Zhittya Medical Innovation Series
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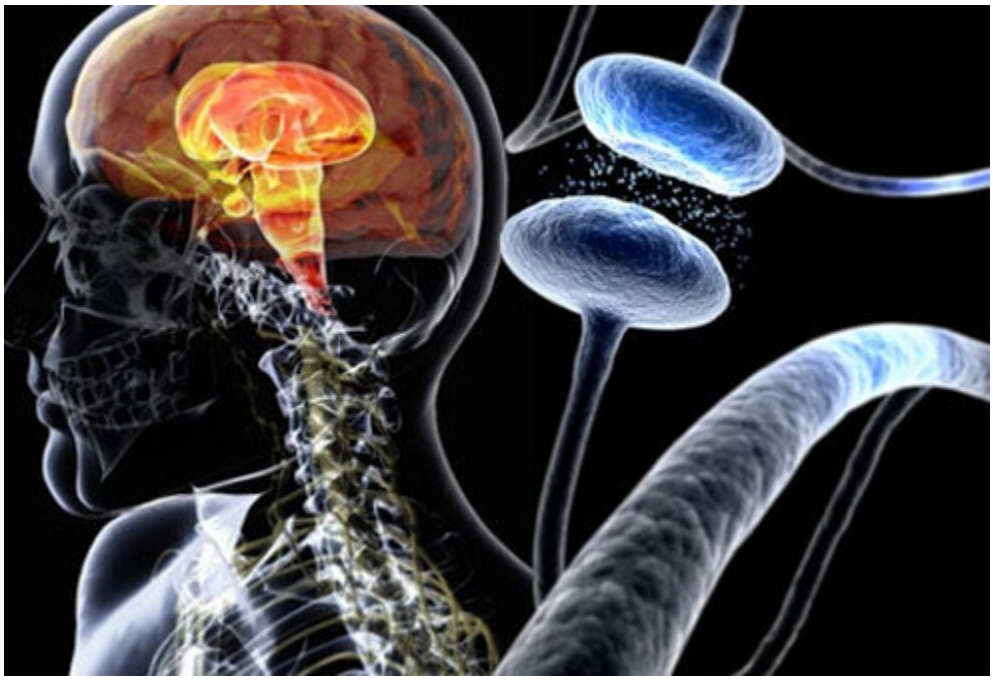
ZHITTYA
GENESIS MEDICINE INC.

Parkinson's Disease:
Therapeutic Angiogenesis as a Disease Modifying, Breakthrough Therapy?



January 3, 2020

Overview: Our Hypothesis as to the Cause of Parkinson's Disease

We believe that vascular disorder and reduced blood perfusion in the small capillaries (the micro-vasculature) of the brain leads to the slow starvation of neurons and is the initiating cause of Parkinson's disease, as well as numerous other neurodegenerative diseases, including Alzheimer's disease and multiple sclerosis. As will be detailed in this White Paper, powerful new imaging techniques can accurately quantitate the flow of blood in specific regions of the brain and these techniques have documented the dramatic decrease in blood perfusion in the brain of a person suffering from Parkinson's disease.

We also believe that "therapeutic angiogenesis" or the stimulation of new blood vessel growth by a biological drug can be successfully utilized to overcome this lack of blood flow in patients with Parkinson's disease. Zhittya Genesis Medicine Inc. (Zhittya) is developing a biological growth factor, FGF-1, that is probably the most potent inducer of therapeutic angiogenesis yet discovered. Outside of the brain it has already shown great promise in US FDA Phase II clinical trials in treating a number of medical disorders characterized by a lack of blood perfusion, including coronary artery disease, diabetic foot ulcers and venous leg ulcers.

In primate models of Parkinson's disease, considered the "gold standard" for the pre-clinical testing of potential new drugs for the disease, FGF-1 has been able to halt the overall motor deterioration seen in this animal model and significantly reverse the motor deficits observed in affected animals, restoring motor skills back to close to 70% of normal. Importantly, the generation of new dopamine-producing neurons can be clearly seen in the FGF-1 treated animals, as well as a reduction in the amounts of the neuron-toxic aggregated α-synuclein particles. Together, these results point to the fact that FGF-1 is behaving as a disease-modifying agent, attacking the root cause of the disease in these monkeys.

Based on these promising results, Zhittya has developed a "Proof of Concept" clinical trial in which three ascending doses of FGF-1 will be infused into patients five days a week for a four week period of treatment. Both safety and efficacy parameters will be measured. Applications to begin testing of FGF-1 in subjects with Parkinson's disease are under review in three countries, including the US, Mexico and Estonia and dosing of patients could begin in as little as 3 months from now.

I. Introduction

Parkinson's disease is a degenerative neurological disorder that affects approximately seven million people globally. Those developing Parkinson's disease suffer slow movement, tremors, stiffness, and difficulty walking. As the disease progresses, it may affect thinking and can also cause behavioral and psychological problems, including dementia, sleep disturbances, and depression. The symptoms result from the loss of brain cells generating the neurotransmitter dopamine. Ultimately, people with Parkinson's disease develop progressive disability and die prematurely.

Presently, there is no treatment to slow the inevitable progression of the disease, and it is not known why the dopamine-generating brain cells begin to die off. Although many of these symptoms can be ameliorated at first with dopamine-like medications, the efficacy of these drugs to treat Parkinson's often wanes over time.

A review of the medical literature supports the potential role for FGF-1 to be a new breakthrough treatment for Parkinson's disease. As detailed in this paper, a number of studies conducted in animal models of Parkinson's disease indicate that FGF-1 can stimulate the regeneration of dopamine-producing cells in the brain leading to an improvement in the classical Parkinson's disease symptoms that these animals display.

In addition, recent research by a number of investigators has established that impaired blood flow in the brain drives the neurodegeneration seen in diseases such as Parkinson's disease. These researchers believe Parkinson's disease is initiated by a gradual reduction in blood flow in the small capillaries of the brain that supply the dopamine-producing neurons in the substantia nigra region of the brain. It is this micro-vascular dysfunction which leads to a slow choking off of the blood supplying the dopamine neurons and also allows the accumulation of the toxic α-synuclein aggregates seen in the brains of humans with Parkinson's disease. It is known that the brain is critically dependent on healthy blood vessels to support the nourishment of its neurons, as well as to remove toxic waste products from the organ. When the brain's micro-vascular perfusion system begins to break down due to age, environmental factors or other insults, disease results.

