ZHITTYA
REGENERATIVE MEDICINE INC.

Suicide Prevention in the Military Following Major Depressive Disorder and Traumatic Brain Injury: Treatment with Fibroblast Growth Factor 1 (FGF-1)?

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Executive Summary

Some 8,000 veterans are thought to die by suicide each year, a toll of about 22 per day, according to a 2012 VA study. The Armed Forces Health Surveillance Center reports that mental disorders are the leading cause of hospitalizations for active-duty forces. The rate at which troops are being hospitalized for mental health illness has risen 87 percent since 2000 and this has led to over a doubling of the suicide rate in active duty soldiers. What are the factors that have contributed to this dramatic increase in mental illness and suicide within the military? A RAND Corporation study reported that at least 20% of Iraq and Afghanistan veterans have post-traumatic stress syndrome (PTSD) and/or depression, prime predictors for a subsequent suicide event. In addition, military service members are at an increased risk for traumatic brain injuries (TBIs), predominantly from blast exposure. Estimates of the prevalence of TBI among returning service members range from 15.2% to 22.8%, and the physical brain damage from a TBI is known to lead to PTSD, depression, and suicidality.

What are the treatment options for soldiers with these impairments? Current strategies are focused on decreasing anxiety and depression in these patients in an effort to limit suicidal ideation. This has mainly involved psychosocial intervention, including psychotherapy and cognitive behavioral therapy. Pharmaceutical intervention with anti-depressant therapy can also provide benefit, although it should be noted that Clozapine, an antipsychotic medication used primarily to treat individuals with schizophrenia, is the only antidepressant with a US FDA indication for reducing the risk of recurrent suicidal behavior.

As will be detailed in this report, a large body of work has underscored the surprising role of a growth factor family, the fibroblast growth factor (FGF) family, in modulating many facets of emotional and motivated behavior and playing a key role in major depressive disorders. For decades, this family has been known to play a pivotal role in neural development and neuroprotection, as well as stimulating new blood vessel growth in damaged tissues, a process known as angiogenesis. This newer role of FGFs in mediating emotional behavior points to additional therapeutic possibilities for the use of FGF to treat individuals suffering from anxiety and depression and at risk of suicide.

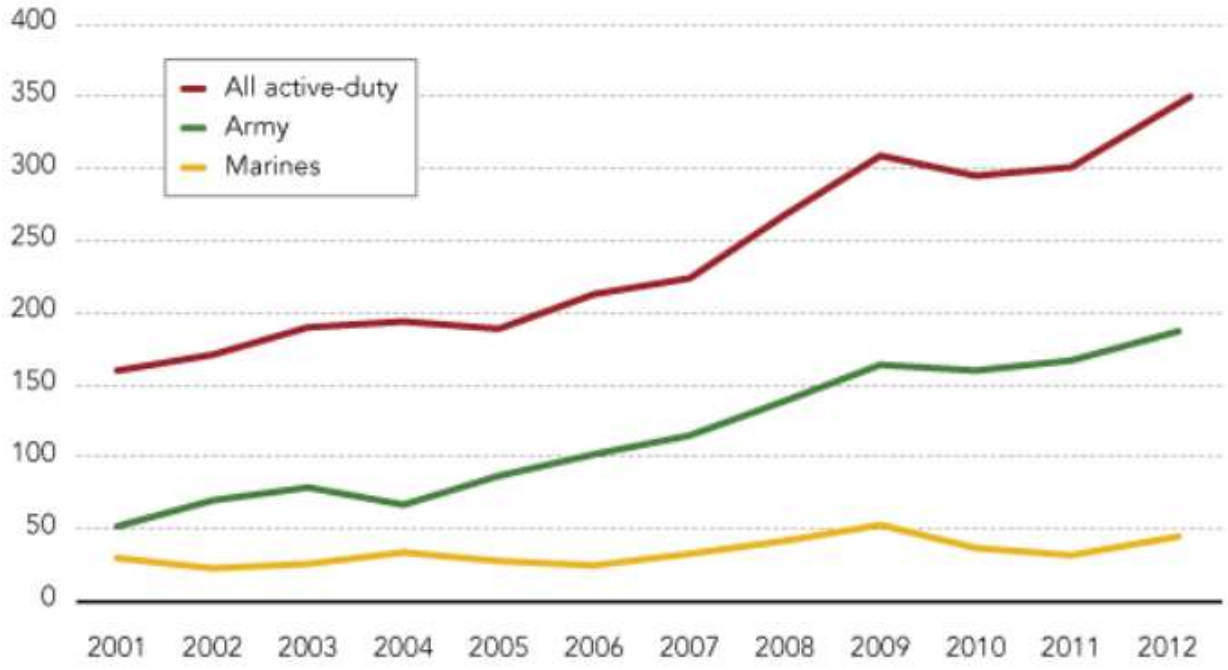
Zhittya Regenerative Medicine's (ZRM) is advancing research and development activities on one of the most potent members of the FGF family, FGF-1, in a number of medical indications. In the past, FGF-1 has been evaluated in several US FDA-approved clinical trials, including Phase I clinical trials using FGF-1 to stimulate angiogenesis in the hearts of patients suffering from severe coronary artery disease, as well as separate trials where angiogenesis stimulated by FGF-1 led to the successful closure of chronic diabetic foot ulcers. Zhittya has recently initiated new drug development efforts to address if FGF-1, with its known angiogenic and neurogenic properties, can also be used as a potential treatment for vascular disorders of the brain, including stroke recovery, Alzheimer's disease, Parkinson's disease and chronic traumatic encephalopathy (CTE). In this report, we will summarize the rationale and promise for FGF-1 to be investigated as a potential treatment for suicide prevention in patients suffering from major depression and/or traumatic brain injury.

