Moser Center for Leukodystrophies
at Kennedy Krieger Institute

ALD 2021 Updates

STAFFING UPDATES

Our clinical and research teams continue to grow, and we are delighted to announce the recent recruitment of highly talented and dedicated individuals who have joined us within the last 12 months.

Our clinical team now includes a total of 19 professionals who are evaluating patients at the Moser Center on a weekly basis. We evaluate around 100-150 individuals affected by ALD per year. Our most recent recruits since last year include:

Dr. Melanie Brown, an experienced pediatric palliative care physician

Dr. William Ide, a pediatric- and adult-trained Physical Medicine and Rehabilitation specialist

Ms. Ayrowyn Tanner, a family Nurse Practitioner focusing on leukodystrophies

On the research side, we now have 16 professionals working on ALD. The research continues to be guided by the leadership of Ann Moser, Dr. Amena Smith Fine, Dr. Christina Nemeth Mertz and Dr. Gerald Raymond. We have recently recruited the following individuals who expand our research activities:

Dr. Manou Amanat, a postdoctoral fellow with expertise in leukodystrophies

Mr. Dandre Amos, a clinical research coordinator

Ms. Inés Garofolo, a laboratory research technician

RESEARCH UPDATES

There are several challenges when it comes to developing a therapy for ALD and other rare diseases. Firstly, we need to discover therapeutic approaches that are clinically feasible, meaning therapies that target the affected parts of the nervous system while minimizing any harm and systemic side effects. Secondly, translating results from the laboratory into clinical trials is challenging in ALD, since the natural history of the disease is not well known and variable rates of disease progression make the unbiased assessment of therapies difficult. There is, therefore, a need to identify markers that can predict disease progression in advance to allow stratification of the right set of research participants into trials. Finally, ALD affects people in all geographical areas, and frequent travel can hamper the enrollment of a sufficient number of research participants into trials. For this reason, here at Kennedy Krieger Institute we have been focusing on:

1) Developing approaches that allow delivery of therapeutics into the nervous system in a targeted manner.
2) Identifying biomarkers and imaging markers that predict disease progression in patients.
3) Remote assessment protocols using wearable technology to minimize travel.

Selective delivery of drugs into the nervous system.

The ALD mouse, which lacks the ALD gene, known as *ABCD1*, has long been used to model the adult form of ALD, known as adrenomyeloneuropathy (AMN). Similar to patients, the ALD mouse develops elevation of very long chain fatty acids (VLCFA), which is the hallmark of ALD. Together with the Johns Hopkins Center for Nanomedicine, and under the leadership of **Dr. Christina Nemeth Mertz,** we repurposed 4-phenylbutyrate, a drug previously shown to turn on the ALD-related protein, resulting in reduction of VLCFA. This drug has been used by other researchers to reduce VLCFA; however, behavior and motor performance were not tested in those studies. The challenge here is that 4-phenylbutyrate requires extremely high doses, which would make a clinical trial in patients not feasible. However, our collaborators at the Center for Nanomedicine have attached this drug to a nanoparticle carrier, called a 'Dendrimer', which allows targeted delivery of the drug into the brain and spinal cord and allows for the reduction of both the dose and frequency, allowing us to test Dendrimer-4-phenylbutyrate in ALD mice.
In our studies, we were able to increase *ALD related protein,* reduce VLCFA in the mouse spinal cord, and improve the animals' gait abnormalities, when drug administration was started early. These data are currently complete and awaiting publication.

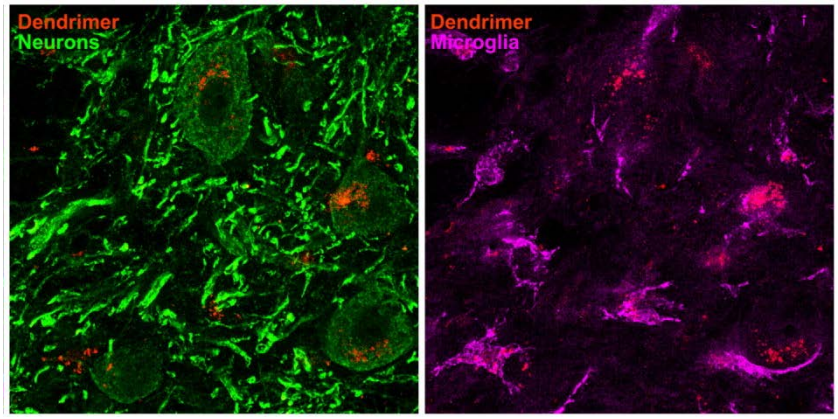
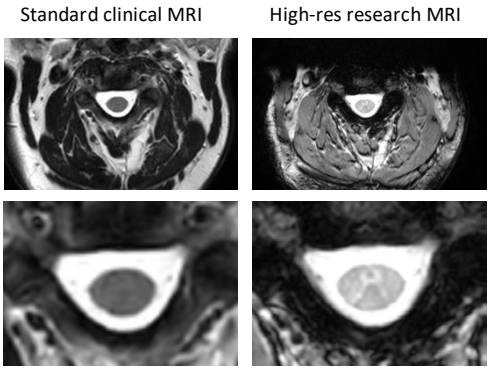


