Zhittya Medical Innovation Series
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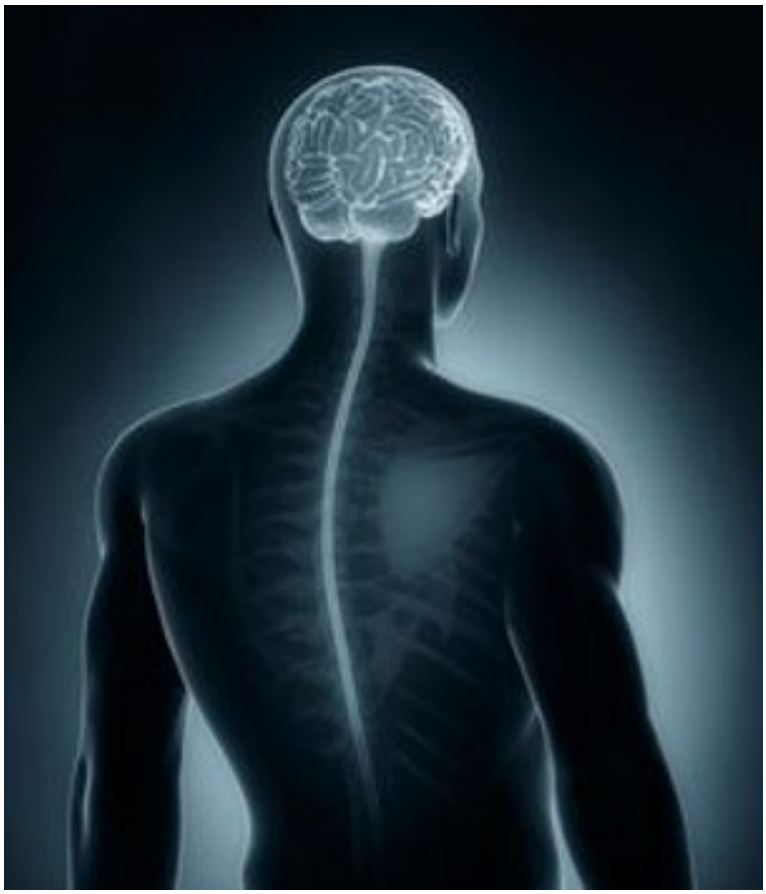
Zhittya
Regenerative Medicine Inc.

Human FGF-1 as a Potential Treatment for Amyotrophic Lateral Sclerosis (ALS)



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I. Introduction

Zhittya Regenerative Medicine, Inc. is advancing a hypothesis that neurodegenerative diseases, such as Parkinson's disease and Alzheimer's disease, are initiated and progress due to a lack of blood flow or perfusion in the brains of individuals suffering from these disorders. This lack of adequate blood flow gradually starves neurons in different areas of the brain leading to dysfunctional neurons and eventually death of the neurons due to a lack of nourishment and oxygen, as well as improper removal of metabolic wastes from the neurons. There is no reason to doubt that similar mechanisms may also be occurring in amyotrophic lateral sclerosis (ALS), a neurodegenerative disease characterized by the selective loss of motor neurons in the brain and spinal cord. A review of the medical literature supports the idea that a chronic lack of blood flow to these motor neurons may underly the development and progression of ALS and that therapeutic agents which can increase blood perfusion in the brain, such as human FGF-1, are potential novel disease modifying agents for the treatment of ALS

II. Prior Evidence of a Blood Perfusion Defect in ALS

The selective death of motor neurons beginning in mid-life is the hallmark of ALS, the most abundant motor-neuron disease of adults. Research as far back as 2001 gave solid evidence that indicated a primary cause of ALS may be chronic deficits in vascular blood perfusion.