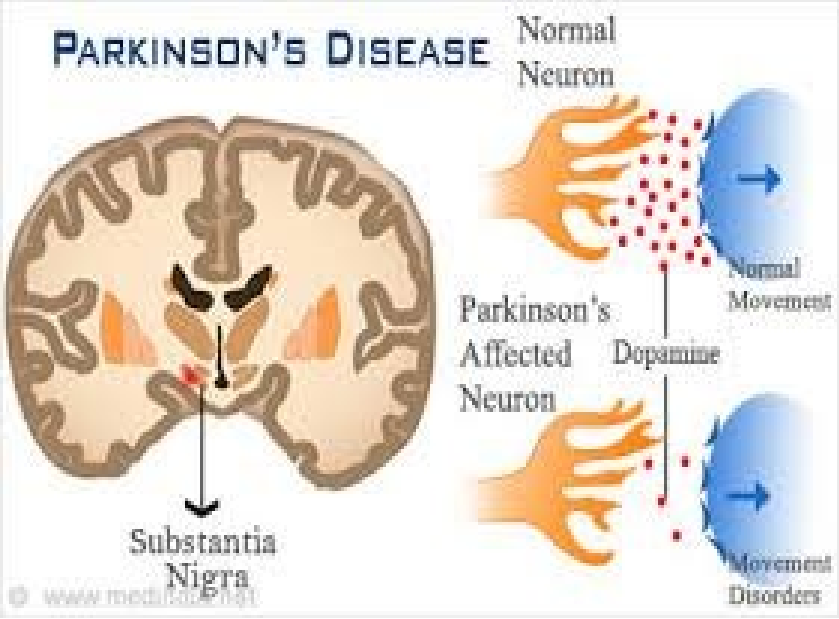
As mentioned above, FGF-1 has been shown to regenerate dopamine-producing neurons in the brains of monkeys with Parkinson's disease. Based on pre-clinical research in animal models of stroke, it is well established that FGF-1 can promote the growth of a healthy microvasculature in the brains of affected animals, leading

to the re-population of the stroke area with healthy neurons. It is this tight coupling of angiogenesis (new vessel growth) with neurogenesis (new neuron formation) which makes FGF-1 such an attractive candidate to move into clinical testing for Parkinson's disease, which will be occurring in 2020.

II. MRI Imaging to Detect Vascular Dysfunction in Parkinson's Disease

Parkinson's disease involves the malfunction and death of vital nerve cells in the brain, primarily neurons in the substantia nigra area of the brain (see Figure 1 below). These neurons produce dopamine, a neurotransmitter that controls movement and coordination. As PD progresses, the amount of dopamine produced in the brain decreases, leaving a person unable to control movement normally.

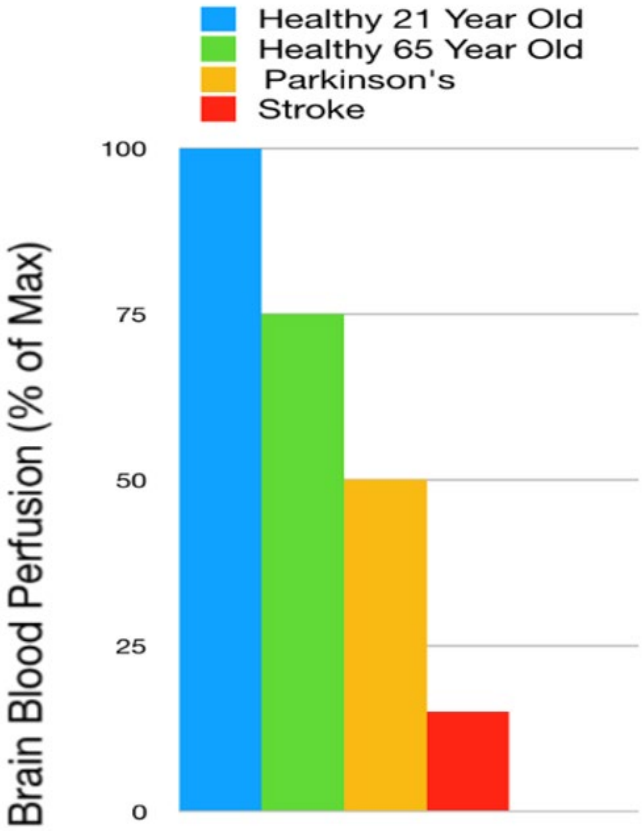
Figure 1. *Left side of figure: location of the substantia nigra in the mid-brain region where dopamine producing neurons are affected by Parkinson's disease. Right side of figure: decreased production of dopamine in the Parkinson's affected neuron leads to movement impairments*



The substantia nigra region of the brain is relatively small, comprising only approximately 500,000 dopamine-producing neurons (the oblong black areas in the diagram above). In spite of this, the newly evolving imaging technique of functional MRI can, in real time, give information on actual blood flow or perfusion rates in the substantia nigra region of the brain. In a recent study, blood

flow was assessed in healthy 21 and 65 year olds, as well as in patients who had Parkinson's disease or had suffered a stroke affecting that region of the brain. Data from that study is shown in Figure 2 below where the dramatic reduction of blood flow by 50% can be seen in Parkinson's disease patients.

Figure 2. *Blood perfusion, as determined with functional MRI, in the substantia nigra region of the brain in healthy individuals and patients with Parkinson's disease and stroke.*

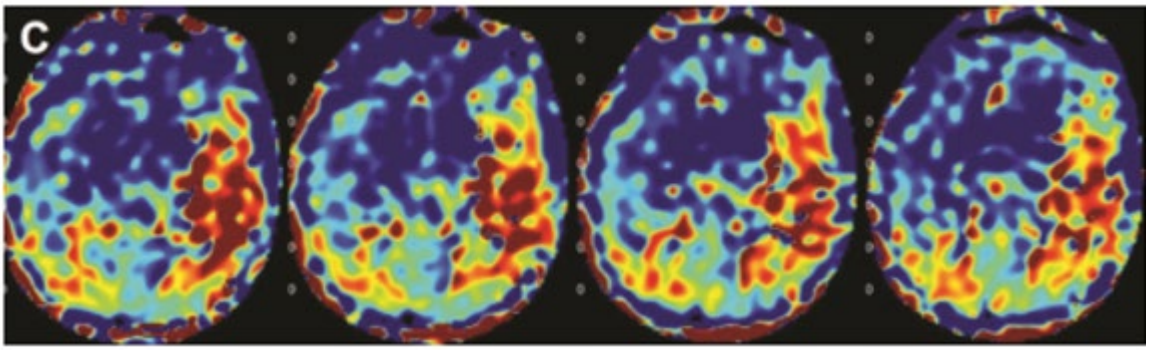


As the data above documents, one potential cause on why dopamine-producing neurons in the substantia nigra region of the brain become dysfunctional and then die is because of a lack of blood flow. This would mainly produce motor disturbances in the patient and give rise to the well-known symptoms seen in Parkinson's disease such as tremors and gait disturbances. However, what about the non-motor aspects of Parkinson's disease that are often seen in this disease, including speech difficulties and cognitive disturbances? Again, we can look at

recent brain imaging studies with functional MRI to try and understand what is going on with these non-motor disturbances.

Below in Figure 3 is an MRI perfusion scan of an individual suffering from Parkinson's disease and with significant cognitive impairment. The scan is of the entire top of the brain which contains the cortical regions of the brain that control cognitive function, memory and executive functioning. In Figure 3, the dark blue areas indicate normal blood perfusion, with perfusion defects going from mild (light blue color) to more significant defects (yellow color) to severe defects (red areas). This patient has a sizable decrease in blood flow (yellow and red areas) in regions of the brain that are critical for proper cognitive functioning.

