The Angel Fund for ALS Research Program

RESEARCH REPORT

submitted by Dr. Robert H. Brown, Jr.

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We are pleased to submit this Progress Report to The Angel Fund for ALS Research. This outlines several major projects in which there has been substantial progress in ALS research over the last year at the UMass Chan Medical School. We feel very fortunate have been the recipients of generous Angel Fund support for our research and therapy programs over multiple years, and we are particularly grateful that support now assists several investigators here.

Pre-clinical Studies

1. Novel toxic spreading substance in ALS

Over the last four years, our lab and four other labs at UMass Chan Medical School (Bosco, Gao, Sena-Esteves and Almeida Laboratories) have collaborated with Prof. Brigitte van Zundert (formerly a post-doc in the Brown Lab, now an independent scientist in Santiago, Chile) to study factors that contribute to the spread of disease in ALS. Prof. van Zundert discovered a substance designated polyphosphate, that is secreted by non-neuronal cells in ALS mice and that impairs the viability of motor neurons. As noted in our report last year, these polyphosphates may facilitate spreading of the motor neuron death process in ALS tissues. The Brown lab has been fortunate to have senior research colleague, Carla Cefaliello, Ph.D., a biochemist from Italy who developed an assay for these polyphosphates in patient spinal fluid. We were delighted that this study was reported in the journal *Neuron* early in 2022 (1).

2. New biomarker to assess function of motor neurons

For more than 15 years, we have pursued development of a new physiological marker to provide an index of motor neuron function. This has focused on the capacity of motor neurons to transport materials from muscle back to the motor neuron cell body in the spinal cord. This fundamental property of neurons, labelled “retrograde axonal transport,” has not been measured in humans or even in mice repetitively. Thus, we have not been able to quantify how well a transported protein travels from muscle, across the nerve-muscle junction and up the axon of the motor neuron into the motor neuron cell body in the spinal cord. We believe this is important because an early event in ALS is disruption of axonal transport. A quantitative assay for axonal transport may be a way to detect early benefit of an ALS therapy and thus accelerate therapy development. Early this year, we were very happy to publish an assay (2) with which we could use a radioactive but non-toxic component of tetanus toxin to assay axonal transport. This work was spear-headed by Dr. Justin Lee in our team, who has been able to define the rate of net retrograde axonal transport in normal and ALS mice. He reported that this transport process clearly degrades in ALS mice over time. As noted last year, Dr. Zack Kennedy (then completing his Ph.D.) used gene editing to inactivate the

offending mutant ALS gene (SOD1). Zack and Justin showed that the transport assay detected benefits of gene editing in the ALS mice long before conventional assays, such as survival. We are considering approaches to moving this therapy toward application in humans, a process that will be slow but potentially very beneficial in accelerating clinical trials.

3. Expression of ALS protein TDP43 in mice

In another investigation (3), we have been privileged to collaborate with our colleague Zuoshang Xu to study the protein TDP43, which is broadly implicated in the biology of ALS, including most familial and sporadic forms. Zuoshang generated a series of mice that expressed slightly more than normal amounts of the normal TDP43 protein. This lead to neurodegeneration involving motor neurons in the cortex and inflammation and loss of myelin in the spinal cord. The consequence was progressive paralysis and death; motor neurons in the spinal cord showed detachment from muscle, although they did not succumb to the disease process. These data show that low-grade but constant elevations of the normal TDP43 protein can be toxic, leading to paralysis. One implication is that therapies that target TDP43 may, if appropriately calibrated, be beneficial in some cases of ALS.

4. Single cell analysis of abnormal gene activation in ALS.

Over the last year, we have again had the good fortune to extend our collaborations with Dr. Paul Greer (Program in Molecular Medicine) to study diverse cell types in the SOD1-G93A mouse model of ALS. As reported last year, this project involves two graduate students, Kit Mocarski and Abbi Hiller, who are studying panels of RNA generated in multiple different cells in the brain and spinal cord. These sets of RNA are activated at different time points as normal and ALS mice age and, importantly, define distinct pathways or molecular signatures that define how these cells contribute to motor neuron death in the ALS mice. The central goal is to identify targets for therapy in these ALS signatures. Kit and Abbi have highlighted a population of inflammatory, disease-associated microglial cells (DAMs) that are activated in the ALS mice and that serve as a potential target for therapy development. At least in male mice we have determined that the death of motor neurons reflects the activation of a surface microglial cell protein (Ms4a ligand). When the Ms4a proteins are deleted from microglia, the survival in the ALS mice is extended. Our intention in these studies is to understand more thoroughly why the Ms4a proteins are adverse in the male ALS mice and how this information might be translated in therapeutic trials.