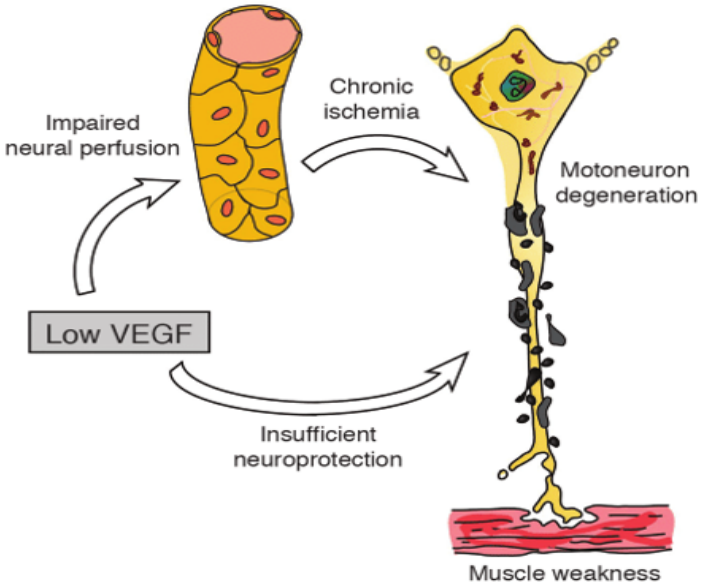
Motor neurons have extremely high metabolic demands, and so it follows that they might be unusually sensitive to a mild insufficiency in the blood supply. Not only are they among the largest cells in the body (their volume is up to 5,000 times that of typical cells), but they can be very long (up to a meter in length in humans), necessitating the transport of proteins and other macromolecules across great distances. Added to this, motor neurons maintain a high rate of electrical firing, placing an enormous demand for an energy supply to maintain this electrical activity. Thus, chronically reduced vascular perfusion could produce deficits in oxygen and glucose, and so fail to meet the energy requirements of motor neurons. The diminishment of vascular perfusion rates with age probably contributes to the selective vulnerability of motor neurons to limited hypoxia or vascular

insufficiency, and along with other genetic and environmental factors, may be the triggering event for ALS.

III. Studies in Animal Models of ALS

In a 2001 publication (1) entitled "Hypoxia and Lou Gehrig" published in *Nature Genetics* researchers from Duke University and UC, San Diego summarized their work and work of their colleagues (2) that showed that a lack of a potent stimulator of new blood vessel growth (in this paper the lack of the angiogenic factor, VEGF, was studied) produced in mice all the classical features of ALS. The lack of VEGF produced degeneration of motor axons and the characteristic denervation-induced muscle atrophy. As in ALS, the neuronal deficits were selective for motor neurons and sensory neurons and neurons in other brainstem nuclei were not affected. In addition, the researchers also established that the ALS symptoms developed not only from a lack of blood perfusion, but also due to a lack of neuroprotection of the motor neurons due to this lack of VEGF. The figure below characterizes the main findings of this research. In Figure 1 below, it is depicted that low levels of the angiogenic growth factor, VEGF, results in impaired blood perfusion due to a lack

Figure 1. *Low levels of the angiogenesis factor, VEGF, in the brain lead to motor neuron degeneration in ALS.*

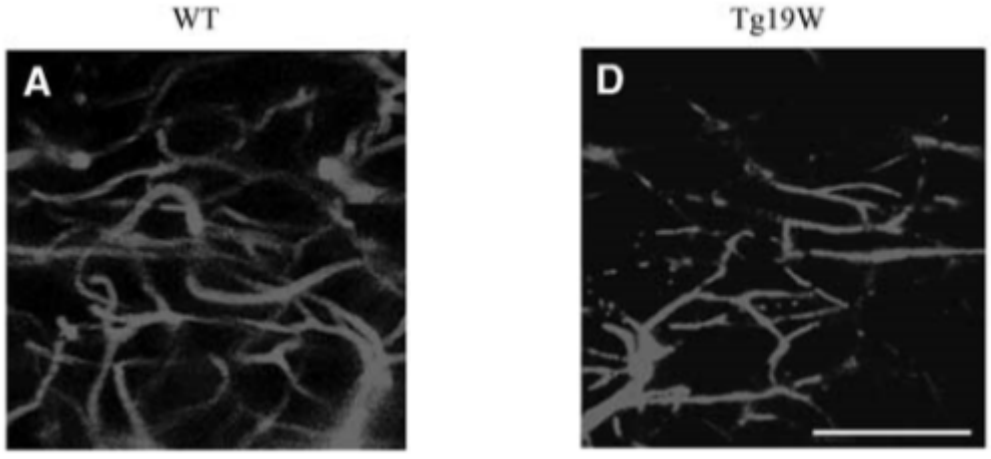


of angiogenesis resulting in chronic ischemia, motor neuron degeneration and muscle weakness. In addition, insufficient neuroprotection could also lead to motor neuron degeneration.