Figure 3. *Functional MRI scan of the brain cortex regions in a subject with Parkinson's disease and dementia. Blue indicates normal blood flow followed by increasing deficits in blood flow going from light blue to yellow and finally to red.*



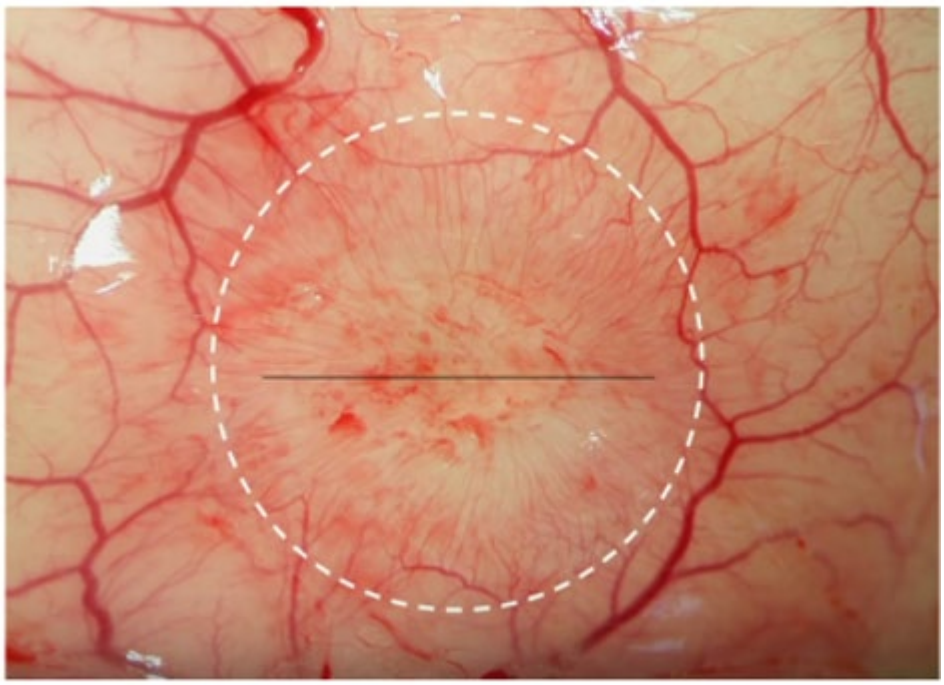
These regions of diminished blood flow in the cortex result from the same processes that we see in the substantia nigra region of the brain containing the dopamine-neurons, namely, the slow choking off of the micro-vasculature leading to neuronal dysfunction.

III. Therapeutic Angiogenesis to Treat Parkinson's Disease

If our hypothesis is correct that microvascular disruption and lack of blood flow initiates and results in the progression of Parkinson's disease, then it would make sense that if you could restore blood flow to the brain by re-establishing the microvasculature, progression of the disease could be halted or even reversed. Let's take a closer look at this process of angiogenesis and how it relates to disease reversal in conditions characterized by a lack of blood flow to a tissue or organ.

We use angiogenesis every day to heal cuts, scrapes or internal damage. It must be stressed that this healing is occurring acutely – within days. Shown below in Figure 4 is "natural" angiogenesis occurring in a skin wound. The fine meshwork of newly formed vessels can be seen radiating into the wounded area of skin. This regenerative process will not only form new skin, but all the other components found in skin as an organ – sweat glands, hair follicles and nerves.

Figure 4. *Natural angiogenesis occurring in wounded skin. The dotted white circle is the area of the wound and as part of the healing process a fine array of new capillaries has formed radiating into the wound area.*

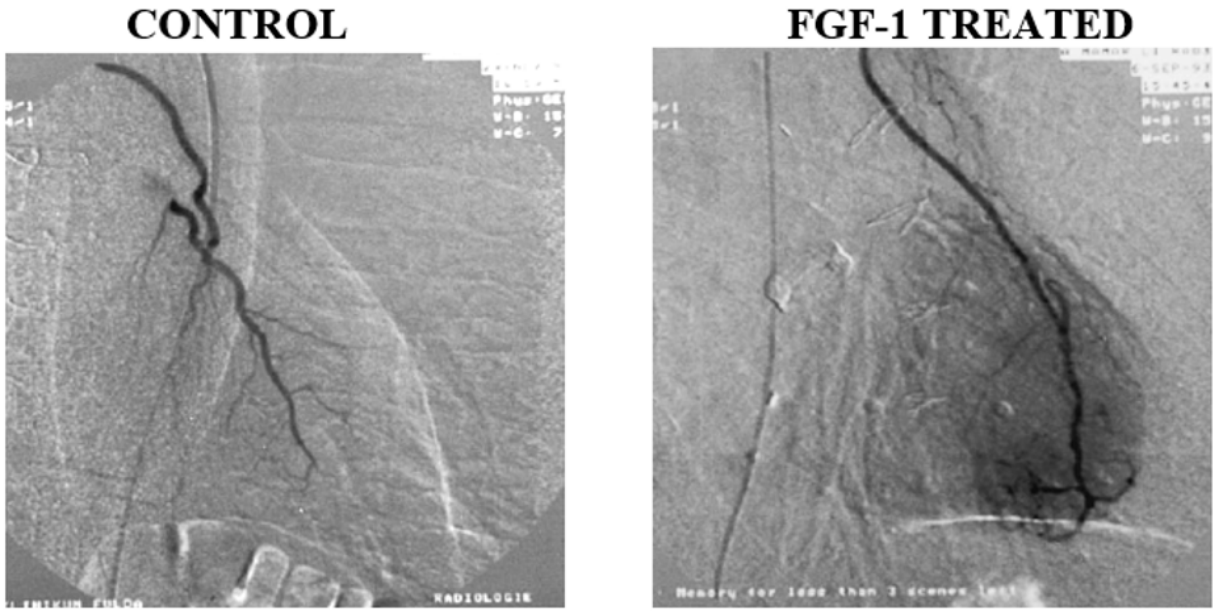


FGF-1 plays a central role in stimulating natural angiogenesis in the picture above. Again, this is an acute response to injury and healing occurs over a period of days. What is happening in neurodegenerative diseases, such as Parkinson's disease, is an entirely different process. This disease develops over decades and it is this extremely slow, but inexorable decline in the microvasculature, that we believe is the root cause of this disease. The body does not recognize this as an acute injury

and thus angiogenesis factors such as FGF-1 are not mobilized to repair the microvasculature. This is where "therapeutic" angiogenesis is required.

We initiate therapeutic angiogenesis by introducing a relatively large amount of FGF-1 directly to the affected organ or tissue. As an example of that, Figure 5 shows the induction of therapeutic angiogenesis when FGF-1 is injected into the heart of a patient suffering from severe coronary artery disease. The figure displays an angiogram where a dye illuminates the coronary vessels and one can see a new "blush" of coronary vessels on the right side of Figure 5, which was seen 12 weeks after the FGF-1 was injected.