I. Depression, PTSD and Suicide in the Military

A study by the Armed Forces Health Surveillance Center found that for all the military personnel medically evacuated from Iraq and Afghanistan between 2001 and 2012, the most frequent diagnosis was not physical battle wounds, but "adjustment reaction," a category that includes grief, anxiety, depression, post-traumatic stress and other mental disorders. In another detailed study of troops' mental health in Iraq by the Army surgeon general, published in 2008, found that three-quarters of young male troops, privates, specialists and sergeants saw someone seriously injured or killed; more than half were attacked or ambushed; 70 percent experienced an IED explode nearby; and 88 percent received incoming fire. One in five reported being "bothered by thoughts that you'd be better off dead" during the previous four weeks.

For a variety of reasons many veterans under stress don't get help. Fewer than half of all the nation's 22.3 million veterans are enrolled with the VA, officials said. Those who do try to get help often find that the nationwide shortage of mental health care professionals translates into long lines and long waits for appointments. All of these factors have led to a dramatic rise in military suicides as shown in the graph below.

Figure 1. *Suicide among active duty soldiers from 2001-2012*



Source: 2012 Department of Veterans Affairs Suicide Data Report

As of September 2014, there were about 2.7 million American veterans of the Iraq and Afghanistan wars. According to the RAND Corporation report, "The War Within: Preventing Suicide in the US Military" published in 2011, at least 20% of Iraq and Afghanistan veterans have PTSD and/or depression, and this number climbs significantly if combined with traumatic brain injury, as will be discussed below. Vietnam veterans of which there are over 2 million, also report high lifetime rates of PTSD and depression ranging from 10% to 31%. PTSD is the third most prevalent psychiatric diagnosis among veterans using the Veterans Affairs hospitals and approximately 50% of those with PTSD do not seek treatment.

Research has shown that there are multiple risk factors for suicide and that these factors may vary with age, gender, physical and mental well-being, and with individual experiences. Treatments and therapies for people with suicidal thoughts or actions will vary as well. The National Institute for Mental Health has focused research on strategies that have worked well for mental health conditions related to suicide such as depression and anxiety. This has included multiple types of psychosocial interventions, such as cognitive behavioral therapy and dialectical behavior therapy where a trained therapist helps the patient to better recognize and deal with disruptive or unhealthy feelings that could lead to self-harm.

