



On July 4, 1939, baseball All-Star Lou Gehrig announced he would leave baseball after having been diagnosed with amyotrophic lateral sclerosis (ALS), commonly known as Lou Gehrig's disease. (Photo by Stanley Weston/Getty Images.)

Joining the Fight to Cure NEURODEGENERATIVE DISEASE

The Laboratory's cutting-edge capabilities and expertise offer new tools to study ALS disease mechanisms and fuel hope for a cure.

IN the late 1930s, beloved Major League Baseball (MLB) All-Star, Triple Crown winner, and record-breaking New York Yankees first baseman Henry “Lou” Gehrig began showing signs of rapidly deteriorating strength and agility. At the time, Gehrig—deemed the “Iron Horse” for his athletic prowess and grit—was at the height of his career. His diminishing performance left fans stunned and confused. Diagnosed with amyotrophic

lateral sclerosis (ALS), Gehrig’s decline was swift. Just a little more than two years after his symptoms began, Gehrig died at the age of 37, a promising life cut short by a cruel and little-known affliction, which thereafter would also be referred to as Lou Gehrig’s disease.

Always fatal, and usually within two to five years, ALS is an adult-onset, progressive neurodegenerative disease in which motor neurons in the brain and spinal cord die. Over time, the loss of these cells prevents the brain from controlling or regulating muscle movement, not just in the arms and legs but in the muscles needed to speak, swallow, and eventually breathe. Yet, while the central nervous system is destroyed, the mind remains fully aware, cognizant of its ever more debilitated state. The cause of ALS is unknown, and no cure has been identified.

Gehrig’s death brought ALS into public awareness, and since then strides have been taken to improve understanding of this insidious disease. However, many

challenges remain as physicians, scientists, and researchers worldwide strive to determine a disease mechanism, identify possible biomarkers, and develop effective treatments.

One of the primary obstacles is that ALS disease pathogenesis is tricky. From patient to patient, how the disease presents itself, the course it follows, and the rate of progression differ. This scientific challenge is compounded by the disease’s rarity. Whereas nearly 2 million people are diagnosed with cancer every year and 19 million are living with cancer at one time, only 6,000 people are diagnosed with ALS every year and 18,000 are living with the disease. Consequently, high-dollar funding opportunities from pharmaceutical companies are limited.

In 2019, Lawrence Livermore joined the fight alongside universities, research institutes, and healthcare partners to better characterize ALS through a strategic partnership between the Livermore Lab Foundation (LLF) and the ALS CURE Project, a nonprofit organization that

identifies and funds promising ALS research studies. (See the box on p. 7.) Sally Allen, LLF's executive director, says, "LLF is dedicated to facilitating the application of the Laboratory's unique capabilities for the public good. The nonprofit is a bridge to a broad range of stakeholders and provides an important mechanism to enable broader society to benefit from the Laboratory's expertise."

In the first year of the collaboration, initial efforts were focused on engagement. Former Lawrence Livermore scientist and strategic partnerships manager Amy Gryshuk led the Laboratory's ALS Research Initiative and was instrumental in identifying multidisciplinary projects with the potential for follow-on funding and fostering relationships with the ALS community. Gryshuk, who now serves as associate director for the Office of Strategic Alliances with Innovation Ventures at the University of California at San Francisco (UCSF), says, "We grew our professional relationships and scientific network and demonstrated what the Laboratory could contribute in the ALS space."

This early groundwork laid the foundation for a growing research portfolio that combines the Laboratory's core competencies in computational modeling, biosciences, engineering, and data sciences with the expertise of external clinicians and researchers. The Laboratory's goal is to help identify the ALS disease mechanism and potential prognostic and diagnostic biomarkers for ALS, establishing a target for therapy development that could lead to a cure.

Insight into "Confounding" Data

According to the U.S. Centers for Disease Control and Prevention, of the 6,000 people diagnosed with ALS each year, only about 5 to 10 percent can trace their diagnosis to inherited genetic factors. What's more, the disease is twice as prevalent among veterans. This

alarming statistic was the impetus for the Laboratory's first ALS project, focused on assessing whether machine-learning (ML) approaches could be used to identify the lifetime risk, age of onset, and rate of disease progression in military personnel.

