impediment for CNS penetration of drugs. In some neurologic conditions, such as epilepsy and brain tumors, overexpression of P-glycoprotein, an efflux transporter whose physiological function is to expel catabolites and xenobiotics from the CNS into the blood stream, has been reported. Work conducted at the Jefferson Weinberg ALS Center has been the first to demonstrate that also in ALS, overexpression of P-glycoprotein and an increase in its activity at the BBB-BSCB drives a progressive resistance to CNS penetration drugs and therapeutics, diminishing their bioavailability and persistence into the nervous system. The Jefferson Health team has clearly demonstrated that over time, as disease progresses, increased P-glycopretien confers pharmaco-resistance and decreases dramatically the efficacy of Rilzuole, one of only a few drugs currently approved for treatment of ALS. They have also demonstrated that, due to the broad substrate specificity of P-glycoprotein, this is a widespread phenomenon that reduces our ability to deliver a large spectrum of therapeutics, thus impacting negatively the opportunity to develop better and more efficient treatments for ALS.

Overcoming drug resistance at the BBB using compounds that regulate expression and activity of P-gp and other drug efflux transporters is still an active area of research. Ideal compounds that would provide a clinically significant inhibition of multidrug efflux transporter activity are compounds that can be safely administered at doses that provide unbound plasma concentration many folds higher than the Ki of the inhibitor. Clinical application of P-gp inhibitors remain challenging with regard to potency as well as specificity. Other possible strategies include targeting signaling pathways that regulate P-gp expression in disease.

The work of Drs. Pasinelli and Trotti has found that a complete understanding of the molecular mechanisms underlying P-gp overexpression in ALS may guide the development of personalized therapy for patients, and could help to improve the selection and inclusion criteria for clinical trials. If the P-gp-mediated pharmacoresistance hypothesis is confirmed in the clinic, then it would be very important to consider testing investigational drugs for P-gp interaction in preclinical stages, thereby improving clinical trial design.

According to Pasinelli and Trotti, examining ALS patients for polymorphisms in the ABCB1 gene that are causative of pathologic P-gp overexpression and drug resistance could be useful in stratifying patients. Patients positive for that particular ABCB1 polymorphism would be categorized as a treatment non-respondent group and considered for an individualized therapeutic regimen.

It is clear to us that changes in therapeutic regimen must include combinatorial therapy of ALS drugs with P-gp inhibitors, as well as adjustments in dose, frequency and length of treatment.

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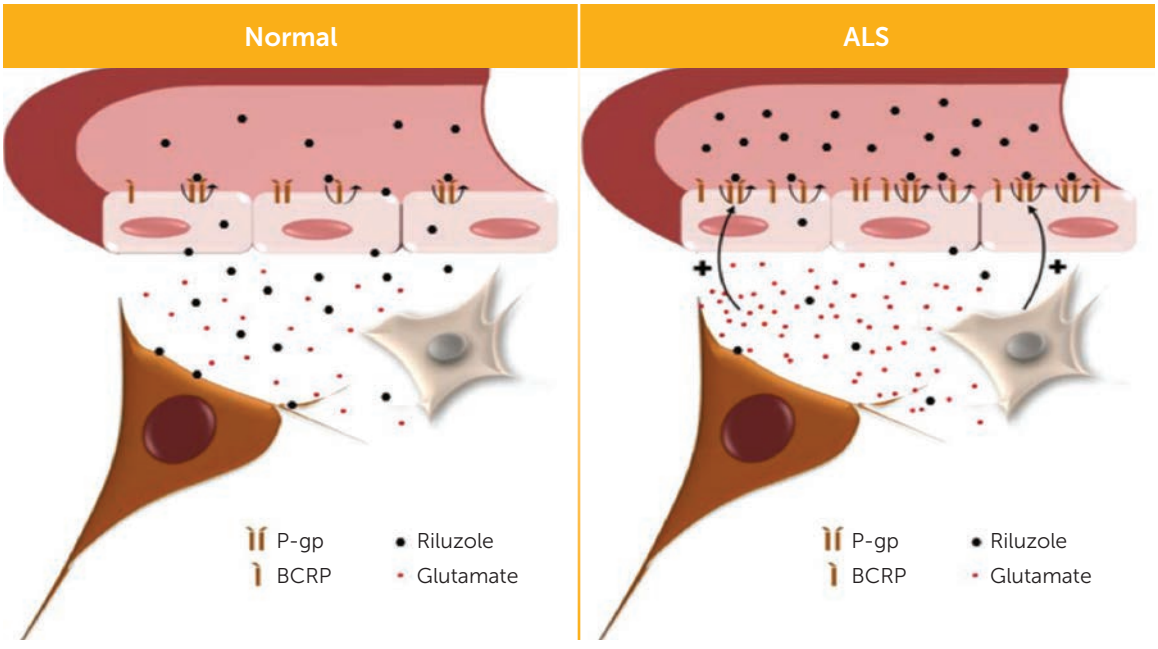