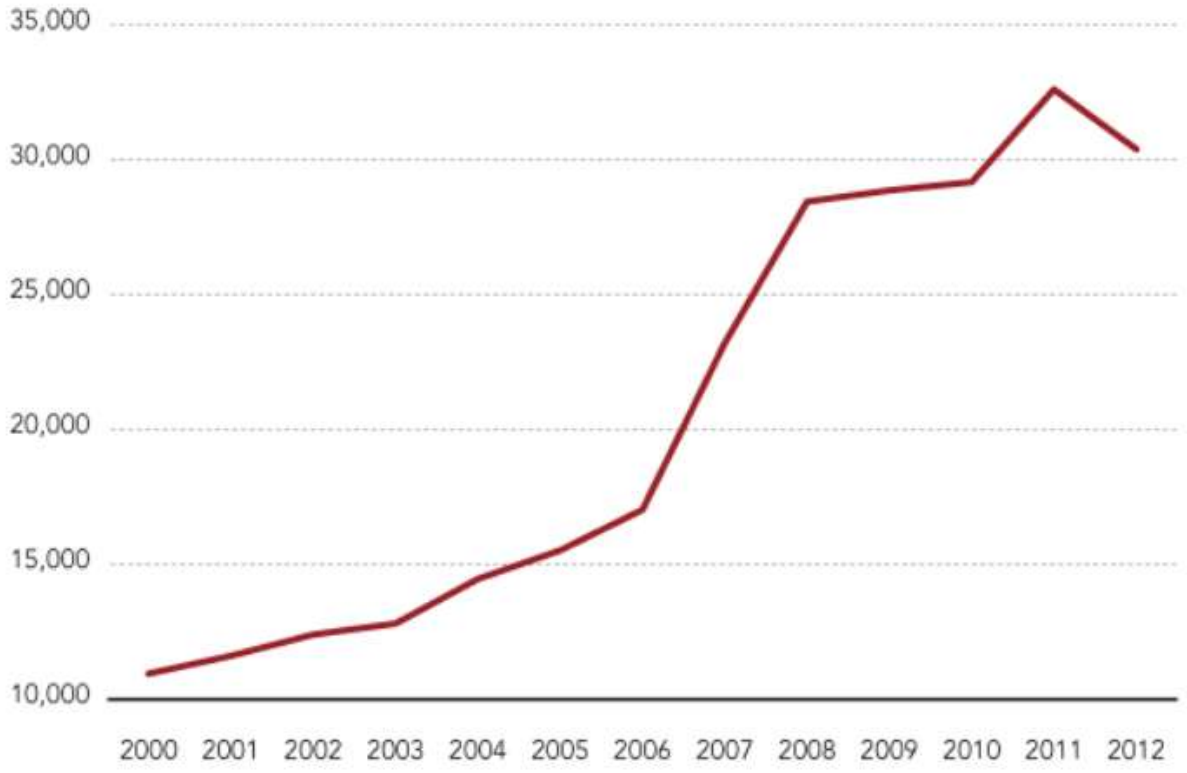
Pharmaceutical interventions generally revolve around the use of anti-depressant medications to control suicidal behavior. Clozapine, an antipsychotic medication used primarily to treat individuals with schizophrenia, is the only medication with a specific U.S. Food and Drug Administration (FDA) indication for reducing the risk of recurrent suicidal behavior in patients with schizophrenia or schizoaffective disorders. Clearly, there is a great need for new medications that could possibly address and resolve the underlying physiological abnormalities that lead to major depression and suicide.

II. Traumatic Brain Injury (TBI)

In military settings, blast exposure is the leading cause of TBI. For U.S. forces deployed to Afghanistan and Iraq estimates of the prevalence of TBI among returning service members range from 15.2% to 22.8%, affecting as many as 320,000 troops. As shown in Figure 2 below, there has been a precipitous rise in reported cases of military TBI. Despite their frequency, the acute and long-term effects of TBI have been a relatively unexplored area of medical inquiry until very recently. Undoubtedly, the "invisible" nature of many TBIs, notably the lack of any external physical evidence of damage to the head or brain, has been a major factor contributing to the impression that these soldiers have an "inconsequential" injury. However, there is good evidence that many individuals develop persistent cognitive and behavioral changes, even after mild neurotrauma.

Depression is a common problem after TBI. About half of all people with TBI are affected by depression within the first year after injury. Even more (nearly two-thirds) are affected within seven years after injury. In the general population, the rate of depression is much lower, affecting fewer than one person in 10 over a one-year period. More than half of the people with TBI who are depressed also have significant anxiety.

Figure 2. *Traumatic Brain Injury in Veterans Returning from Active Duty*



Source: Defense and Veteran's Brain Injury Center, DOD (2012)

A recent report reveals that major depressive disorder (MDD) may be the most common and challenging mental health condition that patients encounter following a TBI with 53.1% of TBI patients in the study experienced MDD at least once in the first year after their injury. Another study showed that suicidal thoughts and attempts are also common reactions to TBI with 23% of the participants had thoughts of suicide, while 17% actually attempted suicide after their injury.