Led by Livermore computational engineer Priyadip Ray, the project was conducted in collaboration with colleagues at the Veterans Affairs (VA) Palo Alto Health Care System's ALS clinic and Center for Collaborative Healthcare Innovation. Ray says, "Over the course of a year, we established a good working relationship with the VA and finalized an agreement that would allow us to use de-identified ALS patient data from the VA's electronic health records, along with clinical data from the Pooled Resource Open-Access ALS Clinical Trials database, to explore the efficacy of machine-learning approaches for predicting factors related to ALS."

Typically, a patient's disease progression is based on the ALS functional rating score, a physician-based assessment of a patient's impairment. "We applied multimodal machine-learning approaches to identify risk factors that could serve as surrogate measures for the functional rating score," says Ray. "We showed that modeling long-range, complex correlations among different factors using patients' vital data could help predict disease progression."

This initial work set the stage for a larger project funded through the Department of Defense Congressionally Directed Medical Research Programs (DOD CDMRP) to identify Food and Drug Administration (FDA) approved medications that could be repurposed to slow ALS progression. Currently, the three FDA-approved drugs for ALS (Riluzole, Edaravone, and the recently added Relyvrio) can extend the survival time of patients temporarily. Ray says, "We know that off-target drugs have been used to treat other neurological diseases, so we're looking for instances in which



ALS CURE Project co-founder Mike Piscotty presents a check to Livermore Lab Foundation representatives Dona Crawford and Sally Allen to fund strategic projects that apply the Laboratory's scientific and technical expertise to meet the challenges of ALS.

off-target drugs could be successful with treating ALS."

The Livermore team, which includes Jose Cadena, Andre Goncalves, Braden Soper, and Boya Zhang, first developed a baseline ML approach using an algorithm that assumes all confounding variables—factors that can affect both treatment and outcome but are not under direct study—are known. "For example, some drugs are not suitable for older patients, and younger patients tend to live longer," says Ray. "If you don't consider age as a confounder, then the data may suggest the medicine lengthens life rather than something else affecting the survival rate," says Ray. With their baseline approach, the team flagged promising classes of drugs for repurposing.

To reinforce the initial drug results, the team applied more sophisticated ML approaches. "We developed two other machine-learning algorithms that remove some of the assumptions embedded in the previous work," says Ray. While the baseline algorithm

Partnering for A Cure

The triumph of the human spirit can enable people to turn tragedy into hope, heartache into purpose. The ALS CURE Project was founded by Livermore computer scientist Mike Piscotty and his son, Major League Baseball player Stephen Piscotty, in memory of wife and mother Gretchen (also a former Laboratory employee), who passed away from amyotrophic lateral sclerosis (ALS) in 2018. The nonprofit raises funds to support promising ALS research initiatives and build strong, international collaborations. Piscotty, who has been a project manager within the Laboratory’s Information Technology Program for more than 30 years, says, “The ALS CURE Project brings independent researchers together to better understand the disease, its cause and progression, and ultimately, find a cure.”

Recognizing the immense scientific capabilities and broad expertise that the Laboratory could bring to bear on ALS, Piscotty began exploring how to bring the two together. He engaged Laboratory management, including former Associate Director for Engineering Anantha Krishnan, who saw the potential benefits of applying the Laboratory’s multidisciplinary approach to ALS research. Krishnan helped pave the way for the initiatives funded through the Livermore Lab Foundation (LLF). Sally Allen, LLF’s executive director, says, “LLF’s partnership with the ALS CURE Project demonstrates the value of having a philanthropic entity that can facilitate public-private partnerships at the Laboratory to help tackle some of today’s most complex scientific challenges.” Piscotty adds, “None of the amazing ALS work being done at the Laboratory would have been possible without the support of LLF and its Board of Directors.”