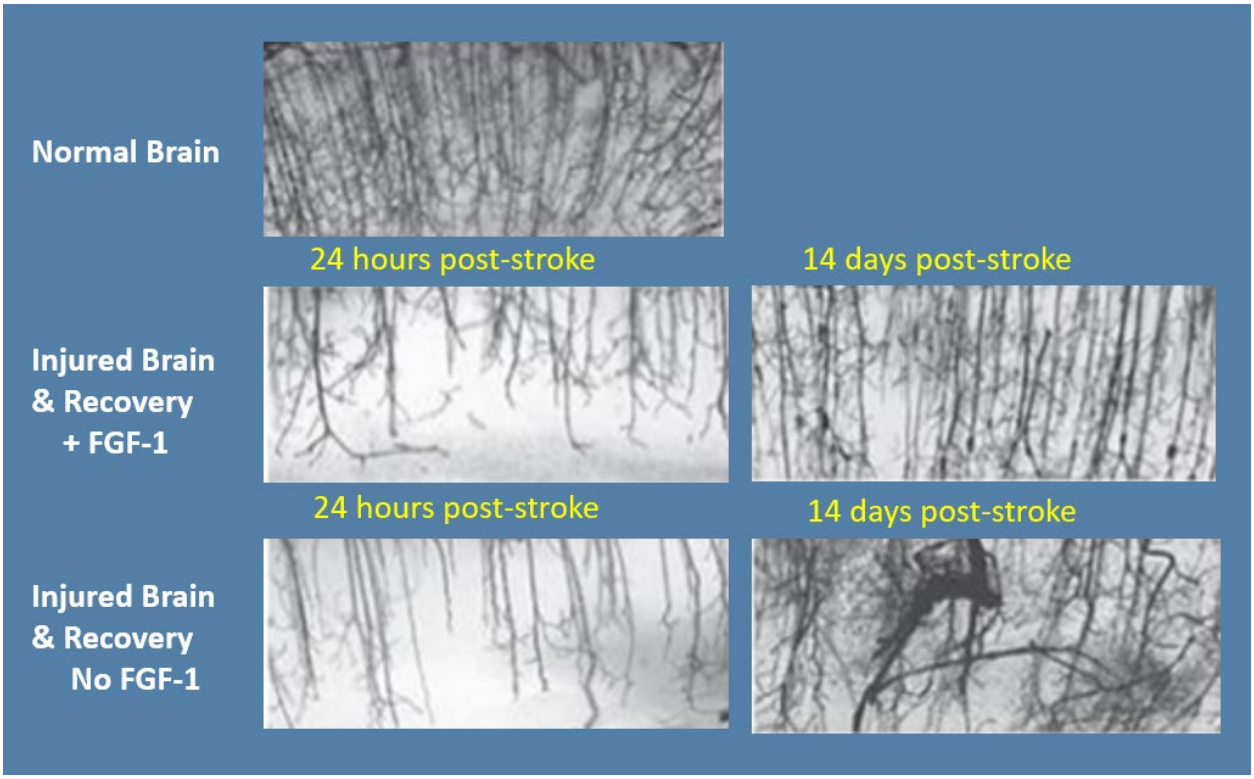
Figure 5. *Growth of new blood vessels stimulated by FGF-1 in the heart of a patient with severe coronary artery disease. Human FGF-1 was injected directly into the heart muscle and angiograms of the coronary vasculature showed a rich network of new blood vessels (right hand panel) when looked at 12 weeks following treatment.*



Now, FGF-1, to date, has not been used to treat any brain disorders. However, in animal models of stroke we can get a good look at the affect of FGF-1 in stimulating the growth of new capillaries (microvasculature) in the damaged brain of those animals. Figure 6 below shows capillary density in the normal rat brain, the density after an experimental stroke is given and the regrowth of new blood

vessels stimulated by FGF-1 over a two week treatment period. It is readily apparent that FGF-1 (middle panels) can stimulate a re-growth of the microvasculature that is decimated by giving the animals a stroke. In the lower panels of Figure 6, where no FGF-1 is given, the brain is trying to repair its vascular system, but does so in a dysfunctional and disordered manner. Motor skill testing revealed that the animals given FGF-1 did significantly better in those motor exercises measured.

Figure 6. *Microvasculature re-growth stimulated by FGF-1 in rats given an experimental stroke. The top panel shows capillaries in the normal brain. The middle panel shows capillary density 24 hours after a stroke and 14 days after receiving daily IV injections of FGF-1. The bottom panel shows rats given injections without FGF-1.*

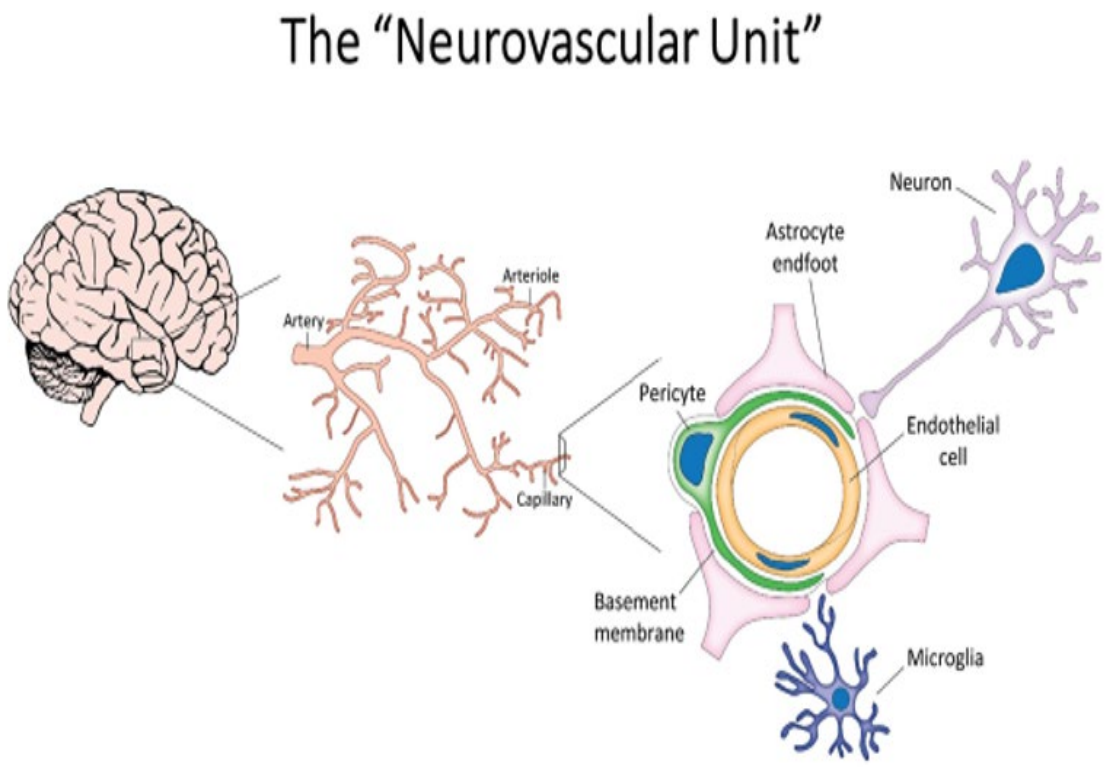


The above is a good example of achieving therapeutic angiogenesis in a brain disorder and we are hopeful that the same type of response will be seen in the brains of humans suffering from disorders brought about by a lack of blood perfusion in the microvasculature of the brain.

IV. The Brain's Micro-Vasculature

FGF-1 does not grow large supply-type arteries. Instead, this molecule stimulates the growth of capillaries and small arterioles in the micro-vasculature. The microvasculature, which comprises 80% of the brain's total blood vessel capacity, is estimated to contain 100 billion capillaries that supply blood to an estimated 86 billion neurons. The vascular and neuronal components form what is referred to as the "neurovascular unit" which is central to how the brain functions. Figure 7 is a depiction of the neurovascular unit which shows how every neuron has a dedicated capillary blood supply to nourish it with glucose, oxygen and other nutrients and also, importantly, to remove metabolic waste products that neurons and other neuron-supporting cells generate.