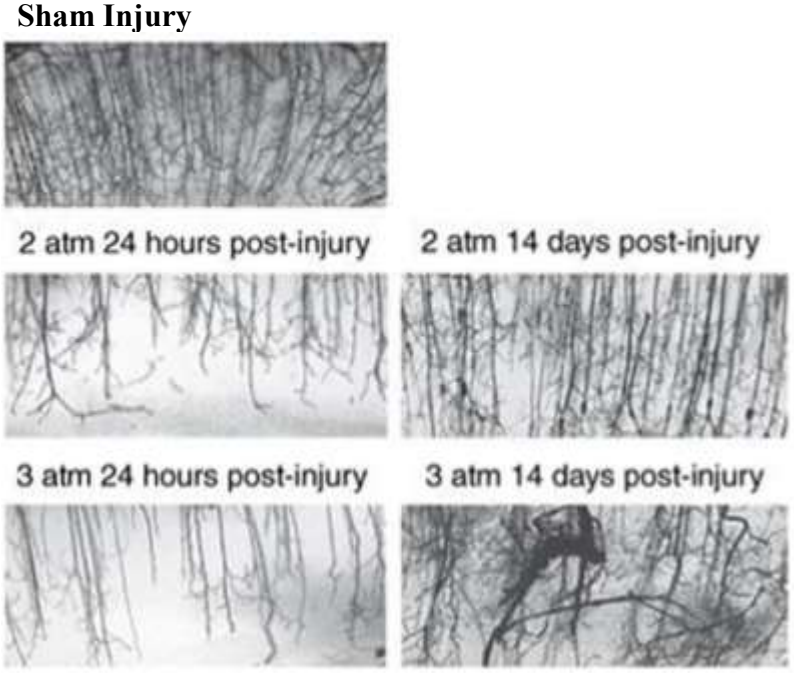
Understanding the effects of military-related TBI presents additional challenges not encountered in studies of neurotrauma seen in athletes or other at-risk groups. With sports-related brain trauma, the injury is generally dependent on the rules of engagement specific to the sport. With military-related TBI, trauma can occur in widely heterogeneous ways, including recreational activities, physical training practices, falls, motor-vehicle accidents, and exposure to explosive blasts. Injury from explosive blasts varies depending on the strength of the explosive and whether the injury occurs in an open field, near buildings or in a motor vehicle. Military TBI is also random and unpredictable, ranging from a single injury to many thousands of traumatic injuries over similar time periods depending on an individual's exposure to blasts and impacts.

The pathology behind traumatic brain injuries has been well studied and multiple animal models of TBI have been developed to examine the pathophysiology of this type of trauma. In general, rapid acceleration, deceleration, or rotational forces cause the brain to elongate and deform, stretching individual cells and blood vessels and altering membrane permeability. Although all cell compartments are affected by the injury, axons are especially vulnerable to shear injury given their relatively long length.

Striking microvascular disordering has also been consistently observed in animal models of TBI. As shown in Figure 3 below, rodents that have been subjected to experimental TBI and then allowed to recover showed a microvasculature that is less dense and more disordered. In these experiments rodents were subjected to pressure-induced fluid percussion TBI, allowed to recover and then the brain microvasculature was examined 24 hours and 14 days post-injury.

On the top panel of Figure 3, a dense and well-ordered microvasculature can be seen in a control animal with a sham TBI. In animals injured with 2 atmospheres (middle panels) and 3 atmospheres (bottom panels) of pressure, depletion of the microvasculature is readily apparent at

Figure 3. ***Recovery from TBI leaves a disordered brain microvasculature.*** *Histological analysis of small blood vessels in brain tissue from rodents subjected to experimental traumatic brain injury. Top panel: sham injury. Middle panels: TBI injury generated with 2 atmospheres of pressure at 24 hours post-injury (left panel) and 14 days post-injury (right panel). Bottom panels: TBI and recovery after 3 atmospheres of pressure. Note the significant depletion of the microvasculature at 24 hours post-injury and the relatively disordered regrowth of the microvasculature at 14 days post-injury (from Park, 2009).*



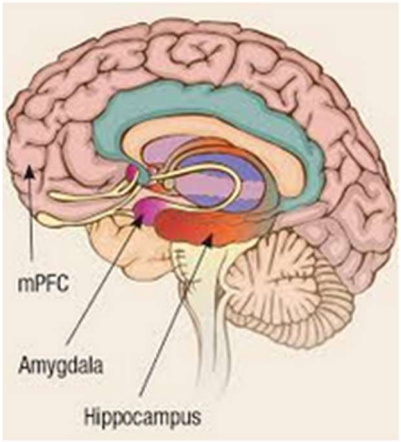
24 hours post-injury injury. Upon recovery at 14 days the microvascular has been reestablished but it is less dense and more disordered than in the control animals.