Annually, the ALS CURE Project holds an ALS Innovation Symposium, where the top ALS researchers from around the world come together virtually to discuss potential research avenues. “We are not concerned with who gets credit but rather that the limited ALS research dollars are directed to the most promising opportunities,”



Mike Piscotty started the ALS CURE Project in honor of his wife Gretchen, who passed away from the disease in 2018. In the background, his son, Major League Baseball player Stephen Piscotty, approaches the batting cage.

says Piscotty. “Through this event, we developed our Roadmap to Cure ALS, which serves as a guide for research grants focused on international collaborations.” To date, the ALS CURE Project has sponsored \$1.2 million in research efforts, and it is committed to seeing a cure become a reality. Piscotty says, “Before Gretchen died, I made a promise that we would continue to work towards a cure.” For Piscotty and all those who have made this work possible, when that day comes, the ALS CURE Project will be complete.

ROADMAP TO CURE ALS

Research pathways

- 1 Multimodal longitudinal machine learning-artificial intelligence statistical modeling and data collection
- 2 Cellular modeling with lab validation
- 3 Organoid/in vitro tissue modeling
- 4 Motor circuit/system dysfunction
- 5 Genetic architecture

Disease progression mechanism(s) and progression biomarker identified

Disease initiation(s) identified

Disease stratified

Diagnostic biomarker

ALS models

Prognostic biomarker

ALS therapies halt disease progression and ALS therapies prevent disease initiation

Increased technology infrastructure over time

The ALS CURE Project’s “Roadmap to Cure ALS” outlines key milestones and objectives in ALS research and serves as a guide for research grants for leading ALS organizations around the world.

assumed all confounding variables were known, the second removes some of the assumptions, and the third considers “deconfounders.” Ray explains, “Imagine trying to determine how much the actors typically cast in James Bond movies will affect a movie’s revenue. A deconfounder machine-learning approach would take all the data and analyze how much of a factor the actors are versus other factors. Perhaps the actors’ level of success in terms of revenue is less about the particular actors than about their being cast in James Bond movies.” Such an approach would allow the team to uncover confounders in a data-driven manner rather than relying on human experts to identify them beforehand, which removes inadvertent biases. The team has run preliminary analyses on all three ML algorithms and has identified several promising drug repurposing candidates. “Our goal is to develop a suite

