BiomediGirls Week 6 Competition

# Analyzing the Biomedical Effects of Potential Therapeutic Treatments in Strokes

Induced Pluripotent Stem Cells and CRISPR/Cas9 Experiment

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**Abstract**

A stroke is an abrupt cessation in the blood accumulation of the brain. A majority of strokes are created by a sudden blockage of arteries pointing to the brain. Other kinds of strokes are created by a blood vessel rupture, thus leading to bleeding within brain tissue. Because of strokes' unexpected occurrences and severe health danger, if not treated quickly, strokes are also described as a brain attack. Ministrokes are characterized when symptoms of a stroke only last for a small period or less than an hour. A stroke's effect is determined on which part and how severely a part of the brain is injured. Some symptoms of a stroke are unexpected weakness, lack of sensation, or difficulty with speaking, seeing, or walking. Since several parts of the brain manage diverse areas and functions, it is usually the area enclosing the stroke that is affected. Strokes can induce headaches, but other strokes can be completely painless.

Forkhead box F2 (FOXF2) works as a transcriptional control and regulates downstream gene representation in embryonic development, metabolism, and in some common diseases, such as stroke and gastroparesis. New researches have revealed that unusual expression of FOXF2 is incorporated with a diversity of tumorigenic methods, such as reproduction, invasion, and metastasis. Since FOXF1 is the central gene concerning strokes, we think that extracting it could hinder the strokes from transpiring. Furthermore, CRISPR/CAS 9, a gene-editing device, could try to eliminate that gene by substituting it with a salutary gene. This is our in vitro study. For the in vivo, we will use induced pluripotent stem cells as a way to target the cells that might carry the stroke gene.

We hypothesize that if GWAC is conducted before the injections of iPSCs cells, then the probability of finding a drop in the connection between COVID - 19 and strokes will rise because GWAC allows the perception of genetic markers which can help in evolving potential therapeutic treatments. We developed the hypothesis by looking at past research examples and thought of legitimate ways to experiment on mice.

**Introduction**

Biomedical research has inevitably become the catalyst to insight profound discoveries and breakthroughs of scientific advancements. The evolvingly emerging fields have executed IND-enabled treatments for strokes, but only 10% of stroke victims recover completely, which directly correlates to stoke diseases being the second deadliest disease in the world. Until recently, scientists have broken the clinical stalemate, realizing that tissue plasminogen activators (tPA), the leading medication for treating strokes, could conceivably deprive certain cells of oxygen, leading to the cultivation of even bigger strokes[1]. Furthermore, experts and doctors have discovered that SARS-CoV-1 and SARS-CoV-2, also known as Coronavirus (an ongoing virus in various components of the world), could potentially lead to long-term damages such as blood clots, which allows strokes to be 8 times more likely to occur compared to the flu. By utilizing a retrospective environment, ideologies of challenging the development of thrombi clots could lead to the promotion of expanding our perception of the therapeutic treatments in strokes. Therefore, the research proposal will be analyzing the biomedical effects of potential therapeutic treatments in strokes through the statement problem of an in vitro and in vivo experiments to minimize the long-term effects of strokes. Instead of trying to just repair the stroke-related cells, this research proposal enables the utilization of stem cells as a way to help repair and remove the damaged cells in the brain. It pushes past the boundaries of surgeries like Carotid Endarterectomy (CEA) and medical treatments such as anticoagulant and antihypertensive drugs and permits there to be a safer route to removing the stroke cells. Not only that but by using the CRISPR/Cas 9 device, to remove the stroke cells from mice, it allows there to be an enhancement of knowledge on the disease itself.

Strokes are the second deadliest disease in the world, with a 44% increase in Americans who were hospitalized in the past decade due to strokes. Due to the alarming rate of growth for stroke diseases, scientists have discovered ways to treat these issues through endovascular therapies, management of increased intracranial pressure, hemicraniectomies, and much more. Additionally, strokes have been classified as Ischemic strokes, Hemorrhagic strokes, and Transient Ischemic Attacks (TIA). In Ischemic Strokes, there is a clot or blockage in the artery causing there to be a lack of blood flow, resulting in damaged brain cells. On the other hand, hemorrhagic strokes happen when a weakened blood vessel bursts causing there to be a leakage of blood to the brain. This is the most deadly type of stroke because it can lead to an onset headache that could potentially take your life. Lastly, TIA stokes are “mini-strokes” and are the least harmful because it has only arisen from a temporary clot.[2] When studying about strokes, scientists have made a recent breakthrough discovering that FOXF1 and/or FOXF2 could be the main gene that increases the risk of having a stroke. While FOXF1 and FOXF2 are significant to the proliferation of rhabdomyosarcoma tumors, studies have also discovered that the depletion of FOXF1 and FOXF2 could decrease the likelihood of rhabdomyosarcoma tumor rates in mice, along with strokes.