5. Analysis of misfolding of chromosomes induced by the ALS gene C9orf72

We are pleased to report that we have had another good year of progress in our collaboration with Dr. Job Dekker in the Program in Systems Biology. As summarized last year, a graduate student in the Brown Lab, Ozgun Uyan, has been co-mentored by Dr. Dekker and Bob to analyze in detail the folding of chromosomes in the motor neuron without and with the C9orf72 mutation. This study compared thousands of interactions between the covering of chromosomes, known as chromatin, up and down all chromosomes. Such a study has not previously been undertaken in pure motor neurons, either in the normal state or with the C9orf72 ALS gene mutation. Ozgun's work has highlighted several interesting points. One is that there are unanticipated patterns of interaction of motor neuron chromatin, even in the absence of the C9orf72 mutation. Another point is that the C9orf72 gene expansion that causes ALS substantially modifies the architecture of that gene, leading to disruptions in some patters of gene expression.

6. Other Preclinical Studies

In addition to the above studies, in which we have been centrally involved, we have been privileged to contribute to studies led by investigators in other laboratories. For example, we assisted an outstanding Israeli scientist, Prof. Eran Hornstein (Wiezmann Institute, Israel) in a study documenting that mutations in a receptor for the protein Interleukin-18 can modify susceptibility to ALS (4). With Professors Ammar Al-Chalabi and Chris Shaw at King's College in London we have shown that in patients with SOD1 gene mutations there is a dissociation between the age of onset and survival (5).

Pre-Clinical Therapeutic Studies

1. Suppression of mutant SOD1 in ALS mice

For three years, we have worked with Anastasia Khvorova in the RNA Therapeutics Institute at UMass Chan Medical School to explore innovative technologies from her lab that provide new methods for inactivating the mutant SOD1 gene. As summarized in our report last year, Anastasia has devised new molecules (called di-siRNAs) that powerfully penetrate into brain tissue and silence the SOD1 gene. The di-siRNAs enhance suppression by comparison with more conventional gene silencing tools (such as antisense oligonucleotides or ASOs). We have now confirmed that Dr. Khvorova's new di-siRNA produce an unprecedented increase in survival in the ALS mice, surpassing any therapy we have previously developed for SOD1 gene suppression. As noted last year, we hope to move this rapidly toward a clinical trial.

2. CRISPR-based editing of the C9orf72 gene in ALS.

Dr. Karin Meijboom in the Brown Lab has been studying the use of gene editing to excise the toxic mutation in the C9orf72 gene as an approach to the long-term goal of correcting that mutation in patients with the C9orf72 form of ALS. The critical mutation in the C9orf72 ALS gene is an expansion of a tract of DNA made of repeats of a stretch of six DNA molecules. Whereas most people have fewer than 30 or so repeats, individuals with C9orf72 may have 100's and even 1000's of such repeats. In the present study, initiated with Dr. Chris Mueller (now at Sanofi) and aided by Drs. Fen-Biao Gao and Erik Sontheimer at UMass Chan, Karin employed CRISPR gene editing methods to excise the expanded repeats from the mutated C9orf72 ALS gene. She was able to accomplish this in ALS cells in a Petri dish and also in ALS mouse models. The data demonstrated shortening of the expanded, mutant stretch of C9orf72 DNA and accompanying findings attested to the truncation of the mutant gene. We are delighted that this study has now been published (6). Like many other teams in ALS research, we and others at UMass Chan Medical School are considering was that this therapy might be moved to human application, process that will likely entail many challenges.