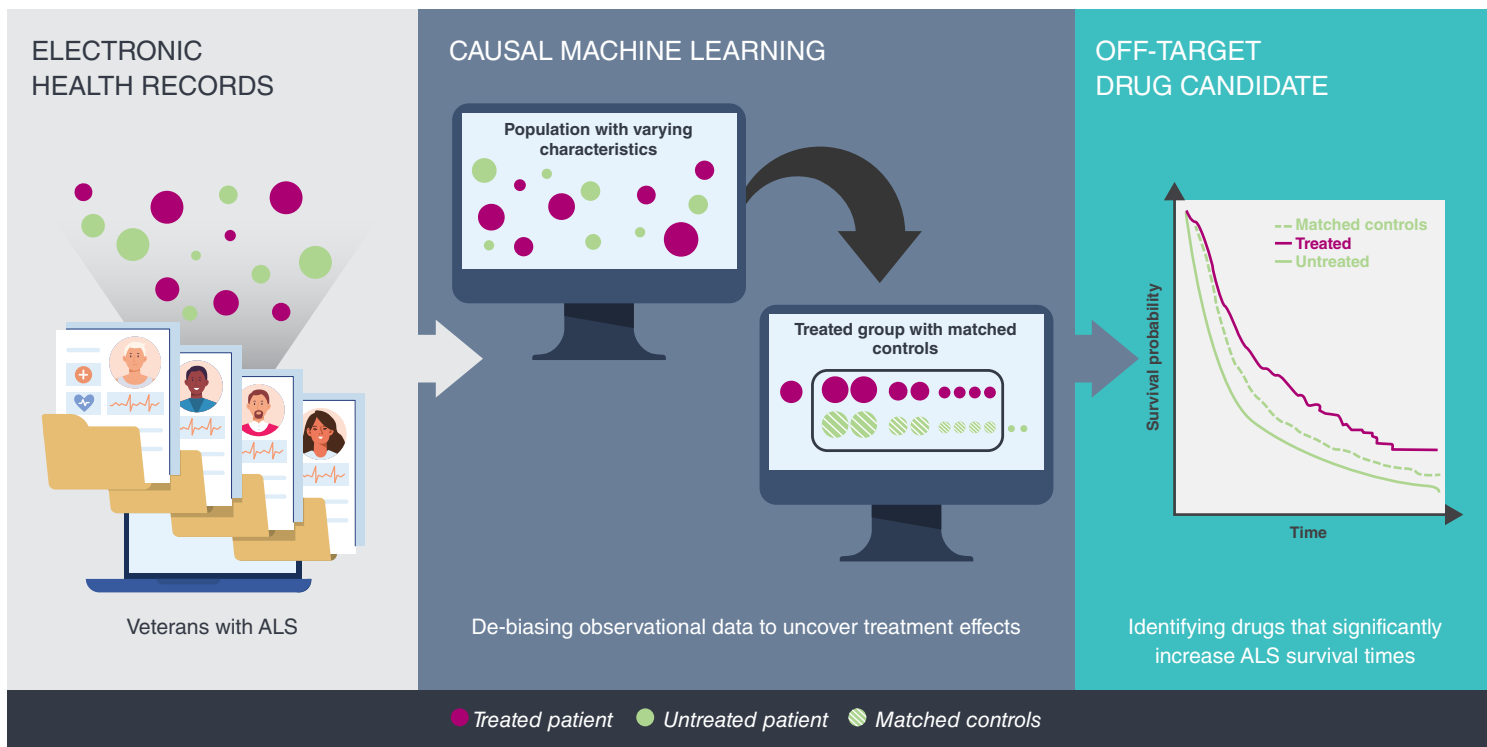
of approaches in which we can reinforce the outcomes to identify specific drugs with confidence.”

While randomized clinical trials are the ultimate gold standard in medical research, they are expensive to conduct. “This work will help us determine if we can emulate a randomized drug trial using retrospective patient data rather than having to recruit trial participants to determine effective drug repurposing. We can then focus limited funding resources on the most promising therapeutics,” says Ray. The research team continues to engage with the VA and UCSF, which has an ALS clinic that could provide a large, separate patient data set for ML exploration. Researchers are also working with collaborators at Stanford University and the University of California at Los Angeles to investigate specific drugs and the biological mechanisms that drive the positive outcomes for ALS patients.

Proteins Could Be Key

Several years ago, as part of the National Cancer Moonshot Initiative, Laboratory researchers began investigating the process of cancer initiation in RAS (rat sarcoma virus) proteins. (See *S&TR*, October/November 2016, pp. 4–11.) The team coupled experimental data with atomic-resolution molecular dynamics simulations to build a model of RAS protein biology in varying types of cell membranes. Mike Piscotty, co-founder of the ALS CURE Project, says, “After reading about this work, I wondered if this same physics-based approach could be used to study the TDP-43 protein, which is present in 95 percent of ALS cases but is poorly understood.”

TDP-43 (transactive response DNA-binding protein 43) is found in cell nuclei, including within nerve cells such as motor neurons, and is involved in RNA-related metabolism and gene expression. The protein is made up of 414 amino acids and



