

## **Angel Fund Progress Report May 2013**

Below is a brief update from Dr. Robert H. Brown, Jr. on the continuing recent progress in the ALS research program. We have made substantial progress in finding new ALS genes and, at the same time, have pursued projects that have direct bearing on therapy discovery. This report will focus primarily on new studies but will also include summarize the report of our major activities over the last 12 – 18 months.

### **I. Stem Cell Studies**

#### ***A. Animal Studies***

In January, we were pleased to publish a report from an extensive consortium that has investigated the possible benefits of implanting neural stem cells into ALS mice. These studies conducted jointly with Evan Snyder from UC San Diego, Ted Teng at Boston Children's Hospital and Jeff Rothstein at Johns Hopkins demonstrated that direct injection of neural precursor cells isolated from mouse embryos modestly prolonged survival in our ALS mice. It was clear in these experiments that a fraction of the donor cells engrafted and persisted in the spinal cords of the host mice. However, even after weeks of engraftment, the incoming cells did not become new motor neurons. Rather, they seemed to exert a positive influence by becoming support cells (astrocytes, microglia) that helped the endogenous motor neurons survive.

#### ***B. Toward Human Studies***

**1. Brainstorm Therapeutics, Inc.** We continue to work closely with Brainstorm Therapeutics (Jerusalem) to develop a pilot trial of stem cells in humans with ALS. The study will involve three centers: UMass Medical School, the Mass General Hospital and the Mayo Clinic. While the final protocol has not been selected, the general plan is to harvest a subtype of stem cells (mesenchymal stem cells) from bone marrow, expand the numbers of cells in cell culture for 4-6 weeks and then re-infuse the cells into the original ALS donor patients. This treatment will likely involve both intramuscular injections and infusion into the spinal fluid. We met with the FDA in the fall for an initial review of this program; a final submission is anticipated in the next 2-3 months, hopefully permitting a start-date before the end of 2013.

**2. Immunosuppression.** A stem cell trial performed at Emory University over the last 18 months produced a clear beneficial effect in one individual (of 14 treated). Because the cells in that study were derived from fetuses, rather than the ALS patients themselves, it was deemed necessary to immunosuppress the recipients, as one would for an organ transplant. Thus, in the one individual who did well in this study, it was not clear whether the favorable response was from

the incoming cells or the immunosuppression. As one approach to dissecting these factors, we are working with Emory and the MGH to initiate a trial of aggressive immunosuppression in a small, pilot group of ALS cases, without stems. We are hopefully that this study will begin by early fall.

## **II. Genetics**

### **A. Massive Gene Sequencing in Sporadic and Familial ALS – 1,000 Exomes**

As previously reported, we have embarked on high-throughput gene sequencing methods to study both familial and non-familial (sporadic) ALS. The initial studies involved sequencing all components of DNA in 50 cases, in a project undertaken with Duke University. We have now expanded that project to target nearly 1,000 cases, focusing on those elements in DNA (known as the exome) that actually are expressed as proteins. In this new project, we are working with Duke University again, but also with multiple other collaborations from Kings College (London), Utrecht University (Holland), Columbia University, McGill University, Stanford University, and a large genetics center in Huntsville, Alabama. We anticipate that by the early fall we will generate close to 800 full exome sequences. This will allow us to test the hypotheses that sporadic ALS may in fact sometimes arise because of gene defects (even if it does not run in families) and that some cases will be a consequence of more than one offending gene mutation. Most importantly, we are hopefully that these insights will help in targeting new ALS treatments.

### **B. Mendelian Genes in Familial ALS**

#### **1. Genes that cause ALS.**

The numbers of gene mutations that can cause ALS continues to grow. In our report in August, 2012, we listed three new genes that we and others reported over the previous year or so (profilin-1 [Dr. John Landers], ubiquilin-1, valosin-containing protein or VCP). We are delighted to say that other teams have now confirmed these findings, reported other mutations in these genes in other families. Working with Dr. Claudio Hetz in Chile, we believe we have identified at least two more candidate ALS genes (studied in progress). Strikingly, mutations in upwards of 30 different genes are now thought to be causal in ALS. While there is a diversity of genes, it appears that they overlap, in that they implicate a small number of pathological processes in ALS. These include protein misfolding, mitochondrial dysfunction, disturbances of RNA biology, and altered axonal transport and growth, among others.