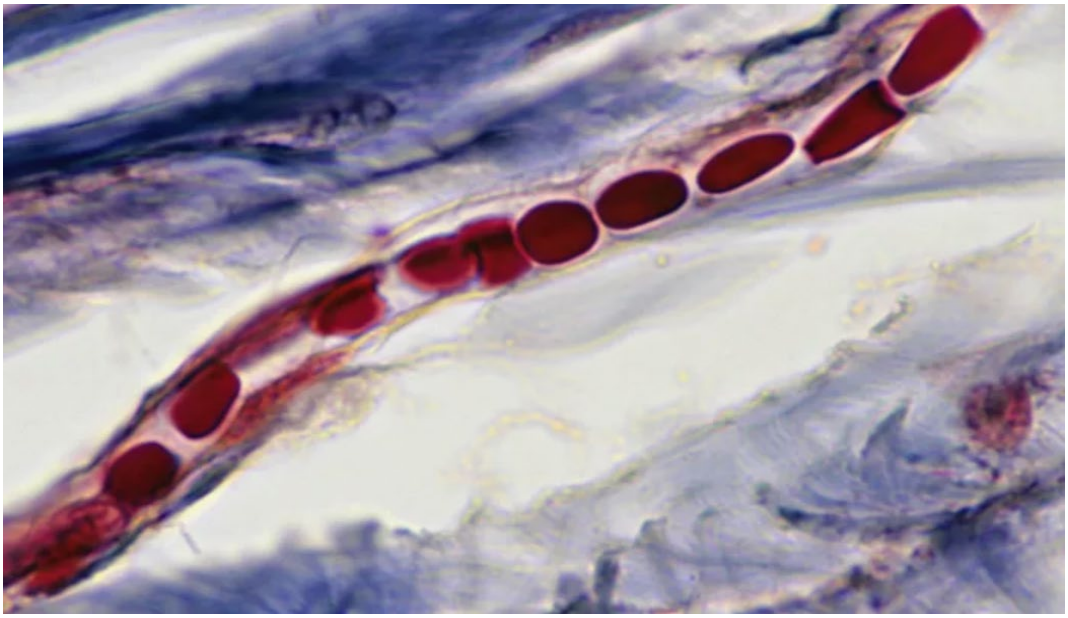
Figure 7 *Depiction of the Neurovascular Unit showing the interaction of capillaries in the vascular system with neurons and supporting cells in the brain.*



As shown in Figure 6 on the preceding page (capillaries in the brains of rats given an experimental stroke), capillaries that make up the brain's microvasculature are slender and fragile with thin walls. Capillaries measure in size from about 5 to 10 microns in diameter (for comparison, a fine strand of human hair has a diameter

of 75 microns). Capillaries are so small that red blood cells can only travel through them in single file as shown in Figure 8 below.

Figure 8. *Red blood cells passing single file through a capillary.*



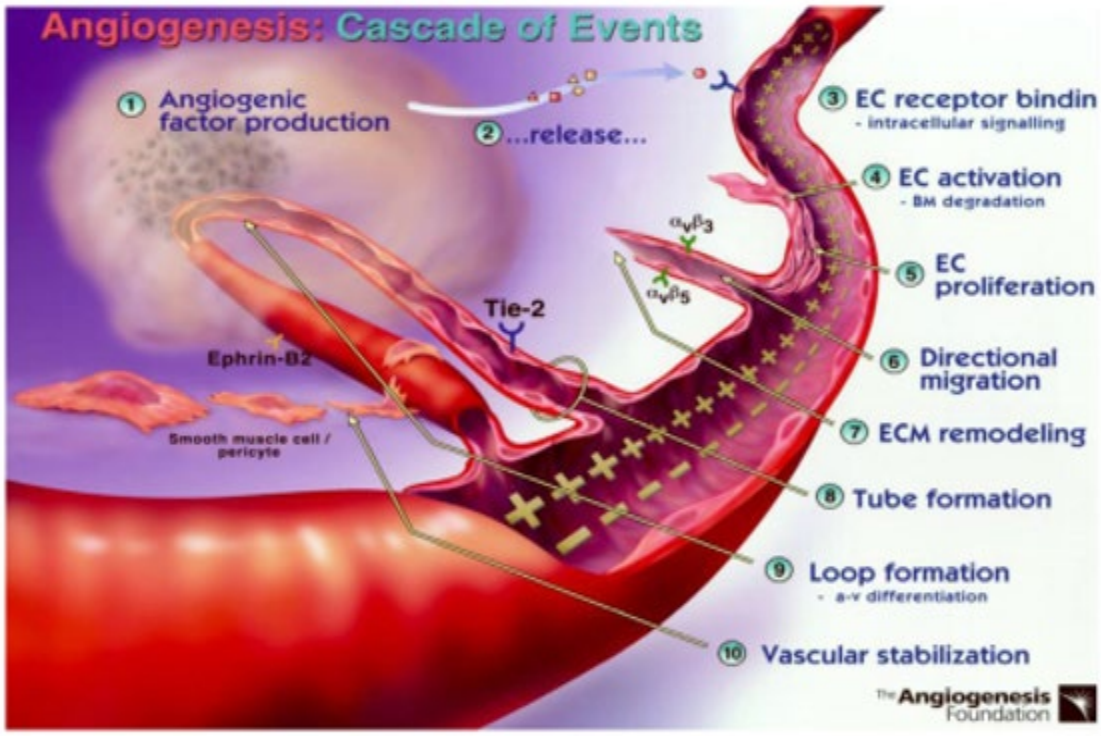
Now, one can readily imagine that it would not take much narrowing of that capillary above by atherosclerosis or some other insult to disrupt blood flow or blood perfusion leading to an oxygen-starved or ischemic tissue. FGF-1, by virtue of its ability to stimulate the growth of new blood vessels such as the one above, can restore proper blood perfusion in damaged tissues or organs.

V. The Specificity of Therapeutic Angiogenesis Utilizing FGF-1: No Blood Growth in Normal Tissues

It has been repeatably shown, both in animals and in humans, that at doses of FGF-1 that stimulate therapeutic angiogenesis in ischemic or damaged tissues, no blood vessel growth is observed in the adjacent healthy tissue. Injection of FGF-1 into normal heart or leg muscles causes no response (one has to go up to 100-fold higher concentrations than used for therapeutic purposes to see blood vessels begin to grow in normal tissues). Why is that? A look at the interaction between FGF-1 and its receptor on the endothelial cells that make up capillaries gives us the answer. As shown in Figure 9 below, angiogenesis is a multi-step process that

starts with the interaction of FGF-1 with its Y-shaped receptor (see top right side of the figure where FGF-1 is depicted as a small ball landing on its Y-shaped receptor). This initiates the cascade of 10 events listed in Figure 9 which culminate in the production of a new capillary.

Figure 9. *The cascade of events seen in the process of angiogenesis. The process is initiated when an angiogenic factor, such as FGF-1, binds to its receptor on endothelial cells on preexisting blood vessels.*

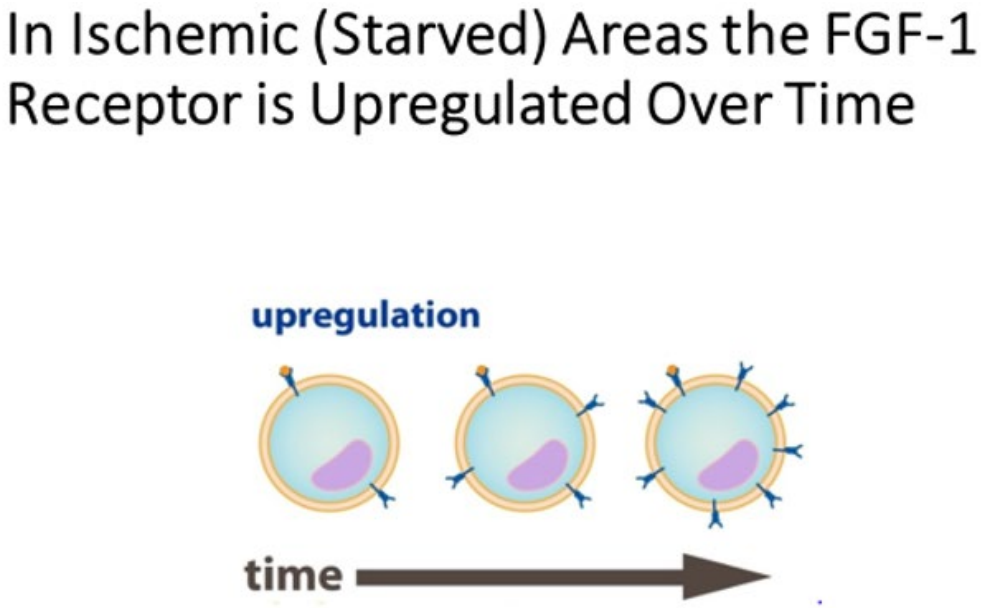


What is remarkable is that in ischemic or damaged tissue, it has been demonstrated that the receptors for FGF-1 have been elevated 100-times that of normal tissue! It is as if the damaged tissue is primed to respond to FGF-1 and giving a therapeutic dose of FGF-1 to the ischemic tissue is like throwing a match in a dry stack of hay. An immediate and explosive proliferation of signals to initiate angiogenesis.

