June 13, 2017

Re: Hsp70 Project Update from Wake Forest

Dear Mr. and Mrs. Blaze,

My name is Miles Lyon and I am the student in Dr. Milligan's lab who is working on the completion of the Hsp70 project. While I know Dr. Milligan does her best to update you with the progress of the project, she is currently being pulled in several directions as head of the neuroscience graduate program, principal investigator of her own lab, and mentor to her students, and even though she is managing that feat day in and day out I asked her if I could email you myself to keep you informed about our progress here at Wake. She thought this would be a good idea because you will know that not only has your support allowed this project to move forward, it has also allowed me to pursue my training so that I too will become a Blazeman Research Warrior. Before I get into the science, however, I'd like to take a little bit of time to introduce myself so you get the chance to know the person working on this project.

I started in the neuroscience graduate program in August of 2015 as a Masters student in Dr. Milligan's lab. I originally applied to Wake Forest to work in this lab and on the Hsp70 project as my senior year of college was coming to a close. Why Dr. Milligan's lab? In all honesty, I had never met any of the scientists at Wake Forest, I didn't know what to expect coming into the program, nor did I know if she even had space for students, but I had been keeping up with her research on motor neurons as a college student and admired her creativity and innovation in the field. Why the Hsp70 project? This is a more straight forward answer – it's a unique, simple, and intelligent concept. As a concept I'm sure you are unfortunately all too familiar with, small molecule treatments for ALS have been extremely ineffective in treating the disease for a variety of reasons, but the one that sticks out to me is complexity. A pharmaceutical compound is designed to activate a cellular signaling pathway with the hopes of increasing or decreasing the target protein product at the end of the cascade. I thought the idea of skipping the whole pathway and providing the target protein directly was brilliant, simple, and evidently effective as David and Mac proved over the past 10 years.

As someone who grew up in a family affected by a motor neuron disease, I knew from an early age I wanted to make an impact in both the patients and families suffering from these traumatic disorders. I know from first-hand experience that these disorders affect people mentally as much as they do physically, and I have the utmost respect for the way your family has fought to raise money and opportunities for this institution to work towards developing therapeutic strategies to treat motor neuron disease.

Now, for the science. I had originally predicted that we would have completed a dose-response curve to determine the effectiveness of Hsc70 on maintaining neuromuscular innervation by May, but as ambitious scientists, we sometimes forget just how many hours and days it takes to finish the job. We are continuing to do a lot of important work in the process to confirm that the science is consistent, accurate, and most importantly correct. So while we are a bit behind schedule, which I am working very

hard to compensate for, I wanted to give you a shortlist of goals that we have completed in the last 6 months:

- Development of an accurate and consistent protocol for the purification of recombinant human Hsc70 (A single batch takes 4 days to make, but after a comparison of multiple single-batch-purifications we have a product that yields great levels of purity and activity compared to what can be bought from scientific corporations) – this protocol and the protein product alone is of great value to investigators in the scientific community who wish to continue studying Hsc70 as a potential therapeutic for any disease.

- We have completed treatment for 90% of the dose response curve for Hsc70 –the main part of this study. This is one of the largest data sets for a single drug dose-response curve that I have come across in the ALS literature. We have completed treatment on 113 of 128 animals spanning 4 different doses of Hsc70 across both sexes. I chose to use a sample size of 8 animals per sex/per dose to give us enough statistical power to make claims on the protein's effectiveness should there be an effect in one sex but not the other, and should that be the case this data set will be very important for further investigation on sex differences in ALS models and patients. I have sacrificed and collected all of the necessary tissue from these animals and I am currently in the process of acquiring and processing the data necessary to examine the effect (the really exciting part!!). To be honest, this part of the project has been the most time consuming. I have beaten myself up for this not being done yet- but as Dr. Milligan pointed out, the gestation period for mice is 21 days- and then we have to hope we get the predicted number of SOD1 mice, to have a treatment group and littermate (and gender) control group within that litter. Mice can have anywhere from 4-10 pups/liter, with half predicted to be SOD1. So while we have multiple breeding pairs going, it still takes time to accumulate the necessary number of animals. Once the animals are born, we wait until day 30 to start treating. And treatments go until day 75 (add 45 days). Once the treatments are done, Dr. Milligan admitted she just thought- "oh, just process the tissue and count the neuromuscular junctions)!" Well, it's a day to perfuse the animals, four days to cryoprotect, another day to embed the tissue, another day to cut the tissue and then two days to do the immunohistochemistry. I take the project from protein production to this point (roughly 109 days from start to finish per treated animal). As discussed below, Because we are doing a blinded study, Dr. Milligan and our MD/PhD student are counting the tissue. Dr. Milligan seems to like to do this because it keeps her connected to the experiments. We are looking for this portion of the study to be completed hopefully mid-July.

- Collection of fresh frozen tissue from a sub set of treated animals to identify where injected Hsc70 ends up in the body – an important bit of science for us, because we need to know where the protein is most effective. If we can systemically treat patients with a compound that doesn't have to cross the blood-brain-barrier, and instead works at the neuromuscular junction in the muscles, we will have a safer and easier target to hit if we can move to human patients.

These are the major milestones in this project that I am proud to have completed, but this project, like all projects in our lab has been a team effort. Our lab technician Pop has helped me breed all the animals and taught me how to dissect the necessary tissue and Marlena an M.D./PhD student in the lab and Dr. Milligan are helping to count the fluorescently labeled neuromuscular junctions I stain to provide me with an un-biased and accurate account of the protein's effectiveness. I am happy to tell you that we have pushed hard and completed the large majority of the work we need to finish this project.

In conclusion of this progress report, I'd like to touch on one more thing – the intangible. It has not been lost on me that this project is very important to your family. I was not fortunate enough to meet your son, but by all accounts he was an incredible person and I consider myself extraordinarily fortunate to be working as part of a project that represents his strength, desire, and passion. I, however, often find myself most fortunate to have your support. What you may not think about when you provide the lab with money is what it is going toward. While it's obviously being used to buy the necessary reagents and supplies we need to complete the work, it is also providing us with opportunities we would not otherwise have. For example, following completion of this project I have been accepted into the PhD tract here at Wake, and I will continue to work in Dr. Milligan's lab studying motor neuron disease- and even maybe helping to move this forward to clinic. Your support has provided me with a chance to work with an incredible group of people under the guidance of a wonderful mentor. As someone who wants to dedicate their lives to studying motor neuron disease, I cannot thank you enough for your support and the opportunity I have been given because of it. I come to work every day, 7 days a week, to repay that favor in kind by ensuring that the work we publish in the coming fall is accurate, complete, and done correctly. You have given me the chance to chase my dreams, and I have made it my personal mission to make the completion and publication of the Hsc70 project the first step of my scientific career.

Once again, you have my unwavering gratitude and I look forward to providing you with positive results shortly!

-Miles

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