Kennedy Krieger Institute
Research. Treatment. Education.

3/29/2023

To: Beth & Mike McGinn
CureLBSL Foundation
3520 S. Wakefield Street
Arlington, MD 22206

Re: LBSL RESEARCH PROGRESS REPORT
(Period: 10/1/2022-3/31/2023)

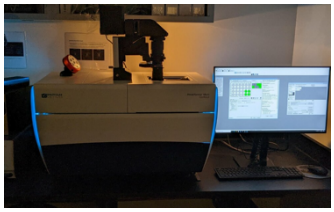
Dear Beth and Mike,

We are delighted to provide you our first six-month progress report on the Moser Center's LBSL Research Program. I am pleased to say that we have essentially accomplished all our Milestones for the last 6 months (with one pending hire) and have in fact achieved more in certain areas than expected.

Please find below a list of our Milestones for the past 6 month's benchmark.

MILESTONE 1. Purchase and install high content screener and undergo staff training for use ✓

The Confocal Micro4 from Molecular Devices has been purchased and was installed in late fall. Our lab staff members have all received the necessary training and we are already heavily using the device for the below experiments.



MILESTONE 2. Hire 3 additional staff members to join existent team (ideally 2 technicians and 1 postdoctoral fellow)

We have been working hard on recruiting additional staff members. We are excited that just this week we successfully recruited two new technicians who will be graduating from College in May and join us as research technicians, Ms. Bayley Lindsay, University of Maryland, College Park 2023 (to start early Summer 2023), Mr. Brett Ratajczak, McDaniel College 2023 (to start early Summer 2023).

Our goal is to have two additional technicians and one postdoctoral fellow. The postdoctoral fellow would be someone with a background in neuroscience who would be able to provide additional intellectual and methodological contributions to our program. However, the current postdoctoral market is in existential crisis as a record low number of new PhD are pursuing academic careers. We currently have an offer out to a candidate who also has several other offers at hand. Meanwhile, we have used our network of friends and collaborators, nationally and internationally, to recruit a postdoctoral candidate. If we are not successful by the end of Spring 2023, we will instead hire a third technician for our program.

With the addition of Bayley and Brett, our group will consist of 3 Principal Investigators (Fatemi, Mertz and Fine), 4 laboratory technicians, 2 postdoctoral research fellows (Turk, Amanat), 2 clinical research coordinators, one PhD student in Computer Engineering, 1 research Nurse practitioner, and two research physical therapists, and two rotating undergraduate students.

MILESTONE 3. Scale up mouse colony to be ready for gene therapy and ASO treatment studies ✓

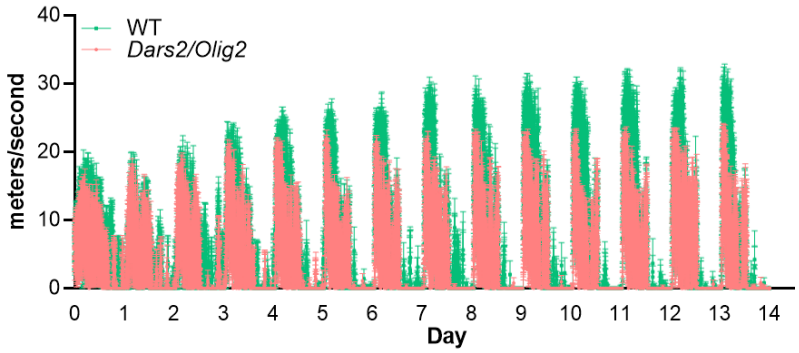
We have made significant progress in not just scaling up our colony, but now also have two additional conditional knock-out animal models which may more accurately reflect the disease. We currently have 62 mouse cages in the animal vivarium dedicated to this effort with a total of **over 300 animals** at any given time point.

We are pleased to report that in addition to the conditional neuronal *Dars2* deletion mouse (published Nemeth et al., 2020), which shows a severe neurodegenerative phenotype, we have since developed two additional models which may more accurately represent the patient condition. Our original model was the *Dars2/CamKII* where *Dars2* is missing in some brain neurons. We now have mice where we knocked out *Dars2* in oligodendrocytes, which produce myelin, and in the sensory neurons of the spinal cord. We hope to use these mice to further validate potential therapies. Currently, these animals are being bred and their deficits/phenotypes are being characterized; some have already started to receive gene therapy as outlined in this table.

	Description	Bred	Phenotype	In treatment
Dars2/CamKII	Deletion in cortical neurons	✓	✓	✓
Dars2/Olig2	Deletion in early oligodendrocytes	✓	*In progress*	
Dars2/Advillin	Deletion in sensory neurons of spinal cord	✓	*In progress*	

Our initial studies show that the *Dars2/Olig2* mouse (the mouse missing *Dars2* in oligodendrocytes) appears slower in a complex wheel running task (figure on the top, A.). In addition, mice missing *Dars2* in spinal cord neurons have a pronounced gait disorder, where they are grossly ataxic and barely able to move. These mice referred to as the '*Dars2 /Advillin'* die within a few weeks after birth (B, picture showing knock-out mouse compared to littermate control, and impaired ataxic posture of an affected mouse on the right).