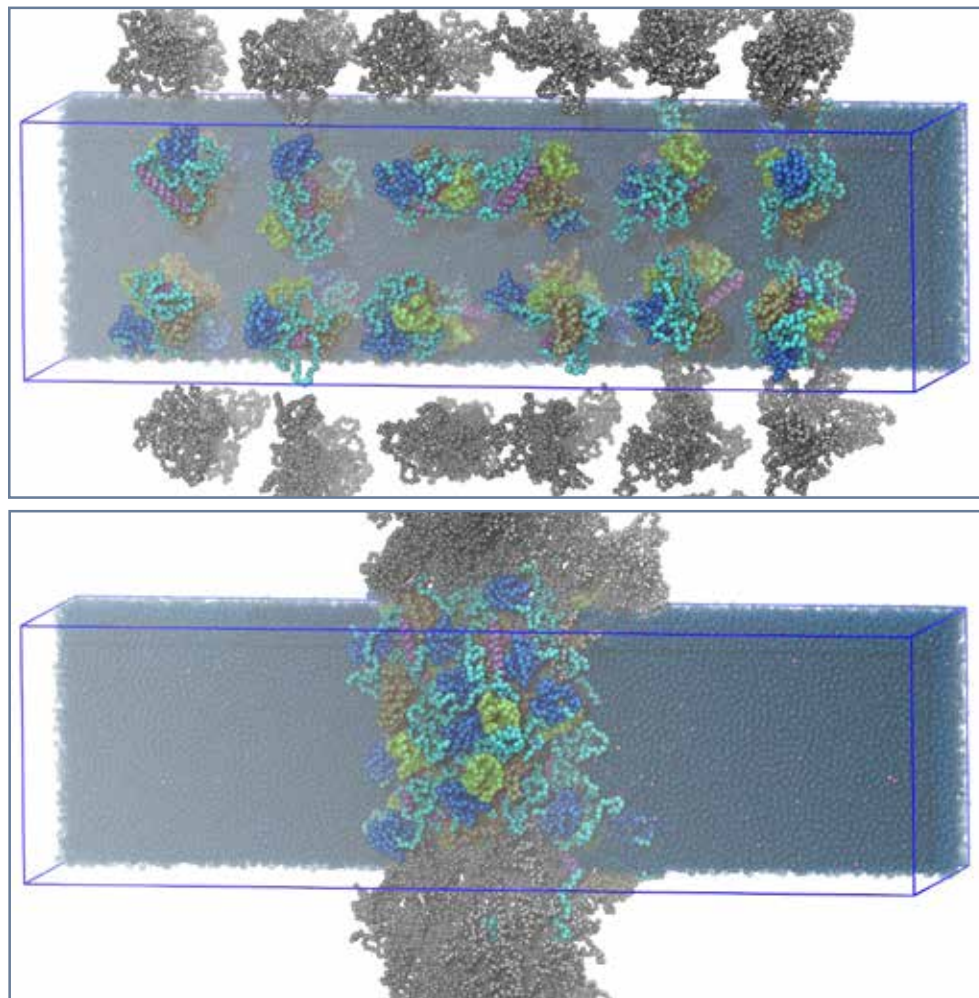
Livermore researchers are using machine-learning algorithms to find effective off-target drugs to treat ALS patients.

several functional domains— independent units of the polypeptide chain that allow the protein to perform its primary function. Although the amino acids dictate the folded 3D structure of the protein, structural rearrangements can occur. Previous research has shown that TDP-43 protein aggregation, wherein proteins misfold or cluster together to form stress granules, leads to plaque formation between nerve cells. These plaques cause motor neurons to die and are one of the few consistent observable indicators in ALS and other neurodegenerative diseases.

As a result, another project within the ALS research portfolio focused on developing an experimentally informed and validated physics-based multiscale modeling framework to understand the physics of TDP-43 protein phase separation and aggregation intra- and extracellularly. “Think of an egg with the yolk and surrounding white membrane,” says Piscotty. “When TDP-43 stays in the nucleus of the cell, in this case, the yolk, the cellular process works well. But, if the protein moves out of the yolk and into the egg white, and then finds other proteins to bind with, the cell behaves differently. What we don’t know is whether neurodegeneration is causing TDP-43 aggregation, or the other way around.”

To help unravel this mystery, Livermore computational physicists Joel Berry and Helgi Ingolfsson are delving into the molecular dynamics of TDP-43 self- and cross-interactions. Berry’s work focuses on developing macroscale models to simulate the liquid–liquid phase separation (LLPS) of TDP-43 proteins. “TDP-43-rich clusters are intracellular condensates—micrometer-scale, membraneless protein- and RNA-rich bodies that are typically liquidlike and are thought to commonly form and disassemble through cell-wide phase transitions,” says Berry. “TDP-43 condensates and stress granules may form when these phase transitions are not properly regulated.”