From the above results, it could be anticipated that neurons would most likely be under-perfused in those areas of recovery which display a disordered microvasculature. This condition has been termed traumatic cerebral vascular injury and is thought to not only underlie the many deficits seen in TBI, but also in its chronic form, to actively participate in the pathogenesis of neurogenerative diseases including Alzheimer's disease, Parkinson's disease and chronic traumatic encephalopathy.

III. Brain Microvascular Disease and Major Depression

Although it generally accepted that microvascular deficits contribute significantly to the pathogenesis of TBI, it is less well appreciated that major depressive order may also have brain microvascular abnormalities in its etiology. It is now well documented that chronic stress has a significant impact on the cellular integrity and function of certain brain areas, most notably the limbic structures which include the hippocampus, amygdala and prefrontal cortex (PFC), as shown in Figure 4 below. These areas of the brain communicate with each other and are vital to consolidating memory, decision making and emotional responses.

Figure 4. C*omponents of the brain limbic system.* *The limbic system includes the hippocampus, amygdala and prefrontal cortex, areas vital to memory, decision making and emotions.*



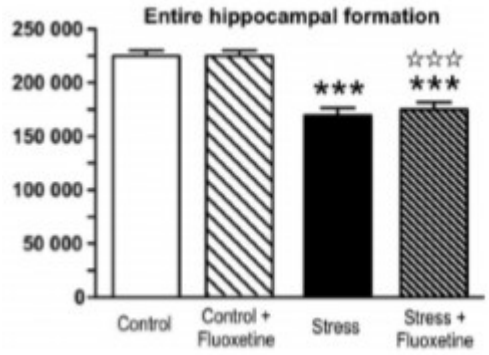
Numerous studies have demonstrated that chronic stress exposure results in reduced hippocampal volume and suppressed adult neurogenesis in the hippocampus. Parallel to these findings, a growing number of studies demonstrate that many of these stress-induced cellular changes can be reversed by antidepressant treatment. In contrast to the intensive efforts that have been made to reveal stress-induced molecular and cellular alterations of neurons, little attention

has been paid to possible changes in vascularization, which is the major structure for supplying nutrition and oxygen to neurons in the limbic system.

The exact pathway for how stress influences the survival of injured neurons is unclear. It has been proposed that stress is harmful, because stress releases glucocorticoids which impair the capacity of neurons to survive. Another possibility, which has come from more recent studies, indicates that chronic stress may impair vascular supply and damage neurons in a process very similar to traumatic cerebral vascular injury which was noted above in the context of TBI. In these recent studies, careful analyses were conducted to examine possible changes in the vascularization of hippocampal tissue after exposure to chronic stress.

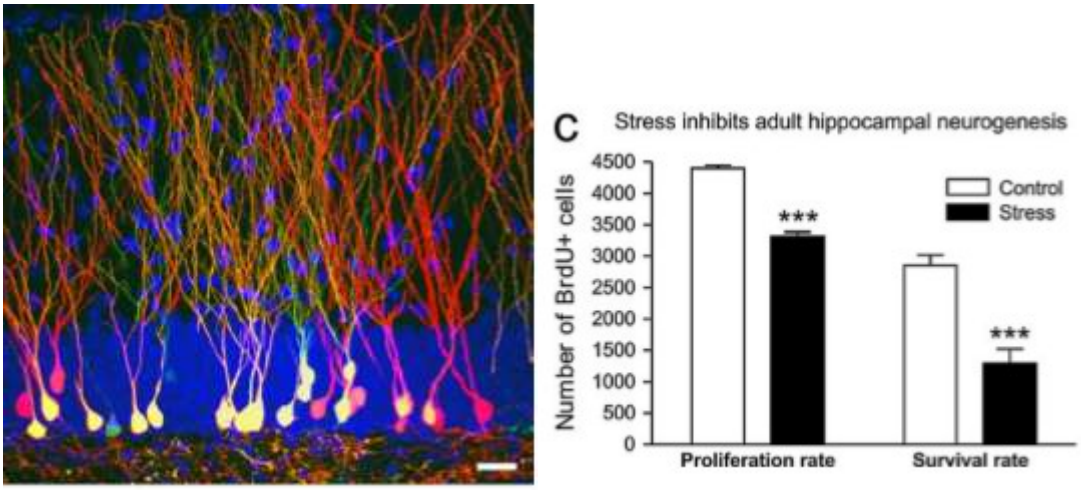
In an animal model of major depression, investigators showed a consistent decrease in the number of small capillaries which supply the hippocampus. As shown in Figure 5 below, there was a significant decrease in the number of capillaries throughout the entire hippocampus in animals that have a depressive disorder brought on by chronic psycho-emotional stress. It should also be noted that as mentioned above, stress results in the release of corticosteroids from the adrenal gland as part of the "fight or flight" syndrome. As corticosteroids are known to be very potent inhibitors of angiogenesis, these steroids could be contributing to the observed decrease in hippocampus capillaries following chronic stress and depression.

