Why it is Important to Develop Clinical Trials for UMN Diseases
Update on NU–9 (AKV–9)
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What are the bottlenecks as we move forward with clinical trials? What is the biology/pathology/draggable target? What is the biomarker?

The Current Picture is that:
• HSP and PLS are rare diseases. (Are they really? Just because patients do not come forward, does not mean that they do not exist. We need to reach out to more patients, early diagnosis, recruitment, engagement and personalized medicine approaches.
• Disease mechanisms are heterogeneous and not fully understood. (This is true, but we are making important progress. (spastin and profilin)
• Druggable targets are not well defined or established. (Not so true anymore. There are common cellular defects, as druggable targets.)
• Drug companies do not show interest. (That is not true anymore. There is AKAVA Therapeutics, the only company that gives priority to upper motor neuron diseases.)
• There are no clinical trials. (Well, that may be changing. Trials may be coming up soon for AKV-9)
• There are no biomarkers (This is a problem that important progress is taking place to remedy).

AKAVA Therapeutics does show interest in UMN diseases. They are developing first in class small molecule therapeutics that inhibit protein aggregation, inhibit enzymes and inhibit cancer for a variety of neurodegenerative diseases and cancers. If you go to their website you will find that they define PLS as follows:

Primary lateral sclerosis (PLS) is a very rare neuromuscular disease that causes slow, progressive upper motor neuron degeneration that leads to muscle stiffness, weakness, and pain in the legs that moves to the arms, and then neck muscles at the base of the brain. PLS can develop at any age, but usually occurs between ages 40 and 60. One rare sub type of PLS, inherit-ed and known as juvenile primary lateral sclerosis, begins in early childhood. In many cases, adult onset does not shorten life expectancy, but gradually affects the quality of life. The mechanisms of PLS are unknown, but are thought to include both environmental and genetic factors. Presently there are no approved treatments to slow the vibration or reverse the disease. Treatment options for patients with PLS focus on managing symptoms and improving muscle flexibility.

AKAVA Therapeutics is also very interested in HSP and HSP patients. On their website they define HSP as follows: Hereditary Spastic Paraplegia (HSP) refers to a group of rare inherited neurological diseases, characterized by progressive weakness (paraplegia) and stiffness (spasticity) of leg muscles. Symptoms worsen, as degeneration continues. Additional symptoms vary and may include impaired vision, ataxia, cognitive, impairments, deafness, epilepsy, peripheral neuropathy and urinary urgency and frequency. HSP is also sometimes referred to as familial Spastic Paraplegia (FSP) or Strumpell Lorraine syndrome. Mutations in different genes occur for the different forms of HSP. The genetic and Rare Diseases Clinical Research Center estimat-es between 80,000 and 800,000 people are affected globally with HSP, and there may be up to 30,000 people affected in the Uni-ted States. The mechanisms of HSP are unknown, but are thought to include both environmental and genetic factors. There are no specific approved treatments to slow, prevent, or reverse HSP.

So there is a company working on treatments for HSP and PLS. Is that not wonderful? We feel confident that more drug companies will gain interest in working on our rare diseases as our SP -

CERN develops and strengthens. The SP-CERN will lay the foundation for clinical trials by developing longitudinal patient registries and deciding on effective biomarkers.

Why AKV-9 clinical trial is important?
• AKV-9 was developed by a cell-based and mechanism focused drug discovery effort.
• Translation is at the cellular level.
• Diseased neurons speak, they tell us what they like and what they do not like. We just have to listen to them.
• This is the first clinical trial to improve the health of diseased upper motor neuron's.

A diagram of an Upper Motor Neuron was displayed. She said the malfunctions may include: Axonal problems, Mitochondrial disintegration, protein aggregation, ER problems or loss of cellular integrity each of which can be caused by different HSP genes. The Spastic Paraplegia Foundation has spent years researching and learning about these different malfunctioning mechanisms of the upper motor neuron. Now, we have a very good understanding of why they become vulnerable. Now, we have targets to work on for remedying the problems.

Dr. Ozdinler then displayed a diagram of an upper motor neuron after it has been treated with NU-9 after 60 days. It showed that the Axon was restored, Mitochondria was stabilized, protein aggregation was reduced, the ER was stabilized and cellular integrity was restored. If NU-9 had been able to improve just one or two of these malfunctions, it would have been a huge step forward but it was able to improve all of them. =AVA Therapeutics has expressed great interest in licensing this compound and taking it forward for clinical trials.