This is schematically shown in Figure 10 below which shows the upregulation of FGF-1 receptors in nutrient and oxygen-deprived tissues. Over time (and this can be years) the lack of blood flow in an ischemic area of the body gradually elevates the FGF-1 receptor on endothelial cells that line the capillaries, almost in a cry for help that more blood vessels are needed in this area. For some reason, and perhaps

these changes in receptor levels are so gradual, there is not enough FGF-1 around to kick off the angiogenesis response to make more blood vessels. Along comes a large dose of FGF-1 administered through therapeutic angiogenesis and the growth

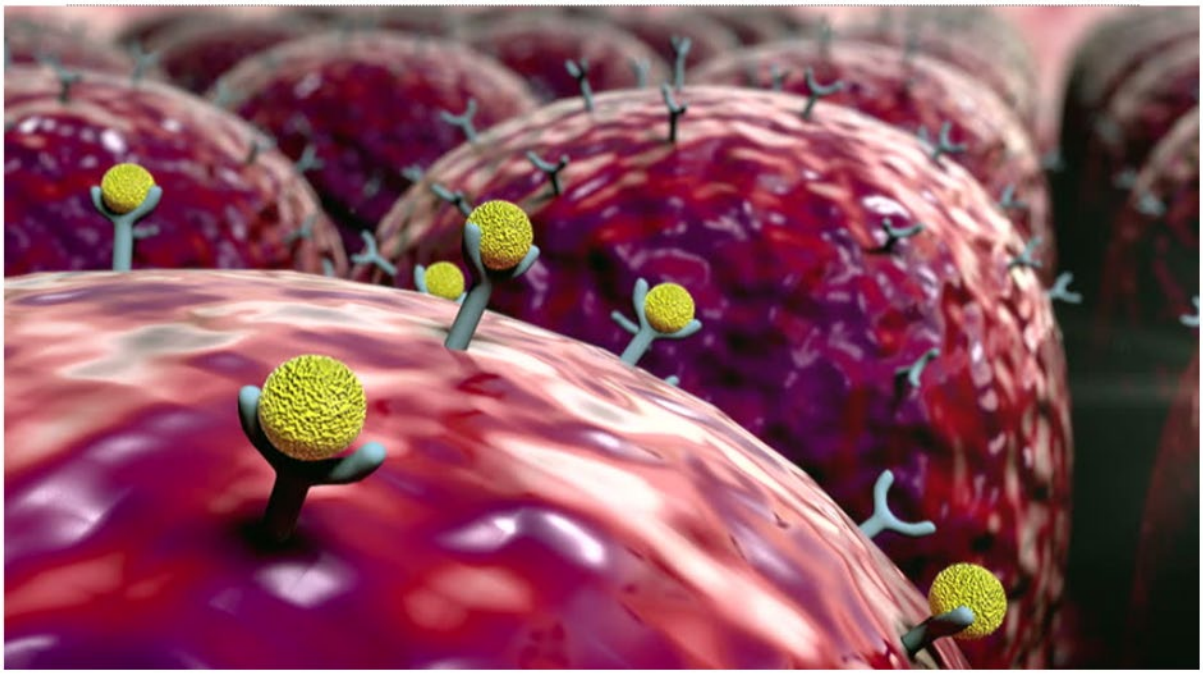
Figure 10. *Upregulation of FGF-1 receptors in ischemic tissues*



of new blood vessels is jump started. Researchers have established that you need approximately 10,000 of those Y-shaped receptors occupied with FGF-1 molecules on an endothelial cell to kick off a round of angiogenesis and form what is called a "foci" of new capillaries. On the following page is an artistic rendition (Figure 11) of what we believe occurs in the brain of an individual with Parkinson's disease brought on by a lack of blood perfusion and then treated by therapeutic angiogenesis with FGF-1.

We envision that the repetitive dosing strategy we will employ in our clinical trials should bring "waves" of FGF-1 to ischemic regions of the brain, stimulating inactive, under-nourished endothelial cells (with upregulated numbers of FGF receptors on their cell surface) into action to form additional blood vessels.

Figure 11. *Artist's rendition of therapeutic angiogenesis in the ischemic brain. The upregulation of the Y-shaped FGF-1 receptors is seen on the endothelial cells that line the capillaries in the brain's microvasculature. FGF-1 (yellow balls) bind to the unoccupied receptors to stimulate therapeutic angiogenesis.*



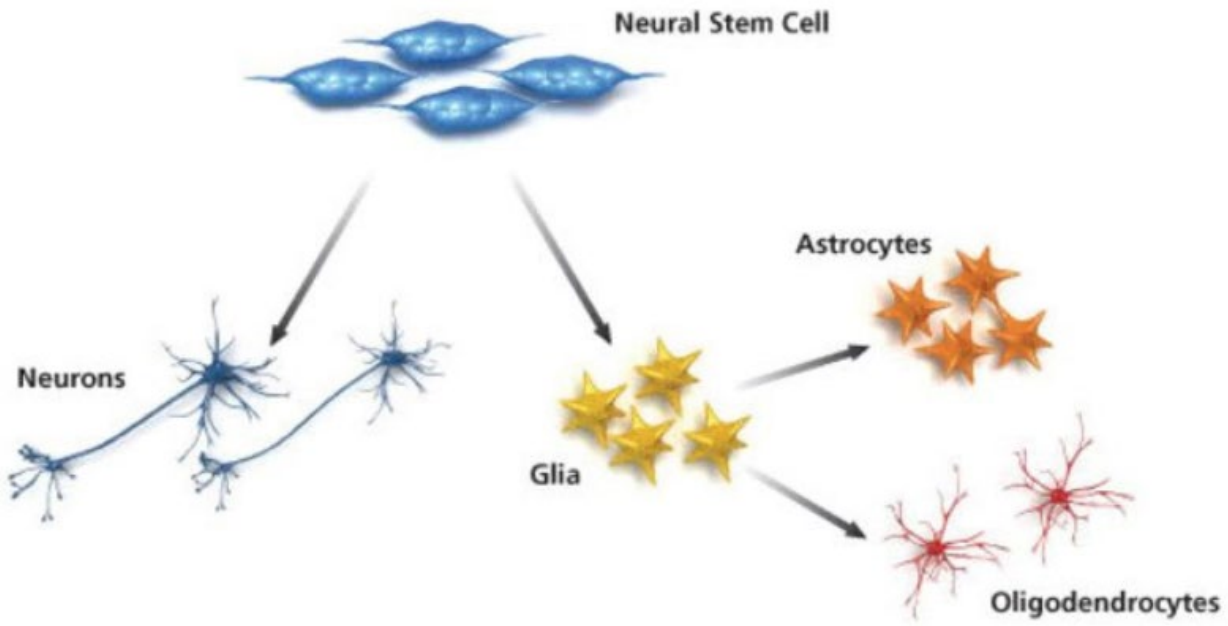
VI. The Role of Brain Stem Cells in Aging and Disease

As discussed above in studies done with rats given an experimental stroke, the process of new blood vessel growth, angiogenesis, is tightly linked to new neuron growth, or neurogenesis. In more chronic, wasting brain disorders such as Alzheimer's disease or Parkinson's disease, we believe the slow choking off of the blood supply to selected areas of the brain results not only in existing neurons becoming dysfunctional, but also the inability of the brain to generate new neurons from the pools of neural stem cells that we all possess.