Figure 5. ***Stress Reduces Capillary Numbers in the Hippocampus.*** *Effects of chronic psychosocial stress on the number of microvessels in the hippocampus. Repeated stress significantly reduced the number of capillaries throughout the hippocampus. Fuoxetine (Prozac) treatment had no effect on microvessel number.*



In this animal model of depression, five weeks of daily psycho-social stress resulted in about a 30% decrease in microvessel number in all hippocampal subareas. Such a dramatic change is likely to have a significant impact on the oxygenation and nutritional supply of the neurons in that area of the brain. Subsequent studies in this animal model of depression established that, indeed, this decrease in capillary number also resulted in a significant decrease in neurogenesis or new neuron formation in the hippocampus. By confocal microscopy, new neuron formation and their survival could be detected and quantified in the hippocampus, as shown in Figure 6 below.

Figure 6. ***Stress Inhibits Neurogenesis in the Hippocampus in an Animal Model of Depression.*** *On the panel on the left side of the figure, new neurons in the hippocampus can be visualized by confocal microscopy. This can be quantitated as shown in the panel on the right where it can be seen that stress inhibits neurogenesis (neuron proliferation and survival) in the hippocampus.*



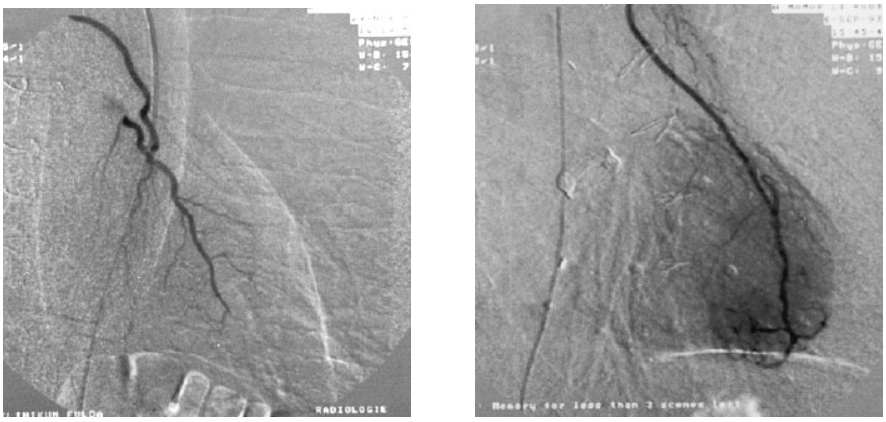
Thus, these and other studies clearly demonstrate that microvascular dysfunction in the brain brought about by stress and depression can have a powerful impact on the proliferation and survival of neurons in the hippocampus, an area of the brain critically responsible for proper emotional and cognitive functioning of individuals. Although most of these recent studies have focused on the hippocampus, there is no reason not to suspect that a similar mechanism may also be in play in other emotional and decision-making centers of the brain, including the amygdala and the prefrontal cortex.

IV. Human Fibroblast Growth Factor: A Potent Co-regulator of Angiogenesis and Neurogenesis and Its Role in Major Depression Disorders

Zhittya Regenerative Medicine, Inc's. management has researched human FGF-1 for over 20 years and has excellent clinical trial outcomes exploiting a more traditional biological activity of FGF-1, namely the ability of this growth factor to stimulate new blood vessel growth or angiogenesis. In FDA-cleared Phase II clinical trials, FGF-1 stimulated the growth of new blood vessels in the hearts of patients damaged by severe coronary artery disease, as well as stimulating angiogenesis and accelerating the healing of diabetic foot ulcers. Figure 7 below shows the characteristic "blush" of newly formed blood vessels that is seen when FGF-1 is injected into the hearts of patients with coronary artery disease.

Figure 7. ***FGF-1 stimulates angiogenesis in the human heart.*** *Angiograms in a patient receiving a coronary bypass procedure who received an injection of either heat inactivated FGF-1 (control heart, left panel) or FGF-1 (right panel). (from Schumacher 1989).*

Control Heart **FGF-1 Treated Heart**



In addition, Zhittya has exploited the neuroprotective properties of FGF-1 and has strong positive data in animal models of stroke that FGF-1 is a potent neuroprotective agent.