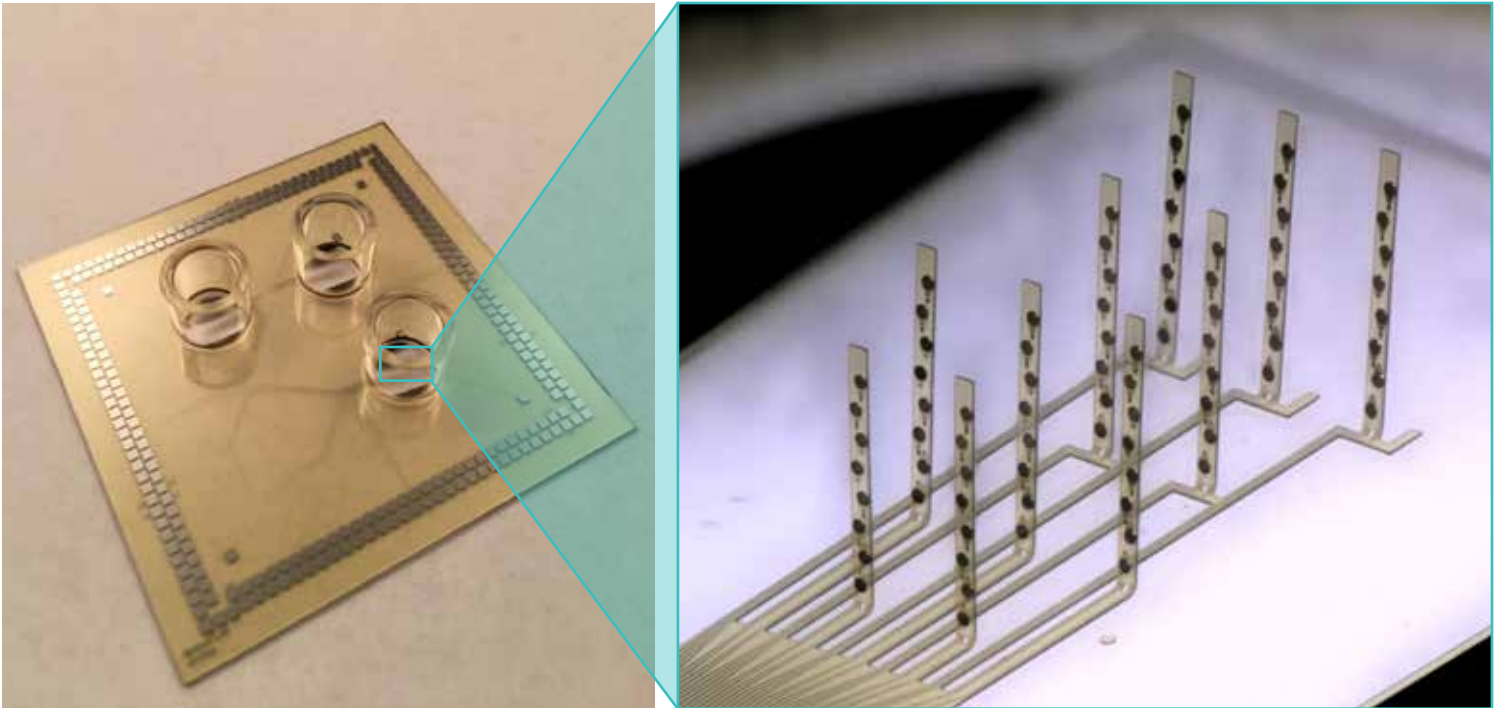
The team developed a full-length molecular model of TDP-43, including all



To better understand transactive response DNA-binding protein 43 (TDP-43) protein–protein interactions and assembly, Livermore computational scientists developed multiscale molecular dynamics models of a full-length TDP-43 protein (top). The coarse-grained molecular dynamics simulation shows 24 full-length TDP-43 proteins that are initially placed apart. As the simulation runs, the proteins quickly associate, forming larger assemblies that coalesce into a single assembly (bottom). Colors denote different protein structural domains.