A.



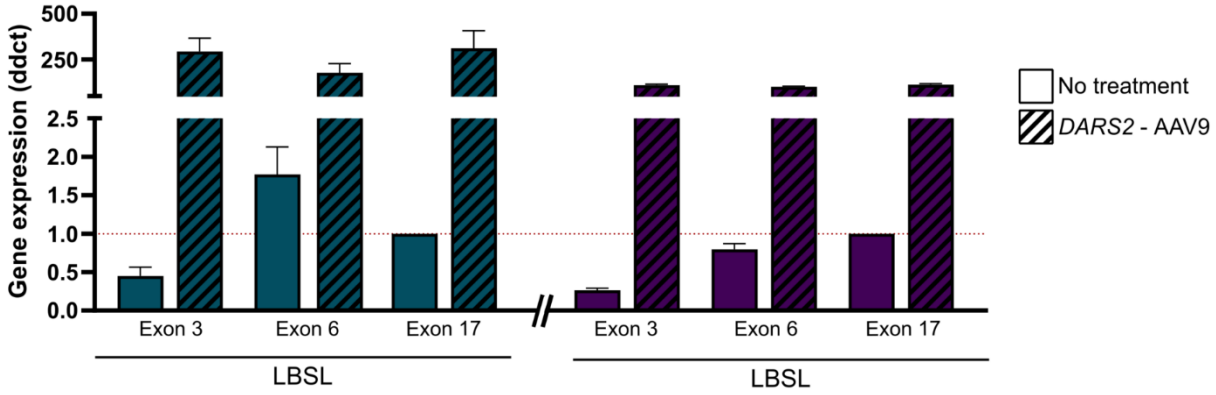
B.

MILESTONE 4. Gene Therapy: Complete production of sufficient AAV9 for animal and cell studies ✓

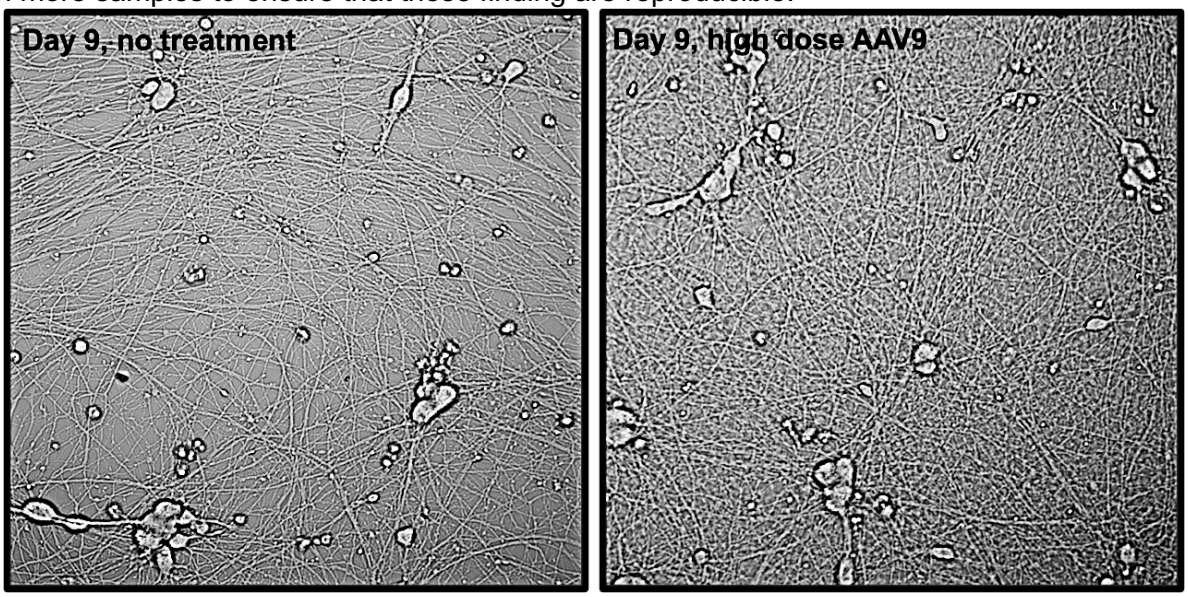
In the fall of 2022, we contracted the University of North Carolina, Chapel Hill to mass produce our AAV9 product made in collaboration with the University of Maryland. This product has since been

received and is in use for *in vivo* and *in vitro* studies.