It is known that FGF-1 sits above VEGF in the angiogenesis cascade and that FGF-1 is a potent stimulator of VEGF production and release in ischemic tissues. FGF-1 is also neuroprotective for motor neurons (3) and so should be able to completely replicate all of these earlier findings seen with VEGF in mice.

Moving ahead 10 years, researchers began examining the early and progressive impairment of spinal blood flow in motor neuron degeneration seen in ALS mice (4). In that study, the researchers performed *in vivo* capillary imaging and directly measured spinal blood flow and glucose metabolism in the spinal cord. The *in vivo* capillary imaging showed a progressive decrease of capillary diameter, capillary density, and spinal blood flow during the disease course (from the pre-symptomatic stage to the end stage of the disease). It was established that there was a strong correlation between decreasing capillary diameter and density which led to the disruption of the "neuro-vascular unit" in the regions of the brain and spinal cord affected by ALS. This can be seen clearly in Figure 2 below where capillaries in similar regions of the spinal cord were imaged in live animals, without ALS on the left and with ALS on the right. There is a significant decrease in the diameter and density of capillaries seen in the ALS mouse on the right, which would be

Figure 2. *Capillary changes seen in the spinal cord of ALS mice*



expected to contribute to the motor neuron degeneration and muscle weakness seen in these animals.

This is exactly what we postulate is occurring in Parkinson's disease, but in those areas of the brain affected by Parkinson's disease, such as the substantia nigra region which contains dopamine-producing neurons. The researchers went on to conclude that this vascular pathology could be profoundly involve in the whole disease process of ALS and represents a potential target for therapeutic intervention of ALS. We believe it represents an excellent therapeutic target and plan to propose a study in human subjects with ALS subjects who will be given IV infusions of the FGF-1 molecule over a period of months, similar to what we have proposed in our planned Parkinson's disease trial.

IV. Studies in Patients with ALS

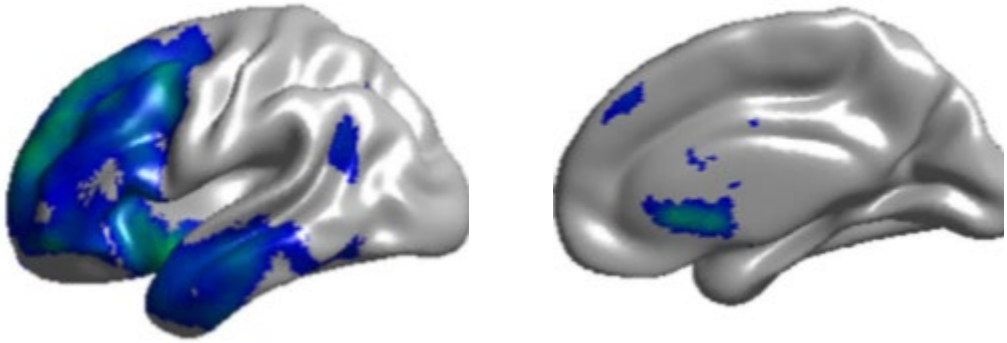
Turning to research in human subjects with ALS, sensitive brain imaging technology has been used to attempt to match ALS severity with the level of blood flow in the brain (5). Medical researchers from UC, San Francisco analyzed brain blood flow in the brains of ALS patients by using an MRI method referred to as "arterial spin labeling", a non-invasive form of magnetic resonance imaging. To correlate the perfusion results with symptoms, they used the ALS Functional Rating Scale to categorize the severity of motor symptoms, and used forced vital capacity to measure breath volume. Remarkably, there was a direct correlation between a decrease in breath capacity and a drop in brain perfusion, as well as a decrease in motor skills involving upper motor neuron involvement and decreased blood perfusion. "The current findings, while preliminary, are promising," the authors write. "Brain perfusion may be a useful tool for monitoring disease progression and assessing treatments in ALS." Certainly, therapeutic agents that may be able to increase blood perfusion in the brain, such as FGF-1, merit a clinical assessment in patients with ALS.

Finally, more recent work (6) in humans with ALS and dementia has also shown a strong correlation between decreased blood perfusion in the cerebral cortex and the development of dementia in patients with ALS. The researchers, as was done above, utilized the MRI technique of arterial spin labeling to characterize the patterns of brain atrophy and blood perfusion in ALS patients with varying levels of cognitive deficit, including ALS with frontotemporal dementia (FTD). They

established that the more severe the lack of blood perfusion was in certain areas of the cortex, the more profound was the patients' dementia. This can be seen in Figure 3 below where blood flow in the cortex is measured in real time by MRI in ALS patients with and without dementia. The areas of diminished blood flow are represented by blue and green regions and it clearly be seen that the areas of decreased blood flow in the patient with dementia cover a much broader area of the cortex, areas involved in cognition and executive functioning.