Therapeutic Studies

1. Afinersen for the C9orf72 gene – toward a second study

This project has been reviewed previously in detail. Briefly, afinersen is the anti-sense oligonucleotide (ASO) generated by the UMass Chan Medical School team (Drs. Jonathan Watts, Helene Tran, Michael Moazami, others) that has looked promising in a single patient study, which was published this year (7). As we design a possible larger study of afinersen, we are balancing several considerations. On the positive side, we now have a full report from Charles River Laboratories on a second, higher dose safety study of afinersen in monkeys. This study, which was recommended by the FDA and undertaken jointly with our colleagues at the MGH, did not show

adverse effects after 3 months of treatment. Also, we have also now published the data (mentioned last year) obtained in collaboration with Dr. Fen-Biao Gao showing that three different biomarkers document suppression of the C9orf72 gene by afinersen (8). On the other hand, in spring of this year, an analysis by Biogen of a comparable ASO targeting C9orf72 failed to show benefit and, regrettably, at higher doses over a six month period showed evidence of exacerbation of ALS in a series of C9orf72 patients. After reviewing these data, we feel that an additional trial of afinersen should be undertaken, an option we are pursuing.

2. ASO to correct the gene STMN2

As we reported last year, Dr. Jonathan Watts and I have worked with Dr. Kevin Eggan (BioMarin, Inc) to generate an ASO that has a beneficial effect in human motor neurons on axonal outgrowth. After review by the FDA, we are dosing a single ALS patient with this ASO. We are awaiting full assessment of the status patient, based on a combination of clinical well-being and blood tests.

3. Second stem cell trial in ALS

As outlined in previous Angel Fund Progress Reports, we (and other centers) have now completed a second trial of stem cell therapy in 95 ALS cases, jointly with the Israeli company Brainstorm. The results of this study have now been reported (9). The stem cells failed to meet their major trial endpoints but did have a remarkable effect on improving molecular markers of disease in the patients' spinal fluid (corresponding to less inflammation and higher levels of neuroprotective factors). The company is now considering avenues for further advancement of this therapeutic approach.

4. Tay-Sachs Gene Therapy

Over the last year, it has been an honor to perform neurological examinations on a series of young patients with Tay-Sachs Disease that our dean, Dr. Terry Flotte, is now treating with gene therapy. Dean Flotte has published an initial report on this ground-breaking project (10).

5. Accelerator Project

Over the last eighteen months, we have continued to develop a new initiative to develop multiple ASOs to treat the most common ALS genes. This project entails close interaction with colleagues at UMass Chan Medical (Drs. Jonathan Watts, Anastasia Khvorova, Daryl Bosco) and will be clinically partnered with our colleagues at the MGH (Drs. Merit Cudkowicz, James Berry).

Selected Recent Publications

Brown Lab

1. Arredondo C, **Cefaliello** C, Dyrda A, Jury N, Martinez P, Diaz I, Amaro A, Tran H, Morales D, Pertusa M, Stoica L, Frit E, Corvalán D, Abarzúa S, Méndez-Ruette M, Fernández P, Rojas F, Kumar MS, Aguilar R, **Almeida S**, Weiss A, Bustos FJ, González-Nilo F, Otero C, Tevy MF, **Bosco DA**, Sáez JC, Kähne T, **Gao** F-B, Berry JD, Nicholson K, **Sena-Esteves M**, Madrid R, Varela D, Montecino M, **Brown RH,** van Zundert B. Excessive release of inorganic phosphate by ALS/FTD astrocytes causes non-cell autonomous toxicity to motoneurons. Neuron 2022 May 18;110(10):1656-1670.

2. **Lee** JP-T**, Kennedy** Z, Wang Y, Lu Y, **Cefaliello** C, Uyan O, Song C-Q, Godinho BM, Xu Z, Rusckowski M, Xue W, **Brown RH Jr**. Imaging net retrograde axonal transport in vivo: a physiological biomarker. Ann Neurol 2022, 91(5):716-729.

3. Yang C, Qiao T, Yu J, Wang H, Guo Y, Salameh J, Metterville J, Parsi S, Yusuf I, **Brown RH**, Cai H, **Xu Z.** Low-level overexpression of wild type TDP-43 causes late-onset, progressive neurodegeneration and paralysis in mice. PLoS One 17(2): e0255710. 2022 https://doi.org/10.1371/journal.pone.0255710.