The illustration that Dr. Blackstone showed this morning identified the cellular problems of the HSP upper motor neuron has the Mitochondria affected by HSP6, Spartin. Golgi are affected by

SLC33A1. Anterograde transport are affected by KIF1A and KIF5A. Microtubules are affected by spastin (SPG4) and REEP1. Tubular ER is affected by atlastin-1, erlin2, seisin, spastin, REEP1 and reticulon 2. Endosomes are affected by NIPA1, spastizin, spartan, spatacsin, spastin, strumpellin maspardin, AP4, AP5 and alsin. Myelin/oligodendrocytes are affected by PLP, CX47 and FA2H.

When they published their paper with Wiley Publishing with clinical and translational medic one, they expressed an interest in producing a movie about it. The movie identified HSP, PLS and ALS as diseases affected by malfunctions in the upper motor neuron and that Dr. Hande Ozdinler, working with Dr. Richard Silverman has developed a drug showing great promise for an improvement of these malfunctioning mechanisms.

This drug lays a foundation for future cell-based and mechanism focused drug discovery studies. Improving mitochondria and ER stability helps to eliminate upper motor neuron degeneration that occurs due to mSOD1 toxicity and TDP-43 pathology.

The reason a patient goes to a clinic is because they have un-wanted symptoms. For example, a patient with Alzheimer's will have a problem with their memory. A patient with HSP or PLS will go to the clinic due to the symptoms of muscle weakness or spasticity. The neuronal circuitry is not functioning properly.

The neuronal circuitry is made up of neurons. Upper motor neurons are important because they both initiate and modulate movement. Though, patients with different forms of HSP and PLS have similar symptoms, the disease may be caused by different, genetic malfunctions.

As we have learned, more and more, about the underlying causes of different forms of HSP and PLS, we are much better able to target those causes individually to reach out-comes that

the FDA will approve of. For the mechanism-based theory, we can develop drugs targeted to specific mechanism malfunctions.

We may not need to start a clinical trial just for HSP or PLS patients in general. We may include them in other ongoing trials if their disease mechanism is the same. A mechanism-based drug discovery may lead to drugs that can help many different kinds of diseases by curing specific malfunctioning mechanisms that different diseases share. We have to develop bio-markers that reflect each specific mechanism that can be malfunctioning in the upper motor neuron for different kinds of HSP and PLS.

The FDA was developed in the 1940s, at a time when there were many diseases that had clinical manifestations. Each disease was treated as a separate entity. They did not know anything about proteomics lipidomics, or metaboliomics. We did not know any of these sciences so just tried to work on each disease on its own. Now we are learning how to help or cure the different malfunctions that are common to many different diseases. By focusing on the malfunctioning mechanisms that many diseases share, clinical trials can include people with that malfunction from many different diseases.

Proteomics: (proh-tee-OH-mix) The study of the structure and function of proteins, including the way they work and interact with each other inside cells.
Lipidomics: is the study of the structure and function of the complete set of lipids (the lipidome) produced in a given cell or organism as well as their interactions with other lipids, proteins and metabolites.
Metaboliomics: is The study of substances called metabolites in cells and tissues. Metabolites are small molecules that are made when the body breaks down food, drugs, chemicals, or its own tissue. They can be measured in blood, urine, and other body fluids. Disease and environmental factors, such as diet, drugs,

and chemicals, can affect how metabolites are made and used in the body. Metabolomics may help find new ways to diagnose and treat diseases, such as cancer.

So, Akava Therapeutics has licenced the NU-9 drug to where it is now called AKV-9. Toxicity studies have been completed in large mammals. Orphan drug designation has been approved by FDA. An IND application has been made to the FDA to start clinical trials on May 5th and they have requested Fast-Track designation for a seamless Phase 2/3. They are raising funds and the goal is to start phase 1 before the summer ends this year. Biomarkers have to be determined before they can move into phase 2 clinical trials. That will be near the third quarter of next year.

SPG4 or Spastin Mutation is the cause of about 40% of people with HSP. Spastin causes microtubule severing and ER shaping.