In addition, we have started to obtain pilot data on the effect of AAV9 in both neurons in the dish and have developed two different routes of administration in our animals. As shown below in neurons treated with AAV9, our initial pilot analysis shows that AAV9 treatment dramatically increases gene expression in two different cell lines as shown here:



In addition, AAV9 treatment promotes growth of dendrites in neurons after 9 days as shown in this figure (the images were taken using our new high-content screener from Milestone 1). We need to obtain more samples to ensure that these finding are reproducible.



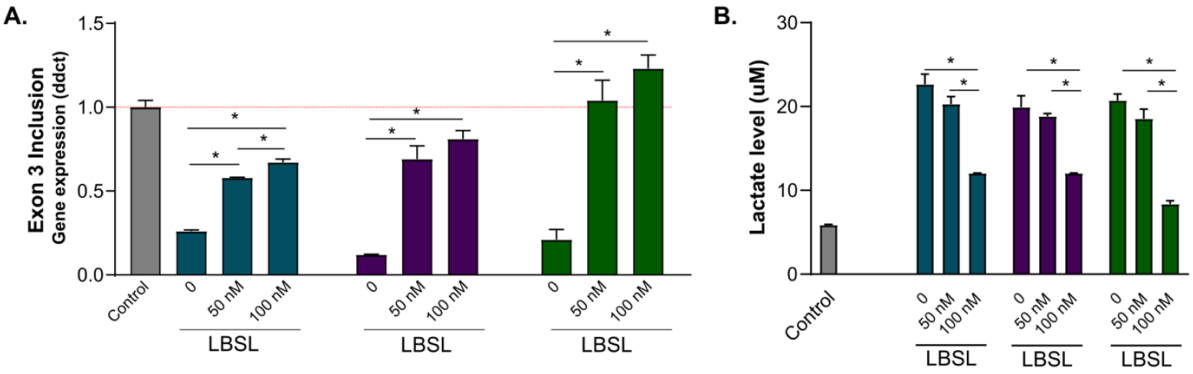
Dars2/CamKII mice have already been treated with *DARS2*-AAV9. Because these mice have a brain phenotype, they were injected with the virus *intracerebroventricularly,* or ICV, during stereotaxic surgery. This relies on specific coordinates to target the right part of the brain during survival surgery. These animals have recovered well and are assessed behaviorally every two weeks.

Finally, since in patients we will likely pursue delivery via the spinal fluid in the spine, we have identified the surgical coordinates for intrathecal (IT) delivery in mice. We now feel confident that we have identified the surgical methods for in vivo therapy in mice.

MILESTONE 5. ASO Therapy: *Complete pilot in vitro efficacy studies* ✓

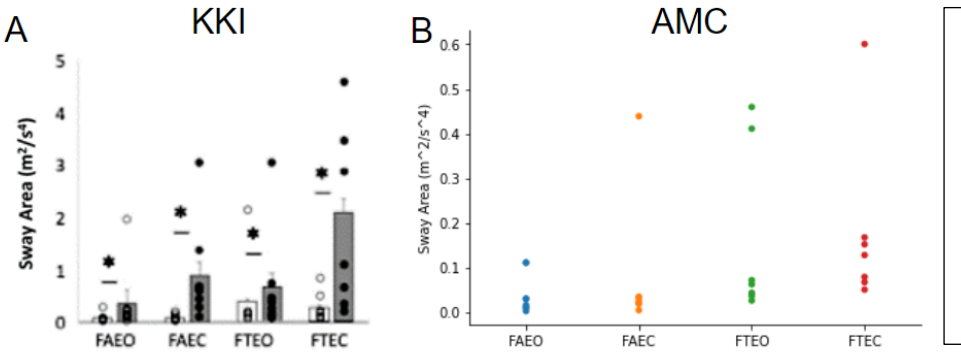
We have pilot data on ASO efficacy in LBSL patient neurons. While studies on dendrite growth are ongoing, we have preliminary data that ASOs can improve gene expression of the corrected (transcripts which include Exon 3) transcript (Figure A, below) in a dose-response manner. Furthermore, we tested the effect of ASO therapy on neuronal lactate production, showing LBSL have increased lactate production compared to control, and treatment with increasing doses of ASO reduce

those levels (Figure B, below). These findings suggest increased DARS2 production and a therapeutic benefit to mitochondrial function; however, more testing is necessary to ensure this data is reproducible and is underway.