For a bit of background, one of the tools that are utilized is the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR/Cas 9) which is a genome-editing tool that could enable geneticists to edit parts of a genome by selecting parts to remove, add, and/or alter in the DNA sequence. By using a protein called Cas9 and a guide RNA(recognizes the

[1] https://www.scientificamerican.com/article/why-the-go-to-stroke-drug-can-fail/

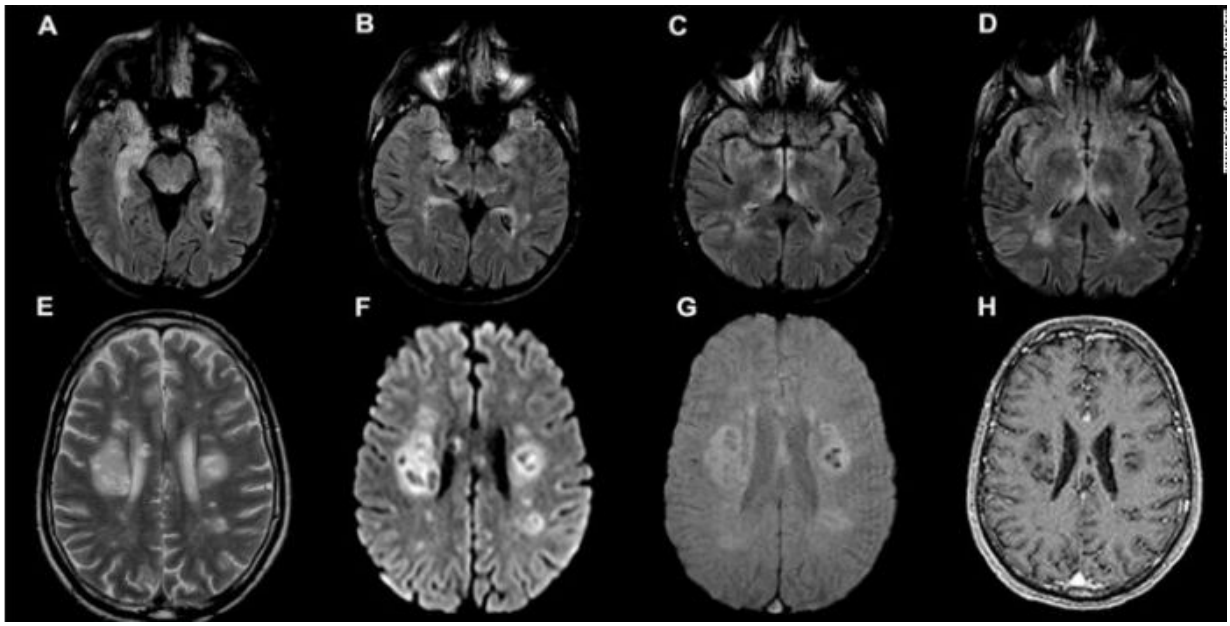
[2] https://www.stroke.org/en/about-stroke/types-of-stroke

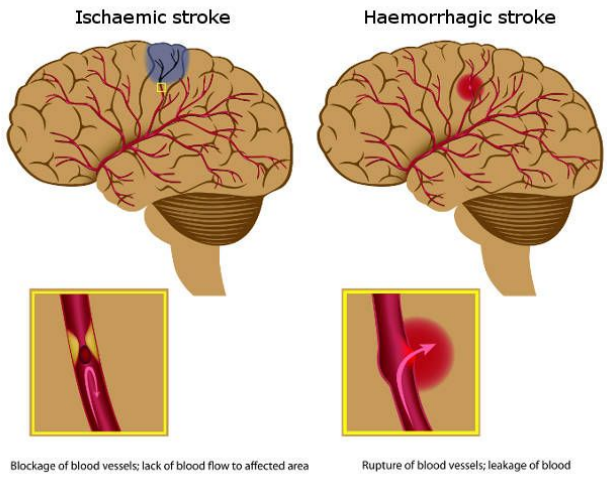
sequence), it allows CRISPR to locate the target cell sequence which provides people with the opportunity to modify the genome. This revolutionary breakthrough discovered by Yoshizumi Ishino could potentially lead to creating new antibiotics for humans. While this does sound transformative to the world, CRISPR testing could be extremely dangerous as it can cause cells to lose the ability to attack foreign cells, leading it to even more damage to genes. Due to this reason, in this research proposal, CRISPR/Cas 9 will only be utilized on mice as a way to learn more about strokes and the outcome of CRISPR testing. Furthermore, another tool that will be used in the in vivo laboratory test is induced Pluripotent Stem Cells (iPSCs). Induced Pluripotent Stem Cells is an embryonic-like figure that is derived from skin/blood cells as a way to help with the therapeutics of regenerative diseases and modelings. This plays a significant role in the research lab because iPSC-NPC transplantation in the brain could increase the rate of proliferation in the subventricular zone and enhance the rate of recovery. Lastly, a method known as the Genome-Wide Association Study (GWAS) can be used to scan genomes as a way to juxtapose the various genetic markers which could be utilized as a way to determine common factors and the presence of a disease. Through the process of GWAS, this approach identifies Single Nucleotide Polymorphisms (SNPs) which can help rapidly scan the various genetic markers which track the different gene variations (Francis S. Collins, M.D., Ph.D.. 2015).