There are currently 18 members of the FGF family and 10 of these growth factors are expressed in the brain. FGF-1 and FGF-2 are ubiquitously expressed in the adult brain with the highest expression in the hippocampus and cortical areas. The prototypical receptor for FGFs, FGF receptor-1, is found mostly on neurons, although its expression has also been demonstrated on neural stem cells. This receptor has been shown to play a predominant role in both the development of the cortex and hippocampus, two key regions involved in the development of major depressive disorder.

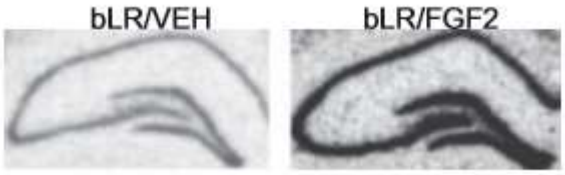
A body of research has now established that the FGF family of growth factors can modify anxiety-like and depression-like behavior and appears to play a significant role in major depressive disorder. This concept emerged from studies of postmortem brains of subjects who had died while suffering from severe clinical depression. Major depressive disorder (MDD) is the most debilitating mood disorder in the United States, accounting for the single greatest psychiatric cause of disability. A better understanding of the pathophysiology of this disease is acutely needed given the high rate of incidence of this disease (25% lifetime incidence of MDD), and that only a 33% response rate is seen in first line treatments for MDD.

The first link between the FGF family and depression came from “gene mining” studies done with brain autopsy specimens from subjects who died from major depression and revealed that members of the FGF family, including FGF-1, were significantly decreased in major depression. These findings have since been extended to other brain regions and have led to a series of studies in animal models that have transformed the understanding of the role of the FGF family in depressive behaviors.

Animal studies were performed in models of depression-like behavior that more closely paralleled depression in humans. The animal models employed focused on the hippocampus, as postmortem studies pointed to this brain region as the most altered by MDD. Moreover, this is an area that is critical in the biology of ‘‘stress-related disorders,’’ including MDD, anxiety, and posttraumatic stress disorder (PTSD). For example, human brain imaging studies have shown that the volume of the human hippocampus decreases significantly in patients with PTSD, consistent with the view that this area is highly responsive to stress-related disorders.

FGFs can control the development and size of the hippocampus and it has been demonstrated that FGFs and their receptors are decreased in these animal models of depression. To ascertain the possibility that FGF could act as an “endogenous antidepressant”, FGF was directly administered into the brains of depressed adult rats and multiple tests of depression-like behavior established that FGF has demonstrable antidepressant properties. In addition, it was also shown that after FGF injection into the brain, specific markers of neurogenesis were up-regulated. As shown below in Figure 8, FGF injections resulted in a robust stimulation of the neurogenesis biomarker, nrk3 kinase protein, which is located within the dentate gyrus region of the hippocampus, an area important for memory retention and exploratory behavior.

Figure 8. ***FGF stimulates neurogenesis in the hippocampus of depressed animals****. The panel on the left shows the dentate gyrus region of the hippocampus from an untreated animal and the panel on the right shows the same region in animals treated with FGF. The dark areas indicate the location and magnitude of expression of a marker for neurogenesis, the nrk3 gene.*