As shown in Figure 12 below, neural stem cells need an adequate blood supply to be active and to mature and differentiate into all of the different types of brain cells

required for healthy brain tissue.

Figure 11. *Stem cells need an adequate blood supply to divide and differentiate into the different types of brain cells that comprise healthy brain tissue.*



In fact, in a landmark paper published recently in the journal, *Cell,* Maura Boldrini and her colleagues at the Columbia University School of Medicine in New York City studied the brains of 28 healthy people who had died suddenly of accidents, but not long term disease. The people ranged in age from 14 years to 79 years, and the researchers looked at the population of stem cells in different areas of the brain, including those areas responsible for memory (hippocampus region of the brain).

What they found was that older people made as many neuronal stem cells in the area of the brain responsible for memory as younger people do. But what is different in the aging brain is the reduced blood flow to nourish these cells. That means the stem cells are dividing less, and generating fewer new neurons in older brains than in younger ones. We believe the exact same process is also occurring in neurodegenerative diseases, such as Parkinson's disease, and that if we can restore blood flow to the brain though therapeutic angiogenesis we have a chance at possibly reversing these devastating illnesses.

VII. The Potential Promise of FGF-1 to Treat Parkinson's Disease: Studies in Primate Models of the Disease

The gold standard for testing potential new drugs to treat Parkinson's disease in humans is a primate model of Parkinson's disease that has been developed in the Cynomolgus monkey pictured below.

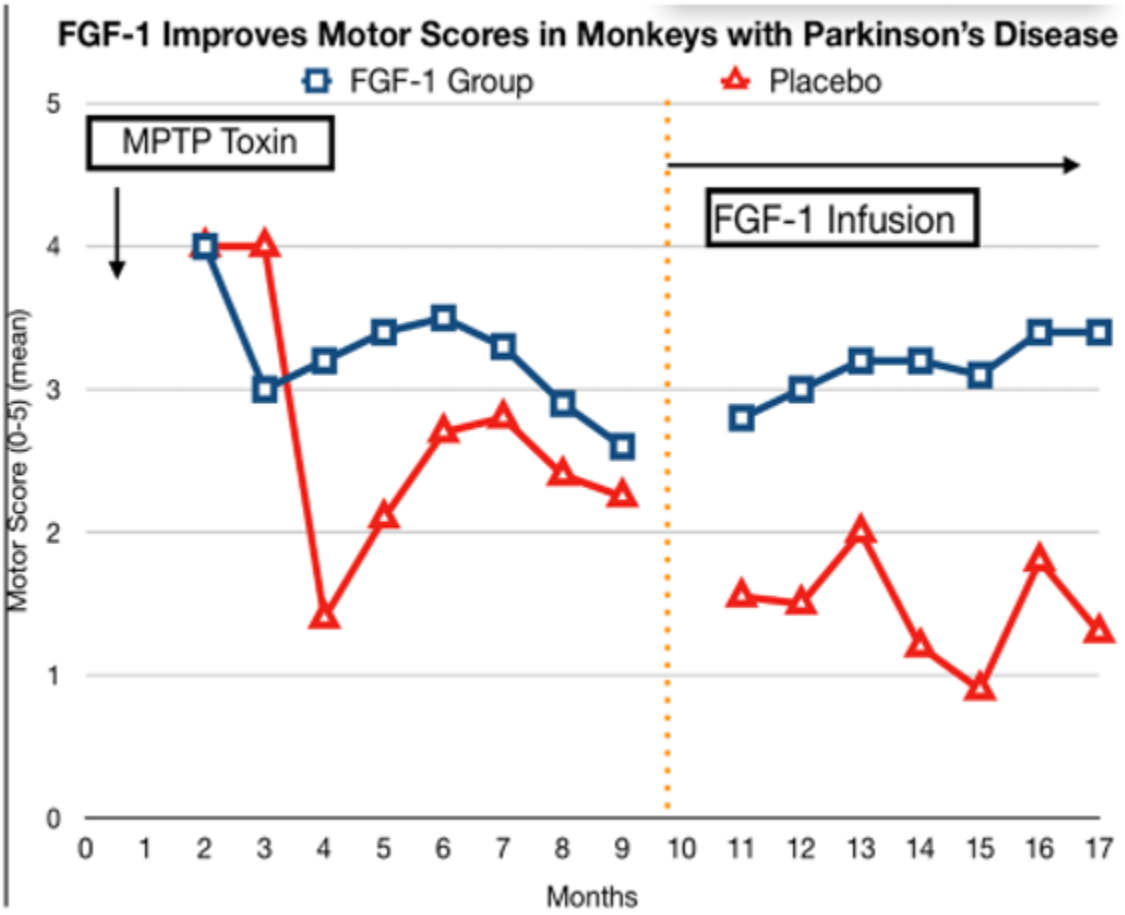
Figure 12. *Cynomolgus monkey used to test new Parkinson's disease drugs*

The animals are treated with a herbicide, MPTP, which is a selective toxin for the dopamine-producing cells of the brain (this activity of the herbicide was discovered in a tragic accident in Washington state, where individuals who had smoke marijuana that had been treated with the herbicide, came down with irreversible Parkinson's disease). Just as in humans, after a period of months following treatment with the MPTP neurotoxin, the monkeys come down with all of the classical symptoms of Parkinson's disease, including tremors of the arms and gait disturbances.

As shown in Figure 13 below, it takes about 6-9 months for the animals to develop these disturbances, at which point FGF-1 or a placebo dose is infused into the animals over a period of approximately 6 months and motor scores are then

measured. As can be seen in Figure 13, FGF-1 was able to slow and then reverse these movement disorders with FGF-1-treated monkeys returning to approximately 70% of normal motor activity.