Frank Davis googled this subject and found this paper written by Dr. Ozdinler where she better explains about both spastin and Profilin:

The movement starts in the brain. Especially for motor neuron diseases, which impact voluntary movement, the brain component requires better understanding and assessment. Upper motor neurons (UMNs), located in layer 5 of the motor cortex, have a unique role for the initiation and modulation of voluntary movement. Their progressive degeneration is the characteristic hallmark of neuropathology observed in hereditary spastic paraplegia (HSP), primary lateral sclerosis (PLS), and amyotrophic lateral sclerosis (ALS) patients. In striking contrast to their clinical importance and relevance, very little is known about the underlying causes of their vulnerability and progressive loss. This lack of information and understanding of the cellular and genetic basis of their vulnerability, as well as the common and unique aspects of their neurodegeneration hinders our ability to develop effective and long-term treatment strategies for diseases of the UMN. In an

effort to bring a mechanistic insight into the cellular and molecular basis of UMN degeneration in two distinct diseases, such as HSP and ALS, we are going to take advantage of the UCHL1-eGFP mice, a reporter line in which the UMNs are genetically labeled with fluorescence. In this proposal, we will focus our attention to the Profilin and Spastin, because like Spastin, Profilin is an actin/tubulin-binding protein, and mutations in profilin results in ALS, whereas mutations in spastin results in HSP. To date numerous mouse models are generated to mimic the human condition. We crossed the hPFN1G118V (generated by Dr. Kiaei) and SPASTC448Y mice (generated by Dr. Baas) with UCHL1-eGFP mice to generate the UMN reporter lines with these mutations. Since we have shown that the human UMNs and mouse UMNs share almost the same aspects of neurodegeneration at a cellular level, we will purify diseased UMNs from the complex structure of the brain as a "pure" neuron population at different stages of the disease, and investigate the dynamic changes in their gene expression profile and the proteins that are present in them with respect to the healthy UMNs. As an internal control, we will use UMNs isolated from TDP-43 mouse model not only because TDP-43 pathology is widely observed in ALS and HSP, but also because this model better represents the spectrum of ALS. Upon completion, our results will begin to reveal the common and unique aspects of UMN vulnerability in HSP and ALS, it will also suggest the distinct set of genes and/or proteins that are responsible for the initiation of their vulnerability. Most importantly our results have the potential to identify druggable targets and pathway of interest for future therapeutic interventions. (This study began in September of last year and will end in October of next year funded by NINDS.)

This model has florescent axons and so they will be able to much more easily see the effects of treatment. Diseased Spastin has short axons. When these are exposed to NU-9 those axons will

lengthen especially in combination with riluzole or endaravone displaying that the upper motor neuron is healing. They have developed a machine for high throughput analysis of actually testing different compounds on both diseased spastin neurons and diseased profilin to see what compounds can help SPG4 HSP or genetic ALS. With this new high throughput analysis machine, they will be available to help other companies quickly identify whether their compounds are effective in treating different forms of HSP and PLS. She thinks these developments are greatly helping to move us in the right direction.

In addition to testing the way the neurons look (i.e., their axon length and shape) they are also testing their functionality. They do this by measuring their cortical connectivity or how well they transmit signals which is the function of them in the first place. They do this through electrophysiology HD-MEA experiments.

Developing Drug Discovery Platforms for HSP through:
- Rapid drug screening, using UM ends diseased with HSP ??? using improved neuron health and function as Ridout
- Drug repurposing?
- Identification of new compounds?
- Getting ready for a drug discovery for HSP patients.
- Identify new compounds or find utility in the old compounds.

In comparing the Spastin and the Profilin genes, they have discovered that the Profilin gene is much more involved in healthy mitochondria and spastin involved in axon transport. Therefore they are much better able to target the malfunction of each gene.

Summary so far:
- There is a drug company willing to develop clinical trials for HSP & PLS patients.

- There is a new compound, AKV-9, moving into clinical trials for affected UMNs .
- We are trying to reveal whether UMNs in ALS, HSP, and PLS degenerate via similar mechanisms.
- We are developing high, throughput drug discovery platforms using HSP UMNs
- We are performing RNA Seg and omics to reveal disease mechanism differences
- Goal: Develop and move AKV–9 forward, lay the foundation for the discovery of other drugs, develop effective clinical trials for HSP and PLS.
- We are developing biomarkers for UMN's.
- We are developing gene therapies directly to UMN's.
- We are developing personalized medicine approaches.
- Goal: find effective and long-term cures for UMNs.