4. Eitan C , Siany A , Barkan E , Olender T, van Eijk K, Moisse M, Farhan S, Danino Y, Yanowski E, Marmor-Kollet H, Rivkin N, Yacovzada N-S, Hung S-T, Cooper-Knock J, Yu C-H, Louis C, Masters S , Professor Kevin Kenna K , Rick van der Spek R, William Sproviero W , Dr Ahmad Al Khleifat AA, Iacoangeli A, Shatunov A, Jones A, Elbaz-Alon Y, Cohen Y, Chapnik E, Rothschild D, Weissbrod O, Beck G, Ainbinder E, Ben-Dor S, Werneburg S, Schafer D, **Brown R**, Shaw P, Van Damme P, van den Berg L, Phatnani H, Segal E, Ichida J, Al-Chalabi A, Veldink J. Whole-genome sequencingg reveals that variants in the Interleukin 18 Receptor Accessory Protein 3'UTR protect against ALS. Nat Neurosci. 2022 Apr;25(4):433-445. doi: 10.1038/s41593-022-01040-6. Epub 2022 Mar 31.PMID: 35361972

5. Opie-Martin S, Iacoangeli A, Topp SD, Abel O, May K, Shatunov A, Fogh I, Bowles H, Limbachiya N, Spargo T, Al-Khleifat A, Williams KL, Jockel-Balsarotti J, Bali T, Self W, Henden L, Nicholson GA, Ticozzi N, McKenna-Yasek D, Tang L, Shaw P, Chio A, Ludolph A, Weishaupt JH, Esselin F, de la Cruz E, Landers JE, Glass JD, Mora JS, Robberecht W, Van Damme P, McLaughlin R, Hardiman O, van den Berg L, Veldink JH, Corcia P, Stevic Z, Siddique N, Silani V, Blair IP, Fan D-S, Camu W, Basak AN, Siddique T, Miller T, **Brown RH,** Al-Chalabi A, Shaw CE. The SOD1-mediated ALS phenotype shows a decoupling between age of symptom onset and disease duration. Nat Commun. 2022 Nov 12;13(1):6901. doi: 10.1038/s41467-022-34620-y.PMID: 36371497.

6. **Meijboom** KE, Abdallah A, Fordham NP, Nagase H, Rodriguez T, Kraus C, Gendron TF, Krishnan G, Esanov R, Andrade NS, Rybin MJ, Ramic M, Stephens ZD, Edraki A, Blackwood MT, Kahriman A, **Henninger N**, Kocher JA, Benatar M, Brodsky MH, **Sontheimer** EJ, Petrucelli L**, Gao** F-B, **Brown RH**, Zeier Z, **Mueller** C. CRISPR/Cas9-mediated excision of ALS/FTD-causing hexanucleotide repeat expansion in C9ORF72 rescues major disease mechanisms in vivo and in vitro. Nat Commun. 2022 Oct 21;13(1):6286. doi: 10.1038/s41467-022-33332-7.PMID: 36271076.

7. **Tran H, Moazami MP**, Yang H, McKenna-Yasek D, Douthwright C, Pinto C, Metterville J, Shin M, Sanil N, Dooley C, Puri A, Weiss A, Wightman N, Gray-Edwards H, Marosfoi M, King RM, Kenderdine T, Fabris D, Bowser R, **Watts JK, Brown, RH Jr**. Potent mixed backbone antisense oligonucleotide safely suppresses expression of mutant C9orf72 transcripts and polydipeptides: first in human pilot study. Nat Med. 2022 Jan;28(1):117-124. doi: 10.1038/s41591-021-01557-6. Epub 2021 Dec 23.PMID: 34949835.