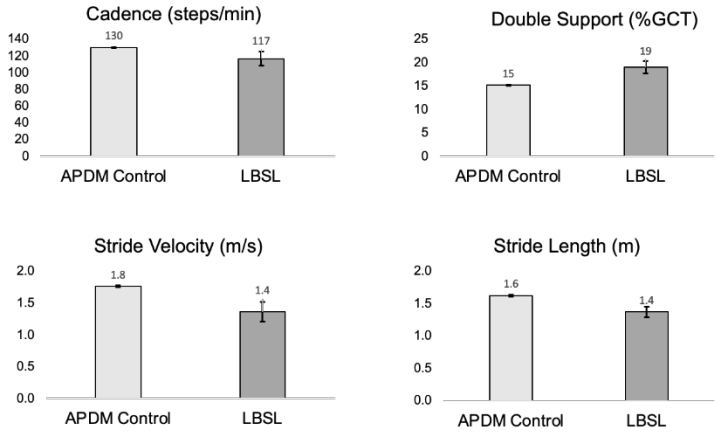
MILESTONE 6a. Complete baseline sensory motor data analysis of Dutch and American patients ✓

We have so far obtained data from 8 Dutch patients who were evaluated in Amsterdam by Dr. Engelen's team. All of these individuals are adults. We have focused on two main outcome measures – sway area, and walking speed. Below is our baseline sway area measurement in comparison to the 8 subjects seen in Amsterdam Medical Center (AMC). Importantly, as has been previously published, we know that sway area decreases significantly with age, therefore, as we can see here children assessed at Kennedy Krieger Institute (KKI) have higher sway measures (note the scale on the left ranges from 0-5 vs the scale on the right is 0-0.5. As expected, it appears that when subjects have their feet together and eyes closed (FTEC) they sway most.



Comparison of Sway between Kennedy Krieger pediatric cases (KKI) vs Amsterdam Medical Center (AMC) adult cases of LBSL. Each dot depicts the sway measure of one patient in these four conditions:
FAEO: Feet Apart, Eyes Open
FAEC: Feet Apart, Eyes Closed
FTEO: Feet Together, Eyes Open
FTEC: Feet Together, Eyes Closed

At Kennedy Krieger, we have determined that the 2-minute walk test is preferable over the 6-minute walk test. While in Amsterdam the initial data included 6-minute walk test, the AMC group is now also collecting the 2- minute walk test. The next figure depicts gait indices obtained from the 6-minute walk test in the 8 adults with LBSL and shows significant differences compared with age matched control published data. We continue to collect data longitudinally on both sites and both sites remain open to enrollment in case new patients are identified.



MILESTONE 6b. Set up natural history study in Finland ✓

Our new research collaborators at the University of Helsinki (PI Dr. Emil Ylikallio) were sent 2 OPALs kits and they enlisted a postdoctoral fellow and a research assistant to contribute effort to the LBSL natural history study. We completed training with their team on the OPALs assessments and the standardized assessment and rating of ataxia (SARA) scale by the end of 2022. They now have IRB approval for the study and will begin enrolling patients this spring.

We also began a research collaboration at the Hospital Pequeno Principe in Curitiba, Brazil (PI Dr. Josiane Souza) in November 2022 to further extend the natural history study participation. The have obtained initial IRB approval and 2 OPALs kits were ordered this month. We anticipate beginning their training in April.

In summary, we are pleased to report that we are on target with our research despite some challenges in recruitment of staff. As outlined in the original Milestone document, our next benchmark will be at 12 months (10/1/2023) with the following expected Milestones and related funding request:

1. For Gene Therapy Project:
    a. Complete efficacy studies in 3 LBSL and 3 isogenic cell lines assessing the effect of AAV9 on DARS2 gene expression, dendrite growth
    b. Complete pilot biodistribution studies for AAV9 in mice
    c. Complete pilot studies on effect of AAV9 on animal behavior in mice

2. For ASO Project:
    a. Complete efficacy studies in assessing the effect of ASO on patient neurons in several LBSL cell lines assessing gene expression, dendrite growth and mitochondrial activity
    b. Complete pilot biodistribution studies for ASO in mice
    c. Engage GMP manufacturer for large scale clinical grade ASO production for human trials

3. For Clinical Research:
    a. Complete interim analysis of follow up data from US and Netherlands
    b. Start obtaining data from Finland
    c. Initial engagement with FDA regarding a Patient-Focused Drug Development (PFDD) vs listening session
    d. Identify regulatory consultant for FDA IND filing

Requested Funding for 4/1/23-9/30/23 period:
$636,000 Salaries and Supplies (for 6 months)
Total: $636,000 direct costs

In order to continue our research we request that you please donate the above requested amount to our program as outlined in our initial proposal.

Payment Instructions:
Checks should be made payable to: **Kennedy Krieger Foundation** and sent to the attention of:
Leslie A. Marsiglia
Office of Philanthropy
707 North Broadway
Baltimore, Maryland 21205

Your gift may also be sent via electronic wire transfer. Please contact Leslie for wire transfer instructions: marsiglia@kennedykrieger.org

Thank you again for all your support and your trust in our work. We are eager to continue our important work and are inspired by your dedication and commitment to support our efforts to find a cure for LBSL.

Sincerely,

Ali Fatemi, MD, MBA