Biomarkers:
This is one of the major bottlenecks that we have and still hasn't been determined despite years of research working toward finding good biomarkers for HSP and PLS. This is a question that the FDA will be asking before they allow us to go into clinical trials.
- What is a biomarker? It is something that is used to accurately measure the progression of a disease and is how scientists can measure how well a drug or treatment is working. It is hopefully a quantitative outcome measure.
- Types of biomarkers: **Diagnostic biomarker** defines the disease itself. Currently PLS and ALS are defined by the time of certain symptoms. They hope to find other forms of biomarkers that better define PLS and separate it from ALS more accurately rather than just measuring time. **Monitoring biomarkers** are measured repeatedly for assessing status of a disease or medical condition or for evidence of exposure to (or effect of) a medical product or an environmental agent.. **Pharmacodynamic (PD) biomarkers** are molecular indicators of

drug effect on the target in an organism. A PD biomarker can be used to examine the link between drug regimen, target effect, and biological res-ponse. A **Predictive Biomarker** is used to identify individuals who are more likely than similar individuals without the bio-marker to experience a favorable or unfavorable effect from exposure to a medical product or an environmental agent. A **Safety Biomarker** is biomarker mea-sured before or after an exposure to a medical product or an environmental agent to indicate the likelihood, presence, or extent of toxicity as an adverse effect. A **Susceptibility/Risk Biomarker** is a biomarker that is associated with an increas-ed, or in some cases, decreased chance of developing a disease or medical condition in an individual who, from a clinical standpoint, does not yet have that disease or medical condition.

- Why do we need biomarkers? This is the only way that the FDA will allow a treatment to be tested. They are a means of measuring the effectiveness of a drug or treatment.

She showed a paper published by Dr. Hiroshi Mitsumoto in April of this year demonstrating the affect of lipidomics. It showed that PLS patients can be accurately distinguished from ALS and Con-trol patients by combining lipidome profile and supervised ma-chine learning without clinical information.

She also showed a recent paper based on a study of two 2 and 3 year old girls in Italy. Both had novel ALS2 gene mutations. These families have sent Dr. Ozdinler their blood samples. By perform-ing many different "omic" tests they are learning what cellular e-vents are effecting these girls. Based on all these tests they have learned that the basic problem that these girls have is their mito-chondrial integrity. There are several FDA approved drugs for mi-tochondrial integrity and so there is some hope for these girls. These drugs were tried on the girls and after 6 months, they re-

ceived blood and plasma again and both of them improved their scores and one of the girls was reported to have actually stood up. They have now continued for almost one year. They will receive the new blood and plasma samples soon and we will then have a longitudinal sample analysis to see if these drugs have continued to improve their conditions.

Innovation and clinical readiness. Need to go hand-in-hand for expedited Success: moving in the right direction.

SP-CERN – Milestones for pilot phase (two years)
Aim 1. To establish Centers of Excellence and organize the CERN consortium. Milestone 1: establishment of an operational plan for CERN.
Aim two. To develop a central research protocol.
Milestone 2: establishment of a central research protocol and IRB approval.
Aim 3. To establish Critical Infrastructure and Test Key Elements in pilot projects.
Milestone 3A: enrollment of 100 individuals with HSP/PLS in a shared clinical is database.
Milestones 3B: biobanking of blood samples from 100 individuals with HSP/PLS in a shared biobank.
Milestone 3C: completion of whole genome sequencing of 15 individuals with suspected HSP/PLS.
Spastic Paraplegia Foundation Centers of Excellence Research Network (CERN)
Achieving trial readiness is the most important unmet need for HSP's/PLS
National international collaboration is essential for creating clinical trial readiness and the rapid development of improved diagnoses and therapies
Innovation, discovery (are your findings translational?) Are you ready for a clinical trial? (Outcome measures, clinical centers.)

Dr. Ozdinler closed by putting up a list of all associates in her lab in Boston and also a list of the scientists who are collaborators with the Ozdinler lab. There were 13 of the former and 15 of the latter. Collaborators included: Richard Silverman, PhD, Marco Martine, PhD, Pave Andjus, PhD, Steve, Vucic, PhD, Peter Baas, PhD, Marcel Mesulam, MD, PhD, Mahmoud, Kiaei, PhD, Neil Kelleher, PhD, Gerardo Morfini, PhD, Mete Altintas, PhD, Paulo Bongiovanni, MD, Roberta Battini, MS, Halil Idrisoglu, MD, Eileen Bigio, MD, Richard Miller, PhD. She also put up a list of all of her sponsors. They included NIH, NIA, DoD, Spastic Paraplegia Foundation, CureSPG4, A Long Swim, ALS Assoc, and the Les Turner ALS Foundation.

She thanked the Spastic Paraplegia Foundation for bringing everyone together, supporting vital research for years, crating the new SP-CERN and moving everything forward so expeditiously.

Dr. Ozdinler then spoke about the large art work that she has donated for a fund-raising auction. The art is really a photo of florescent neurons that she has enhanced with her computer and very attractively printed and framed. (This art was later auctioned and the winning bid was a $5,000.00 donation to The Spastic Paraplegia Foundation.)