Figure 4. Schematics of the blood-spinal cord barrier (BSCB) in normal condition and in ALS. Disease-driven upregulation of P-gp and BCRP in endothelial cells of the BSCB clears Riluzole from the Central Nervous System (CNS) counteracting its potential beneficial effect.
Source: Piera Pasinelli, PhD, Davide Trotti, PhD

CLINICAL RESEARCH AND TRIALS

The foregoing examples of our ALS Center's research will have far-reaching implications for patient care at Jefferson Health and elsewhere, as we lead in both research and treatment of ALS. The Center's research activities investigate the similarities and differences between patients with ALS, thus providing a deeper understanding of the many variants and aspects of the disease.

Jefferson Health collects, banks and studies samples from patients as well as healthy volunteers, for molecular comparisons. Through analyzing blood, urine and tissue we are able to identify biomarkers of disease specific to subgroups of ALS patients.

Jefferson Health will also be involved in a new initiative in ALS, as one of 54 centers nationally selected as a site for an upcoming Platform trial. (See Figure 5) This approach to an interventional clinical trial allows for the testing of multiple therapies simultaneously, dramatically reducing the time taken from drug discovery to FDA approval, and minimizing placebo participants through sharing the placebo data between therapeutic arms.

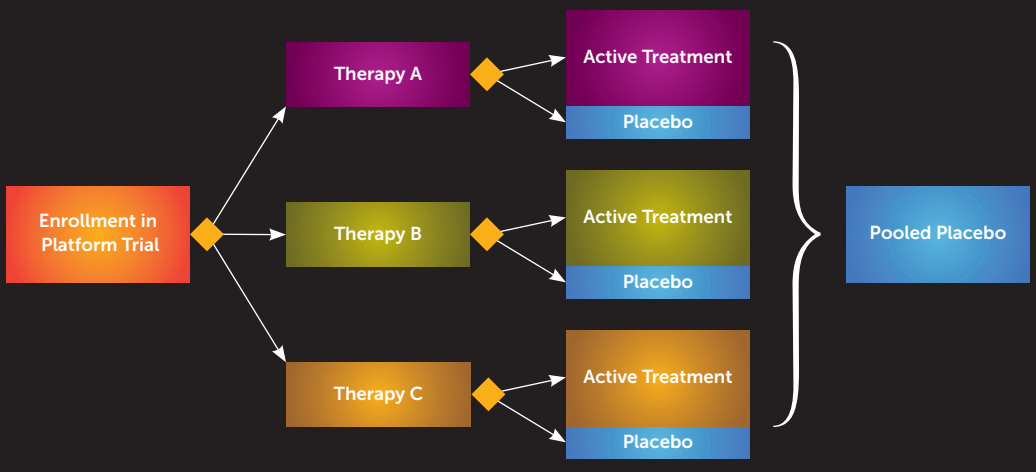
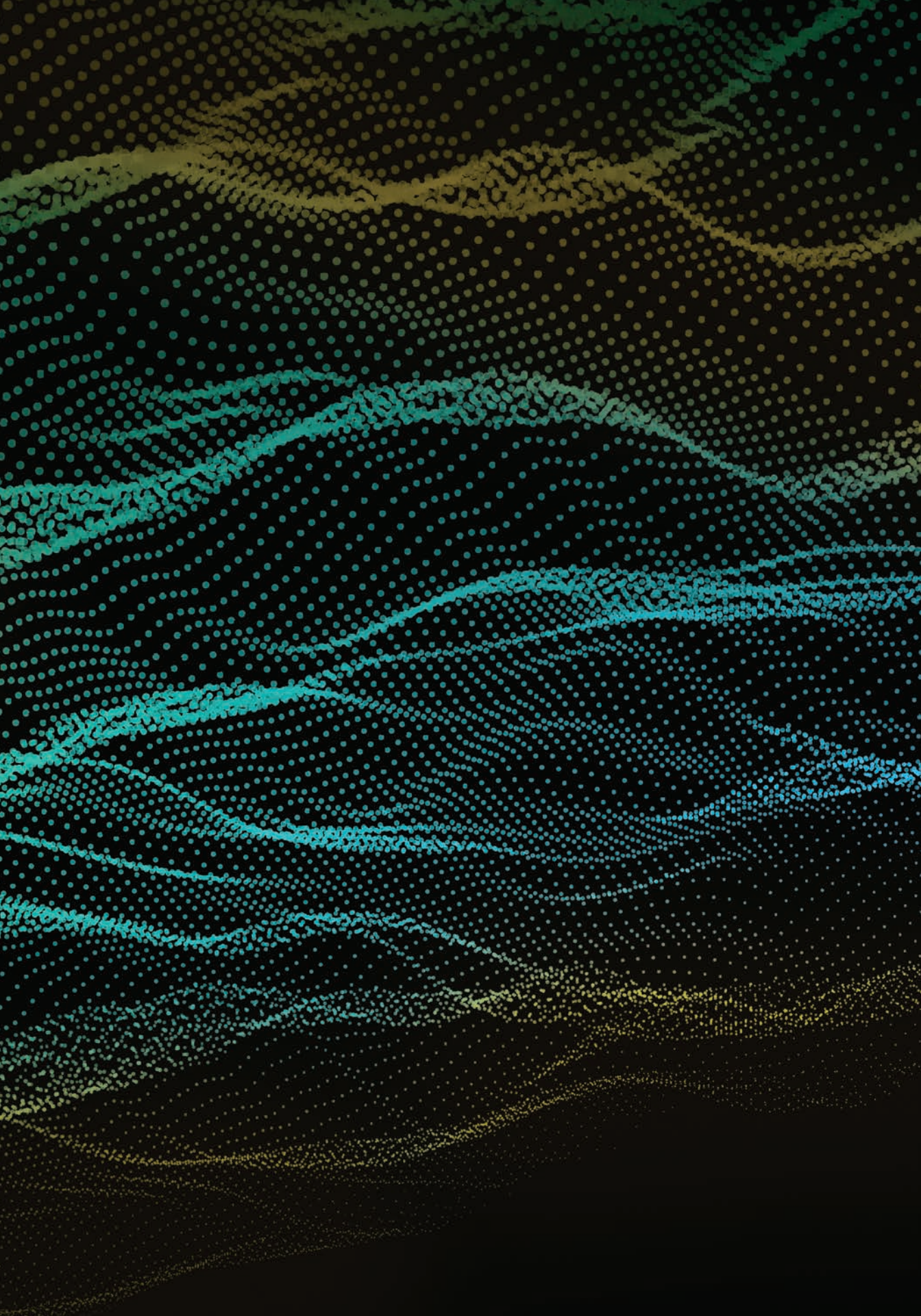


Figure 5. Arms of the Platform Trial. Pooling the placebo groups gives a 1:1 experimental to placebo participant ratio.
Source: Laura Oakley, BS, CRC, Clinical Research Coordinator

Using the newly discovered insights highlighted prior, combined with the identification of new genetic mutations, will aid Jefferson Health's clinicians understanding of the underlying mechanisms that cause ALS. This integration of research and treatment, especially when diagnosis of ALS begins early, expands options and technologies available to ALS patients at an earlier stage of their disease, with the goal of extending life.



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