In a recent study by multiple scientists and geneticists, GWAS used an immunochip array that had 3420 cases of Ischemic strokes and 6821 control groups, they discovered that when GWAS was directly applied to the strokes (HDAC9 was included), there was a risk factor that was later identified as a genetic risk that was specifically for the large artery stroke. This study is significant because it could be utilized as a way to learn more about strokes. Since our experiment is testing CRISPR on the mice, using the GWAS beforehand could deepen our understanding of potential therapeutic treatments. As shown in the data chart below, scientists collected various data based on different fields (Kilarski, 2014).

| Study | Cohort | Controls | All | SVD | LVD | CE |
|---|---|---|---|---|---|---|
| Immunochip | Belgium (Leuven) | 319 | 396 | 49 | 57 | 147 |
| | Germany (Munich) | 2,355 | 421 | 8 | 101 | 127 |
| | Netherlands (Utrecht) | 1,145 | 556 | 232 | 324 | 0 |
| | Poland (Krakow) | 255 | 384 | 28 | 33 | 119 |
| | Sweden (Lund) | 997 | 796 | 183 | 56 | 246 |
| | UK (London & Glasgow) | 1,790 | 867 | 257 | 152 | 130 |
| | Total | 6,861 | 3,420 | 757 | 723 | 771 |
| WTCCC2 | | 5,972 | 3,548 | 580 | 844 | 790 |
| METASTROKE[a] | | 56,032[b] | 8,480 | 1,177 | 1,203 | 1,586 |
| INTERSTROKE | | 852 | 797 | 228 | 165 | 0 |
| VISP | | 1,047 | 1,725 | 0 | 0 | 0 |
| | **Total** | **70,764** | **17,970** | **2,742** | **2,935** | **3,147** |
| ICH (GOCHA) | | 390 | 389 | — | — | — |
| ICH (GOCHA-Warfarin) | | 169 | 181 | — | — | — |
| ICH—Europe | | 529 | 532 | — | — | — |

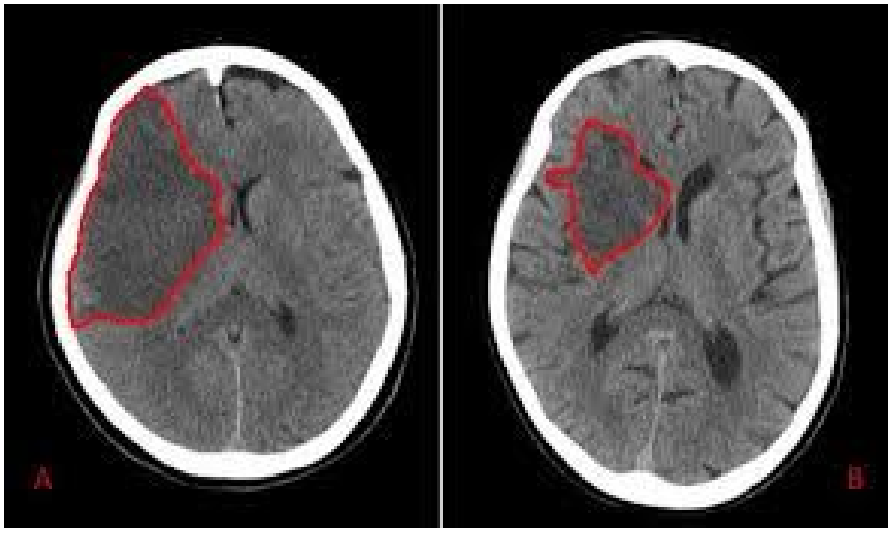
Additionally, another research proposal that was significant to our proposal was published by The Lancet Psychiatry which explained the relationship between COVID-19 and Strokes. The study looked at 125 hospitalized patients with Covid-19 who also had some sort of neuropsychiatric complication. Based on that, 57 of the patients had an ischemic stroke, 39 had an altered mental state (encephalitis), 10 were diagnosed with psychosis, and 6 had cognitive issues. An overwhelming amount of patients suffered from ischemic strokes after COVID-19 which exemplifies that neuroscientifically, there might be a direct correlation between Coronavirus and strokes. This is tremendously important to address because with COVID-19 hitting an all-time high in places around the world, understanding the connections of neuroscience enables scientists to develop potential cures and vaccines. In the diagram below, it displays brain scans from UCL on July 8th, which shows shocking results (Yeung, 2020). The image below shows the brain damage to ischemic strokes (Headway). Comparing the two diagrams below, it represents how strokes and COVID-19 could lead to dangerous long-term effects. Therefore, our hypothesis is that: If GWAC is run before the injections of iPSCs cells, then the likelihood of finding a decrease in the relationship between COVID-19 and strokes will increase because GWAC enables the understanding of genetic markers which can help in developing potential therapeutic treatments.