Figure 3. *Real time MRI imaging of blood flow in the brains of ALS patients with dementia. Regions of decreased blood flow show as blue and green areas.*

ALS Patient with Dementia **ALS Patient without Dementia**



Remarkably, in patients with genetic mutations that lead to ALS/dementia, it was established by this imaging technology that blood perfusion deficits develop during the pre-symptomatic stage of the disease, before substantial neuron atrophy or dementia is present. Thus, this imaging technology can provide early information pertaining to the blood perfusion defect in patients on the ALS/dementia spectrum, even at the pre-symptomatic stage. This points to possible therapeutic intervention with an angiogenic agent, such as FGF-1, to actually prevent the initiation of the disease process.

V. Conclusions:

There is a large and accumulating body of evidence that vascular dysfunction and diminished blood flow in the brain is an initiating factor in the development of neurodegenerative diseases. This has certainly been shown in Alzheimer's disease, where the Alzheimer's Disease Neuroimaging Initiative has provided reams of data in over 1000 patients with Alzheimer's disease that definitively establishes that the first deficit seen in this disease is a decrease in blood perfusion in the brain, long preceding the more familiar symptoms of the disease, including cognitive decline and the appearance of beta amyloid plaque. Similar imaging studies have pinpointed vascular lesions and blood perfusion deficits in Parkinson's disease patients, both in sections of the brain regulating motor function (through dopamine-producing neurons) to cortical regions of the brain where perfusion defects lead to well-known symptoms of Parkinson's disease, including loss of smell, slowing and slurring of speech and cognitive decline.

As outlined in the present report, there is ample evidence in both animal and human studies, that a lack of blood perfusion to brain and spinal cord motor neurons is a critical event in the initiation of ALS. As with Alzheimer's disease and Parkinson's disease, we believe the slow and inexorable decline of angiogenesis and neurogenesis in the brains of ALS patients leads to disease progression, and a factor which can potently stimulate those two physiological processes is an attractive candidate to test in clinical trials. Human FGF-1 is such a candidate. It is a natural factor in our bodies that is constantly put to use to heal and regenerate injured tissues or organs. As shown in studies in the ischemic human heart, we would expect FGF-1 in the brain to hone in to any area of the brain areas characterized by a lack of blood perfusion and ischemia. For example, in an ALS patient who also suffers from dementia, we would fully expect that the FGF-1 molecule would stimulate angiogenesis and neurogenesis not only in the affected motor neurons of the brain and spinal cord, but also in those affected cortical regions that regulate memory and cognition.

We hope to begin our testing of FGF-1 in US FDA-authorized clinical trials in patients with neurodegenerative diseases starting in January 2019. We have chosen as our first two medical indications, patients with Parkinson's disease and patients with ALS. Soon after those trials are begun, we hope to quickly move on to a similar clinical trial in patients suffering from multiple systems atrophy).

References

1. Skene P, Cleveland D. Hypoxia and Lou Gehrig. *Nature Genetics* 28:107-108 (2001).

2. Oosthuye, B. et al. Deletion of the hypoxia-response element in the vascular endothelial growth factor promoter causes motor neuron degeneration. *Nature Genetics* 131–138 (2001).

3. Vargas MR et al. Fibroblast Growth Factor-1 Induces Heme Oxygenase-1 via Nuclear Factor Erythroid 2-related Factor 2 (Nrf2) in Spinal Cord Astrocytes. *J Biol Chem* 280:25571-25578 (2005).

4. Miyazaki K et al. Early and progressive impairment of spinal blood flow–glucose metabolism coupling in motor neuron degeneration of ALS model mice. J Cerebral Blood Flow Metab 32:456-467 (2012)

5. Rule RR et al. Gray matter perfusion correlates with disease severity in ALS. *Neurology* 74:821-827 (2010).

6. Shen D et al. Brain structural and perfusion signature of amyotrophic lateral sclerosis with varying levels of cognitive deficit. *Frontiers in Neurology* 9:1-9 (2018).