414 amino acids, using select structures of the protein functional domains. Ingolfsson’s expertise lies in all-atom and coarse-grained molecular dynamics simulations, which describe individual particles at high resolution. He says, “Data from higher resolution simulations serve as parameters for the larger-scale models.” The course-grained, macroscale models, one of which is optimized for disordered proteins, explore the full-length protein’s

self-assembly process as well as protein–protein interactions—ranging from dozens to hundreds of proteins in assemblies— illustrating the role of protein self-affinity in LLPS and protein conformational evolution. Ingolfsson says, “Liquid–liquid phase separation has been extensively studied for physics and materials science applications but applying it to biology is new, and most work in this area is focused on experiments. Not many people have



Livermore's ALS-on-a-chip technology incorporates a 3D co-culture of motor neuron cells and glia (non-neuronal brain cells) grown and nourished inside experimental wells that sit on top of the microelectrode arrays (inset), which allow for noninvasive interrogation of the cells.

done this type of simulation work and at this scale.” These simulations could play a crucial role in linking molecular-level interactions to aggregate behavior of protein assemblies, potentially giving insight into ALS mechanisms.

The team has secured additional funding through the DOD CDMRP to take this research to the next level. In collaboration with San Francisco-based research groups Gladstone Institutes and Prosetta Biosciences, the team will integrate computational models and data from experiments to study whether small molecule drugs that disrupt TDP-43 aggregation can have a neuroprotective effect. Ingolfsson says, “Establishing that small molecule compounds can restore TDP-43 nuclear localization would support further development of treatment options.” Livermore colleagues working in the biosciences will contribute to this

work, with a new capability for studying neuronal cells in vitro.

ALS-on-a-Chip

In 2014, Livermore researchers unveiled an innovative device called the in vitro chip-based human investigational platform (iCHIP)—an integrated system that combines primary human cells, tissue engineering, and microfluidics—to evaluate the effects of chemicals, viruses, and drugs on humans without relying on animal or human test subjects. (See *S&TR*, March 2014, pp. 16–19.). Through a strategic initiative funded by the Laboratory Directed Research and Development Program, this platform was later adapted to create the brain-on-a-chip technology, which simulates the central nervous system by recording activity from multiple brain cell types deposited and grown onto a small platform embedded

with microelectrode arrays. (See *S&TR*, May 2019, pp. 4–11.)

As part of a third ALS research effort, Livermore biologists Nick Fischer and Heather Enright have further adapted the technology to deliver “ALS-on-a-chip.” The device takes a closer look at the connection between ALS-related protein aggregation and stress granule formation from the experimental side. Fischer says, “Our system may provide a new tool to study the possible mechanism of protein aggregation on ALS disease progression, while also serving as a tool for evaluating new therapies.”

ALS-on-a-chip incorporates a 3D co-culture of motor neuron cells and glia (non-neuronal brain cells) grown and nourished inside experimental wells that sit on top of the microelectrode arrays. The first phase of the project focused on establishing the culture conditions necessary to maintain the cells’ viability and evaluating the network formation, cellular morphology, and functional signatures in vitro. The team will soon begin testing a handful of therapeutic candidates developed by collaborator

Prosetta Biosciences to assess how the drugs affect the network function.

Ideally, the platform will provide a controlled way of analyzing the onset of stress granule formation and tracking how the proteins affect the function of the motor neurons over periods of time ranging from hours to weeks. If the team can demonstrate how the functional signatures of the neural network correlate with structural changes within the motor neurons, the research could open new avenues for developing, testing, and validating promising therapeutics, including ones with potential to prevent or reverse stress granule formation.