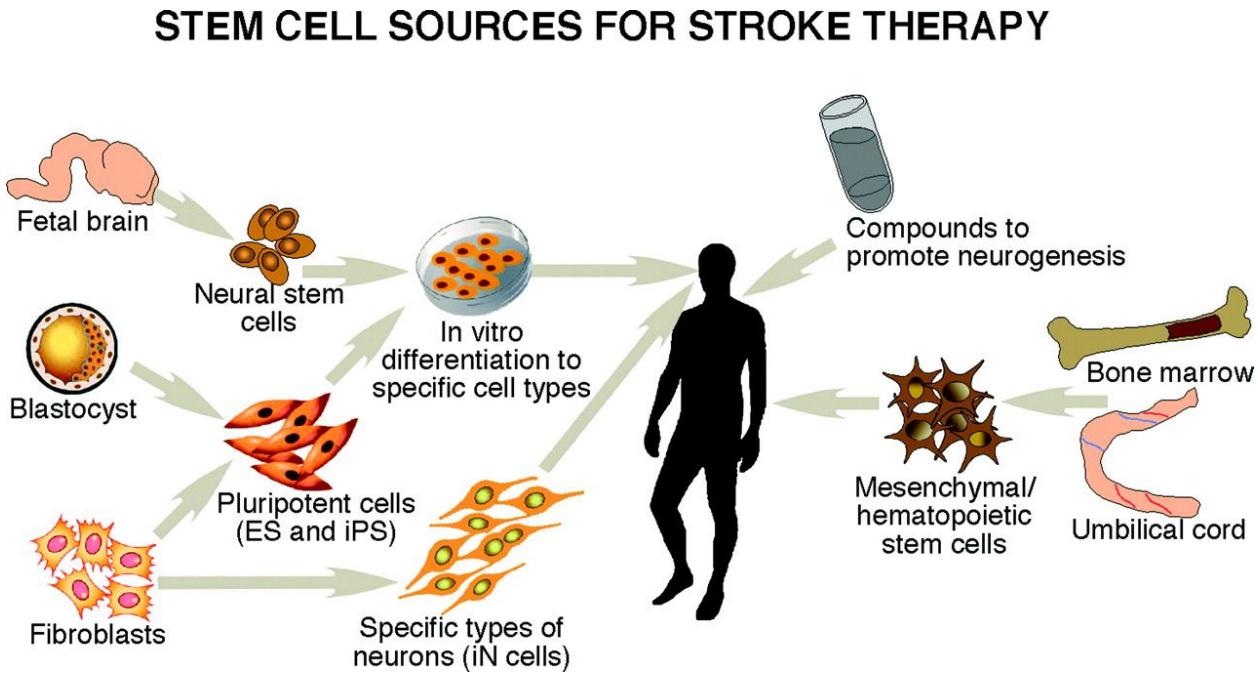



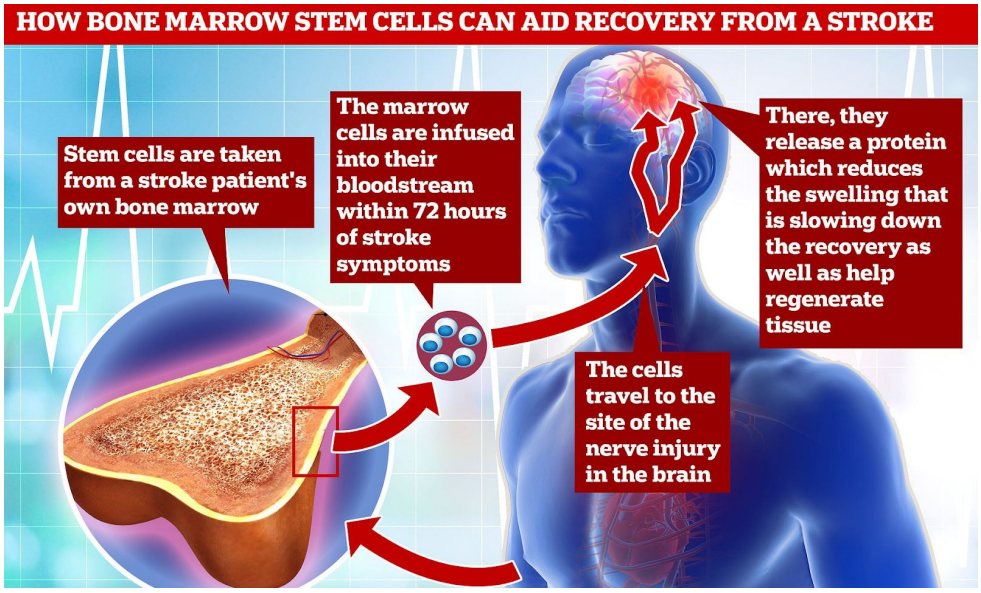
**Materials and Methods**

In order to ensure a thorough understanding of strokes, there will be two experiments (both performed in the lab), an in vivo and an in vitro. For instance, in the in vitro experiment, a GWAC run will occur as a way to understand the stroke that is located in the mouse, and then CRISPR/Cas 9 will be utilized as an attempt to remove the gene of FOXF1. Specifically, the CRISPR machine will have a molecular scissor that enables the modification of genes. There will also be a CAS9 protein that is an endonuclease that runs the CRISPR test and the guide RNA that provides a clear direction to how the genome will be cut. Before the test is officially tested, CAT scans will be utilized to notice the various diagrams of the 10 mice that have a stroke-related gene in their bodies. CAT scans are critical because they allow there to be a pinpoint in the location of a stroke, narrowing down where doctors would have to inject tPA in the body. Firstly the 10 mice would undergo a CAT scan, determining the FOXF1 or FOXF2 gene and how to isolate it. After that, a GWAC run will be used as a way to determine how to narrow down the genomes. Then for the in vitro experiment, CRISPR will be given the guide RNA of the genetic coding for FOXF1. Lastly, CRISPR is coded to replace the damaged gene of FOXF1 with a healthy gene. As predicted in our hypothesis, this will increase the chances of developing a therapeutic treatment because there will be enough data to compare the before and after results. Additionally, the timeline will run for 3 months. Three months is a decent timeline because CRISPR testing requires careful markings to develop a functional test method because tiny mistakes