Figure caption: *Nanoparticles (red) are shown within cells of the ALD mouse spinal cord. Nerve cells are shown in green in the left image, while microglia, the brain's inflammatory cells, are labeled pink on the right. The nanoparticles carrying the drug accumulate both in nerve cells and in microglia after being injected under the skin of the mouse.*

The mouse studies have been funded by the Brian's Hope Foundation and the many other generous donations we have received. We are very thankful for the financial support and trust in our work. We are in great need of research funding to continue similar work in the lab, and have

submitted NIH grant proposals for future work using the dendrimer platform which would combine 4-phenylbutyrate with another drug that targets inflammation and oxidative stress. We believe that combination of these different drugs through our nanoparticle delivery approach will likely have the most powerful impact in ALD.

Identifying Molecular and MRI-Imaging Predictors of Disease Progression.
A major challenge in ALD research remains our inability to predict when the disease will start and what form of ALD an affected individual will develop. While newborn screening is extending quickly across the United States, we still have no way to determine which symptoms will progress and how fast these symptoms will progress.



We have begun MRI scanning AMN and healthy control individuals, looking at experimental high-resolution anatomic and functional myelin imaging of the spinal cord to quantify the biochemical health of the nerve sheath. The goal of the MRI research is two-fold: first, to understand and develop markers of spinal cord health in AMN, and second, to integrate MRI imaging into the artificial intelligence (AI) tools being developed at the Moser Center.

Dr. Bela Turk has previously developed a series of AI applications for large data-sets including plasma biomarkers, clinical measures into the *'Moser Center Neural Network*' which has shown a high grade of accuracy in experimentally predicting the trajectory of AMN symptom progression for research purposes. These networks have been developed in collaboration with a team of machine learning specialists at Johns Hopkins Malone Center for Computer Engineering in Healthcare, with a manuscript in preparation.

In addition to imaging, we are working hard to identify blood markers that correlate with disease severity and disease progression in AMN. This is an ongoing collaboration with Dr. Jaspreet Singh at Henry Ford Medical System, where we have conducted a study measuring thousands of blood metabolites and other small molecules referred to as microRNAs. We have found a series of potential markers that appear to strongly correlate with the severity of neurological impairment in AMN patients.

We are now in need of funding to conduct a prospective study to determine whether these blood biomarkers can in fact predict the severity of the disease in the future. This study would ideally be done to complement the studies using wearable technology. Together with Dr. Singh, we have applied to the NIH for several grants in the hopes of receive funding to conduct this study.

Harnessing Wearable Technology to Remotely Assess Patients in Clinical Trials.
In 2017, in partnership with Dr. Amy Bastian, Chief Science Officer at Kennedy Krieger Institute, we began a new research project utilizing a wearable technology platform that had been used in Parkinson's Disease as a tool to remotely assess patients' balance, walking speed and other gait measures. In 2019, we were able to secure a consortium grant funded by the National Institutes of Health, in collaboration with Dr. Florian Eichler at Harvard University and Dr. Adeline Vanderver at Children's Hospital of Philadelphia, which provides us funding to conduct a longitudinal study in men and women with AMN using wearable technology and AI tools, and to remotely assess their gait dysfunction. An outstanding young physician scientist in our group, **Dr. Amena Smith Fine**, has deployed the wearable platform to evaluate walking and balance in

a natural history study of ALD since February 2021. These tests are performed both on-site during clinic visits and remotely at patients' homes. So far 15 patients have been tested in-person or remotely as part of this study, and we plan to enroll another 15-20 over the next year. This work complements Dr. Smith Fine's ongoing projects using wearable technology to learn about disease progression in childhood-onset leukodystrophies.

Importantly, we have established a strong collaboration with our Dutch partners at the University of Amsterdam, led by Dr. Marc Engelen who has adapted our research protocol, and we are now collecting patient data both in the U.S. and in The Netherlands remotely using the same wearable technology. We have already identified a series of variables that we can assess remotely depicting the abnormal gait findings in AMN.

OPAL sensor, kit and placement for testing gait and balance

In July 2021, Dr. Smith Fine was awarded a grant that supplements the above-mentioned NIH consortium grant and funds a portion of her faculty salary. We collaborate with a group of computer and biomedical engineering specialists utilizing AI techniques to analyze such data.

This data will be very useful for future clinical trials which could be conducted at patients' homes, facilitating access to these trials.

Please contact Leslie Marsiglia at marsiglia@kennedykrieger.org to further support this work by making a donation.