#### **2. Genes that modify the course of ALS.**

In the summer, members of the UMMS team reported two gene variations that do not cause ALS but that appear to modify its course. One is a gene called EphA4, described with our colleague Dr. Robberecht from Leuven, Belgium. Our observation was that reducing the activity of this gene improved outcome in ALS mice and rats, and probably in humans. We also helped Dr. Marc Freeman at UMass study a different gene that triggers death of axons after injury. In normal

motor nerves, genetically silencing this gene (called sarm), greatly improved survival of nerve axons after injury. Over the last six months new colleagues in the lab (Drs. Owen Peters, Nils Heninger) have found that genetically deleting this gene is also slightly beneficial in ALS mice and, in addition, in head trauma. In the same period, we have also undertaken experiments to silence these genes not by genetic breeding techniques but by using inhibitory RNA (siRNA), delivered to the spinal cord via gene therapy using adeno-associated virus (Drs. Guangping Gao, Dan Wang).

### **III. ALS Biology**

The primary importance of the new ALS genes is that they should define targets for ALS therapies. Accordingly, once an important new gene is identified, we attempt to generate both cell and animal models that illustrate the toxicity of the mutant genes. We have previously outlined our studies of SOD1 ALS mice, which are continuing. The main area of progress in this regard over the last six months has been in our efforts to generate cell and animal models of the new gene (C9orf72) whose mutations can cause both ALS and frontotemporal dementia. Briefly, a new investigator on our team, Dr. Xiao-Yang Shan, has been able to reproduce initial evidence of pathology generated by mutant C9orf72 in cells in culture. And, working with Dr. Pieter de Jong in California, we also have generated a series of mice that, in our early assessment, show evidence of C9orf72 toxicity that mimics that seen in C9orf72 patients. We do not know yet if these mice will develop paralysis, but will acquire data on this as the mice age.

### **IV. Therapeutic Trials**

#### **A. Mouse trials**

We continue to test candidate drugs in our ALS mice. We currently have two drug trials underway in the mice. We also are well into a trial of a novel protein as an ALS therapeutic, with cautiously promising early results. The protein we are delivering to the brain and spinal cord using a virus (adeno-associated virus, or AAV) administered by vein when the mice are young.

#### **B. siRNA – moving to monkeys**

##### **1. RXi.**

In our report last August, we noted that we have an active program with a company founded by Dr. Craig Mello's, RXi, to develop modified inhibitory RNA molecules that can be administered to the spinal fluid (with a pump) and potentially permeate the spinal cord to silence genes. We have had some success with this strategy, targeting mutant SOD1 gene. With colleagues at RXi, we have clear evidence that some chemical modifications of the RNAi enhance delivery to the brain tissue from spinal fluid. However, the same modifications enhance toxicity of the agents. In our present studies we are trying to find a

balance between the need to get the compounds into the tissue, where we know they can silence genes, but without toxicity.

## **2. Gene Silencing with Adeno-Associated Virus.**

In parallel studies we are working closely with member of Dean Flotte's gene therapy team (Drs. Guangping Gao, Miguel Sena-Esteves, Chris Mueller) and two graduate students to use the AAV virus as an alternate approach to deliver SOD1 silencing reagents to the spinal cord. This project has become one of the major foci of our lab. We have achieved modest success with this strategy with ALS and so are now planning trials of the same viral delivery system in monkeys. While there are no monkey models of ALS, if we can silence the normal versions of the target ALS genes (SOD1, C9orf72) in monkeys we will be in a position to consider the design of a human trial of this delivery system for individuals with mutations in either of these two genes.

## **C. Drug Development**

We are fortunate to have NIH funding to pursue a third approach to gene silencing, which is searching for chemicals that can silence genes, with a specific focus on SOD1, C9orf72 and the gene FUS/TLS. We have published one molecule that silences SOD1 and are currently seeking others. This study involves multiple ALS investigators at UMMS (including Dr. Daryl Bosco), the drug discovery team at Partners in Boston (headed by Drs. Marcie Glicksman and Adrian Ivinson), and Dr. Greg Petsko at Cornell Medical School. We have a one day consortium meeting May 29 to review our progress.

## **D. Human Trials**

On the clinical front, our ALS clinic has done well over the last year, due in large measure to the recruitment of two outstanding neuromuscular faculty: Dr. Johnny Salameh and Dr. Colin Quinn. Thanks to their expert hard work, and the continuing excellent efforts of Diane McKenna-Yasek, we are engaged in four human trials at UMMS: mexilitine, immunosuppression, a muscle activator from Cytokinetics in San Francisco, and the Brainstorm stem cell trial noted above.