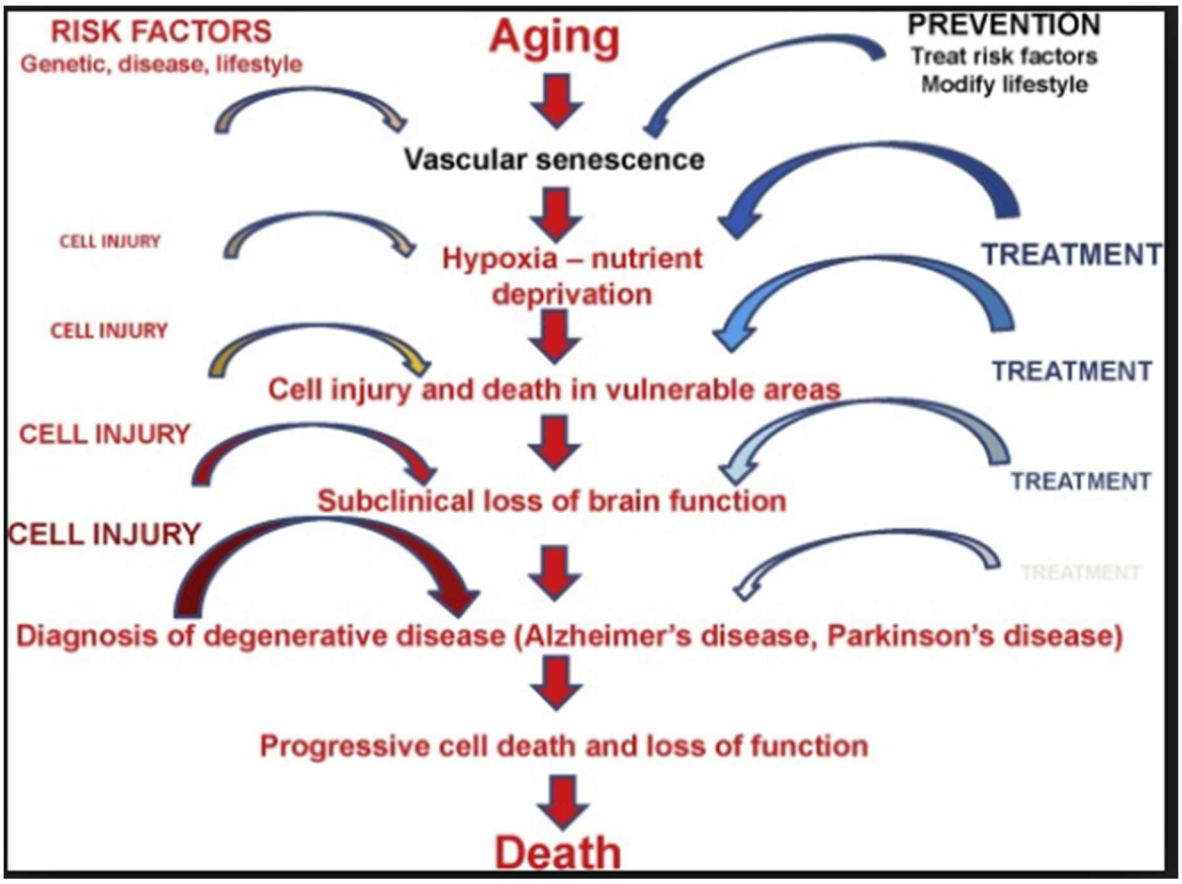
Taken together, this body of reserch points to a critical role of the FGFs in mediating major depression. FGF-1, as the most potent and widely-acting member of the FGF family, and with its ability to co-regulate both angiogensis and neurogenesis, would appear to be a good therapeutic candidate to test in brain disorders that are characterized by a disrution of the microvaculature resulting in underperfusion of brain neurons.

V. Summary and Future Directions

Zhittya Regenerative Medicine is currently manufacturing clinical lots of its FGF-1 drug to be used in clinical trials in patients with diabetic foot ulcers and severe coronary artery disease. It has also developed a protocol for a toxicology study in animals that would support the long term IV administration of FGF-1 for use in vascular disorders of the brain. The company is committed to see if FGF-1 has any potential use in benefitting patients with debilitating neurodegenerative diseases, including stroke, Alzheimer’s disease, Parkinson’s disease and CTE. These diseases are unmet medical needs and we firmly believe that there is a strong body of evidence indicating that these degenerative diseases have as a primary initiating cause, dysfunction in the brain’s

microvasculature. Our thinking is reflected in Figure 8 below where "vascular senescence" can also mean vascular dysfunction or vascular disorder. We believe that FGF-1, as a stimulator of angiogenesis in the microvasculature, could be given early in these brain disorders, to potentially prevent the downward spiral to neuron cell injury and death. Even if given later in the disease progression, we believe that FGF-1, with its ability to co-regulate both angiogenesis and neurogenesis, could also be of possible benefit in later stages of the disease process.

Figure 8. *Vascular dysfunction and senescence as a precipitating cause of neurodegenerative diseases.*



Given our current knowledge, the word "Aging" in the figure above, could be replaced with the words "Trauma" or "Stress" and the same downward spiral would be seen with TBI or major depressive disorder. With this in mind, we believe it would be important and logical to see if long term IV FGF-1 infusions could benefit patients with the TBI, PTSD or major depressive disorder, allowing a proper restoration of blood perfusion to neuronal populations at risk. It is our hope that this would then lead to an improvement in mood and function in these patients, with an accompanying reduction in suicidal thoughts and actions.

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