such as mistakenly introducing errors could lead to harmful damages on the organ's DNA and the body. This is why the mice will be placed in a culturally-appropriate area that enables the reduction of outside factors. The diagram below represents a CAT scan of two patients that had a stroke. The patient on the left side shows hemianopia and the damage of the left cerebral artery. On the other hand, the patient on the right shows a partial occlusion in the right middle cerebral artery territory.



On the other hand, for the in vivo experiment, five mice that have stroke cells and/or genes would undergo a surgery that will insert induced pluripotent stem cells to replace the damaged ones in the brain. To ensure the safety of the mice, they will be fed under a ketogenic diet that promotes healthier brain function as a way to potentially reduce seizures and strokes. After using that diet for a week, there will be conduction of two trials: the phase 1 trials (which test the safety of a procedure) and phase 2 trials (which test for efficacy) underway in stroke. Both are essential to understanding the behavior of strokes in the body. All in all, this diagram represents how stem cells could be utilized and injected into the brain as a way to reduce inflammation and rebuild damaged areas.



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**Reflection**

After thoroughly collaborating on this research proposal, discussions were made as a way to equally distribute the work. In this proposal, Sophie was in charge of creating the cover page, abstract, and works cited page. For Frances, she was in charge of creating the introduction, materials/methods, works cited, and reflection. Both of us were able to participate and efficiently communicate to generate this research proposal.