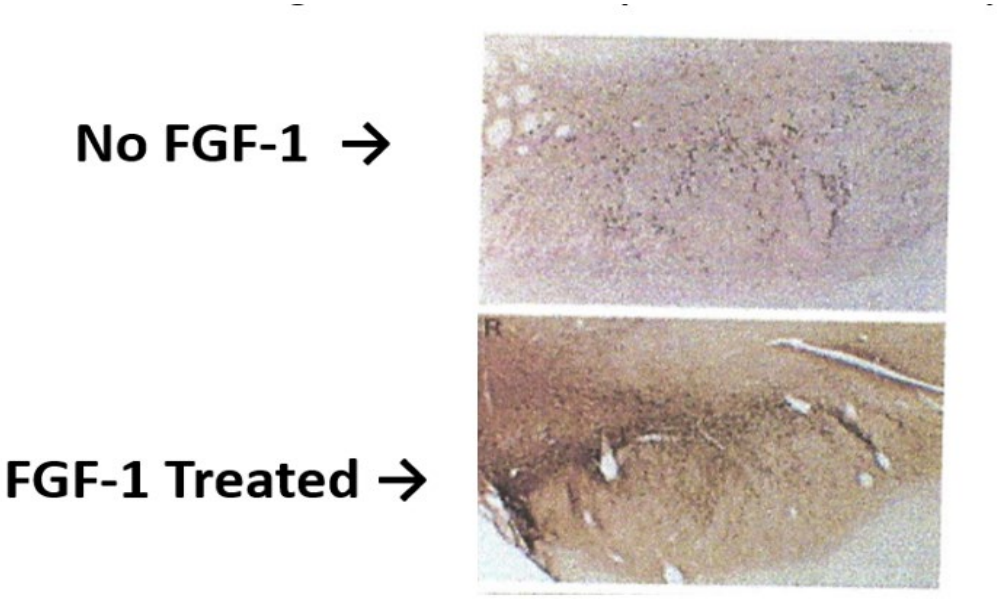
Figure 13. *Testing of human FGF-1 in a monkey model of Parkinson's disease. The left hand x-axis is motor scores with "5" being normal movements and "0" being total lack of the four types of movements that were measured in the study. MPTP toxin is administered to the animals to bring on the motor symptoms of Parkinson's disease over a 9-month period and then animals are given either FGF-1 or a placebo infusion weekly over a 6-month period of time.*



Importantly, at the end of 17 months, the monkeys were euthanized and their brains were examined for the regeneration of new dopamine-producing neurons. As shown in Figure 14 below, new dopamine neurons were regenerated by FGF-1 treatment as evidenced by the brown-staining new dopamine neurons in the bottom

panel. We believe this disease modifying event led to the improvement in the motor scores in monkeys treated with FGF-1.

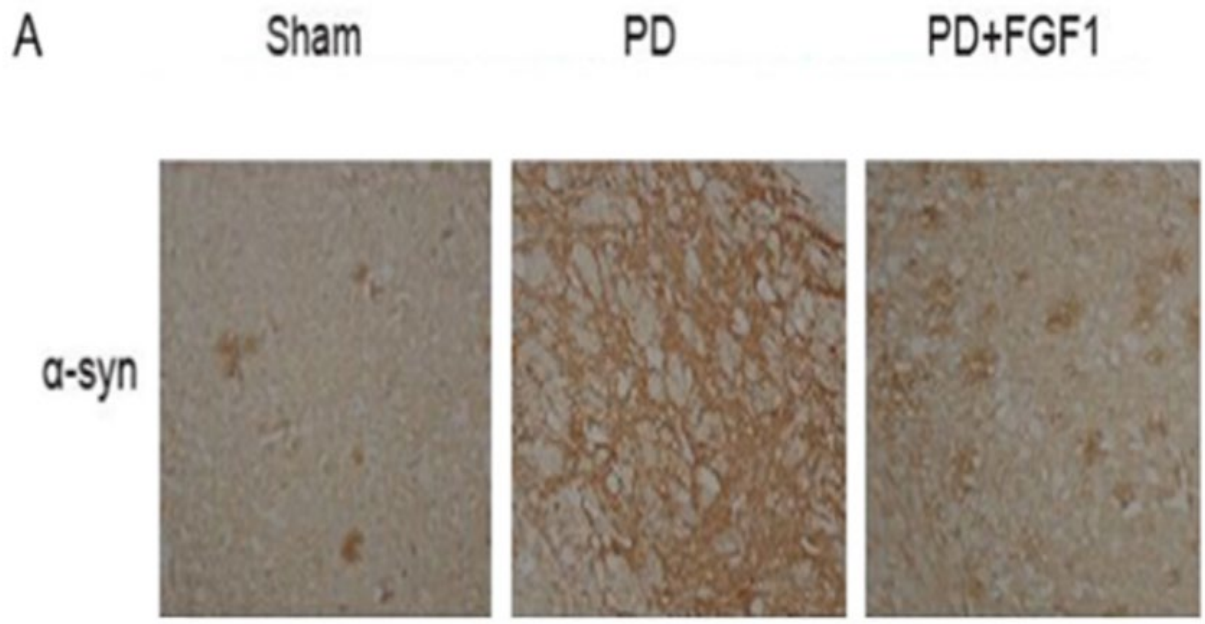
Figure 14. *FGF-1 increases dopamine production in the brains of monkeys with experimental Parkinson's disease. Brain slices through the substantia nigra region of the brain were stained to reveal the presence of new dopamine-neurons (the dopamine cells stain brown).*



Another important aspect of Parkinson's disease pathology can also be examined in these animal models of Parkinson's disease. As has been shown in Alzheimer's disease with the toxic beta-amyloid protein, Parkinson's disease patients develop toxic tangles of aggregated α-synuclein protein in their brains. This is thought to further damage the dopamine-producing neurons that have already been weakened by poor blood perfusion.

Animals with experimental Parkinson's disease also accumulate this protein (see the middle panel in Figure 15 below) and this is significantly reduced with FGF-1 treatment (right hand panel in Figure 15). Again, we believe this is a disease modifying activity of FGF-1 which contributes to the motor improvements that were seen in the treated animals in this study.

Figure 15. *Treatment with FGF-1 decreases the presence of aggregated α-synuclein in the brain's substantia nigra region. The aggregated protein is clearly visible in the central panel, marked "PD" and is greatly reduced in FGF-1 treated animals shown in the right hand panel, "PD+FGF1".*



The above data generated in animal models of Parkinson's disease is why we are so excited and anxious to get our clinical trials underway in patients with Parkinson's disease. We believe our hypothesis is correct and that a lack of blood perfusion to selected areas of the brain is the initiating event in this and other neurodegenerative diseases and a continued lack of adequate blood perfusion is the primary reason the diseases progress.

We believe we are on very firm medical ground, based on the evidence that we have uncovered that a therapeutic, such as FGF-1, that is a potent stimulator of angiogenesis and, subsequently, neurogenesis in the brain, is truly an agent which deserves to be tested in human patients with this devastating disease.

VIII. Planned Clinical Trials in Patients with Parkinson's Disease

The Table below lists the trial design of our first "Proof of Concept" Phase I clinical trial in patients with mild to moderately severe Parkinson's disease. We have identified three different neurological centers where we will be carrying out these first studies which are located in the United States, Mexico and the Baltic country of Estonia.

Table I: Phase I Clinical Trial Design

- **Three ascending doses of the drug will be studied**
- **No placebo group; all patients will receive1 hour intravenous infusions of FGF-1 five days a week for a six week period**
- **Safety and effectiveness will be monitored**
- **Follow-up visits (monthly) to 1 year out**

It should be noted that all subjects enrolled in the study will receive one of 3 doses of FGF-1 which have been determined to be safe in preclinical animal testing. The patients will have a central catheter placed so the infusions of FGF-1 can more comfortably be given over the duration of the clinical study. Qualified neurologists have been identified in each of the 3 countries where the trials will be held and these individuals, who made some very important improvements to the design of our study, will serve as Principal Investigators for the clinical trials.

We hope from these "Proof of Concept" studies we will learn important facts on the safety of FGF-1 given to individuals with Parkinson's disease, something that has never been attempted before. We also pray that we start seeing some sort of positive signal relating to halting or reversing the progression of the disease in these first studies. A signal that we can then build on by testing different doses of our drug or perhaps using a different treatment regime to combat this truly unmet medical need.

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