The work shows promise, and in June 2022, Fischer began collaborating with the University Medical Center (UMC) at Utrecht in the Netherlands on a separate, but related project. The work will integrate the Laboratory's work with ALS-relevant 3D neuronal cultures and 3D multielectrode arrays with the expertise of UMC Utrecht in developing and characterizing ALS-relevant neuronal cells and brain organoids. "This work can give us amazing insight into a part of the body that cannot be evaluated while a person is living," says Piscotty. "Organoid and tissue arrays provide a noninvasive way to peek inside the brain to help us better understand disease mechanisms and accelerate therapeutic development."

Future Possibilities

The success of these early projects has generated momentum as the Laboratory finds more areas in which to contribute. Under a proposed collaboration with UCSF, Livermore researchers will investigate whether environmental factors may play a role in ALS disease mechanisms by studying the microbiomes of ALS patients. Previously, the Livermore team, led by biologist Crystal Jaing, studied associations between pathogens and various forms of cancer and worked with NASA to characterize the microbiomes of astronauts and the environment within the International Space Station. (See *S&TR*,

January/February 2018, pp. 12–15.) "Using both computational and microbial data, we can look at patients' progression scores, diets, and general environment to explore whether there are factors within patients' microbiomes that could be contributing to disease onset and progression," says Jaing. CDMRP funding has been approved for the project, which includes collaborators from UCSF and Stanford. Team members look forward to applying their expertise to potentially identify opportunities for ALS prevention, early detection, and treatment.

Another pilot project recently funded by LLF uses ML approaches to stratify ALS patients based on in vitro drug response. Livermore computational engineer and principal investigator for the project, Alan Kaplan, says, "We will take imaging data from in vitro experiments and assess how a cell's appearance changes, such as shape and morphology, pre- and post-treatment and evaluate how the drugs are affecting the cells."

Image data is being provided by collaborators at the University of Sheffield in the United Kingdom. "My role is taking all the shape descriptions and data from each cell and using machine-learning approaches to distinguish patterns of how the cells change with which drugs," says Kaplan. ML allows the researchers to analyze hundreds of features computed per image. "Drug response is not uniform in the patient population. Therefore, we are developing methods that allow us to assess hundreds of morphological parameters per cell to determine potentially complex relationships that differ between patient subtypes." Ultimately, the team aims to generate an ML model that can stratify patients into subgroups based on correlations between the expression of pathological hallmarks in cells and their drug response to accelerate drug discovery.

Fueling Hope through Science

The breadth of capabilities and diverse approaches that Livermore is applying to tackle ALS research challenges and the

external partnerships with medical providers and other disease experts are leading to new discoveries in disease pathogenesis. Piscotty says, "People should be proud of the work being done here and with strategic partners to advance research in this important area. The work under way to identify disease mechanisms and speed therapeutic development is a testament to what can be accomplished when we work together to achieve a common goal. This research can also be applied to other neurodegenerative diseases."

Since the ALS Research Initiative began, the Laboratory has gained recognition for the tools brought to the ALS fight. "In the Spring of 2021, LLF sponsored a Rae Dorough Speaker Series talk on neurodegenerative disease and what we have learned so far," says Dona Crawford, chair of the LLF Board of Directors. "The Laboratory has become a compelling partner in this space, a natural convener of multiple research efforts serving as a neutral, honest broker. We hope to make faster progress collectively than what would be possible individually." Piscotty adds, "The key for hope in the ALS community is to discover the target—the disease mechanism along with an ALS biomarker—that we can share with pharmaceutical companies to rapidly bring drugs to market to slow or even halt disease progression and in the future may even allow patients to regenerate movement." Bringing great minds together is an essential part of this work. Now, it is time to play ball.

—*Caryn Meissner*

Key Words: ALS CURE Project, ALS-on-a-chip, amyotrophic lateral sclerosis (ALS), biomarker, disease mechanism, Livermore Lab Foundation (LLF), Lou Gehrig's disease, machine learning (ML), microbiome, molecular dynamics, motor neuron, neurodegenerative disease, TDP-43 (transactive response DNA-binding protein 43).

For further information contact Nick Fischer (925) 422-6144 (fischer29@llnl.gov).