8. Krishnan G, Raitcheva D, Bartlett D, Prudencio M, McKenna-Yasek DM, Douthwright C, Oskarsson BE, Ladha S, King OD, Barmada SJ, Miller TM, Bowser R, Watts JK, Petrucelli L**, Brown RH**, Kankel MW, **Gao** FB. Poly(GR) and poly(GA) in cerebrospinal fluid as potential biomarkers for C9ORF72-ALS/FTD. Nat Commun . 2022 May 19;13(1):2799. doi: 10.1038/s41467-022-30387-4. PMID: 35589711

9. Cudkowicz ME, Lindborg SR, Goyal NA, Miller RG, Burford MJ, Berry JD, Nicholson KA, Mozaffar T, Katz J, Jenkins LJ, Baloh RH, Lewis RA, Staff NP, Owegi MA, Berry DA, Gothelf Y, Levy YF, Aricha R, Kern RZ, Windebank AJ, **Brown RH Jr**. A randomized controlled trial of mesenchymal stem cells to induced

secrete high levels of neurotrophic factors in amyotrophic lateral sclerosis. Muscle Nerve. 2022 Mar;65(3):291-302. doi: 10.1002/mus.27472. Epub 2022 Jan 5.PMID: 34890069.

10. Flotte TR, Cataltepe O, Puri A, Batista AR, Moser R, McKenna-Yasek D, Douthwright C, Gernoux G, Blackwood M, Mueller C, Tai PWL, Jiang X, Bateman S, Spanakis SG,Parzych J, Keeler AM, Abayazeed A, Rohatgi S, Gibson L, Finberg R, Barton BA, Vardar Z, Shazeeb MS, Gounis M, Tifft CJ, Eichler FA, **Brown RH Jr**, Martin DR, Gray-Edwards HL, Miguel Sena-Esteves M. AAV gene therapy for Tay-Sachs. NatMed, 2022.

Bosco Lab

1. Traumatic Brain Injury (TBI) as a stress-inducing risk factor for ALS

We know there are gene mutations that cause ALS, but that most cases are sporadic. Therefore, we are looking at environmental risk factors, including the effects of stress and injury on ALS. In collaboration with Dr. Nils Henninger at UMass Chan, who is also supported by the Angel Fund, we are studying how brain trauma, or TBI, contributes to ALS. This work has led to a publication this year that reported correlations between animal weight and severity of TBI (11). The Bosco lab has also uncovered stress response pathways that are activated in TDP-43 mouse neurons after TBI, which may represent a therapeutic targets for stress-induced ALS.

2. Analysis of Microglial Cells in ALS

In terms of understanding what causes ALS, we are also investigating cellular processes that can trigger ALS or worsen the disease process. One way we are doing this is to look at how different cell types within the central nervous system handle stress. A class of cells that we are particularly interested in are immune cells that are supposed to help the brain and spinal cord after injury or during disease, but the field is finding that these cells become dysfunctional and can make the disease worse. The Bosco lab has recently established human induced pluripotent stem cell-derived microglia, a type of immune cell that plays a vital role in disease. This work has led to two publications this year (12-13), and one that has been submitted for publication. We also contributed to the publication on a different glial cell-type in ALS, astrocytes, as mentioned by Dr. Brown above (1). The Bosco lab is extending studies of glial cells into ALS mouse models as described in our request for support last year. Specifically, we are assessing the reactive state of microglia in ALS mouse models and are challenging mice with a drug that induces demyelination to examine mutant microglia function *in vivo*; these studies are currently underway.

Selected recent publications

11. Differential association of baseline body weight and body-weight loss with neurological deficits, histology, and death after repetitive closed head traumatic brain injury. Kahriman A, Bouley J, **Bosco DA**, Shazeeb MS, **Henninger N**. *Neurosci Lett.* 2022 Feb 6;771:136430. doi: 10.1016/j.neulet.2021.136430. PMID: 34973374.

12. Human Microglia-like Cells: Differentiation from Induced Pluripotent Stem Cells and In Vitro Live-cell Phagocytosis Assay using Human Synaptosomes. Funes S, **Bosco DA**. *J Vis Exp.* 2022 Aug 18;(186). doi: 10.3791/64323. PMID: 36063018.

13. A reference human induced pluripotent stem cell line for large-scale collaborative studies. *Cell Stem Cell*, Pantazis…Funes S, **Bosco DA**, …Merkle. December 2022. https://doi.org/10.1016/j.stem.2022.11.004

Respectfully submitted,
Robert H. Brown, Jr., D.Phil., M.D. Dary A. Bosco, Ph.D.

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