Cyclic Co-Delivery of SARS-CoV2 and ACE2 via Lentiviral Vectors to Target Glioblastoma (GBM): *In Vitro* and *In Vivo* Models for Viral-Mediated Fusogenic Therapy and Tumor Suppression

The Failure to Include People with Disabilities in Vaccination Roll Out: Applying Intersections of Power for Comprehensive Analysis

A Thesis Prospectus
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On my honor as a University student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments.

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Research Question: "How do inequalities, especially those related to transportation, socioeconomic status, and nationality, impede access to flu and COVID vaccines for people with intellectual and developmental disabilities?"

Introduction

Innovation is constantly driving new medical treatments to benefit the health outcomes for our society. Cancer is the second highest cause of death in the United States, with an average of around 600,000 cancer related deaths each year (Center for Disease Control and Prevention, 2022, p. 1). An average of 5.5 million dollars are spent on researching cancer treatments each year (Head et al., 2024, p. 1-2). My capstone group and I are conducting upstream research for the creation of an immunotherapy treatment for glioblastoma (GBM) by utilizing SARS-CoV2 spike protein and the ACE2 receptor. We will determine if the combination treatment of these will lead to cell fusion, which will be used as a proxy for cell death. When designing a novel medical treatment, it is important to consider how it would affect all populations and how fair distribution can best be accomplished. One example of poor execution of considering all populations to achieve fairness is the COVID-19 vaccine distribution. While many groups of individuals with pre-existing conditions were prioritized in the rolling out of COVID-19 vaccines, people with intellectual or developmental disabilities were excluded from early vaccination. This was a huge mistake because many people with disabilities have conditions that would make it harder to fight off COVID-19. For example, people with Down Syndrome were up to 10 times as likely to die from COVID-19 than people without Down Syndrome (Wiggins et al., 2021, p. 3).

When creating new medical technologies, it is the engineers' responsibility and duty to not only ensure that the technology helps every population equally but also to ensure that the distribution of that technology is fair and just. Instead of proceeding with what is easiest or

fastest, multiple options should be weighed to yield the best solution. If engineers and people in the healthcare industry neglect to take into consideration these ideas, certain populations could be left out or potentially harmed by the new technology.

Technical Research Question

GBM, a World Health Organization (WHO) grade 4 glioma, is the most common and most aggressive primary malignant brain tumor, accounting for 47.7% of all central nervous system brain tumors in adults (Thakkar et al., 2024, p. 1). The incidence rate is 3.19 per 100,000 people in the U.S., with males having a 1.6 times higher incidence than females. Despite advances in treatment, survival rates remain dismal, with fewer than 5% of patients surviving beyond five years after diagnosis (Tamimi & Juweid, 2017, p. 3-4). The median life expectancy is 15 months, largely due to GBM's rapid proliferation and invasion into the healthy brain parenchyma, complicating effective removal or targeting (Stahl et al., 2022, p. 1). There is a substantial clinical need for different treatment modalities for GBM. Current treatments primarily serve to delay the tumor progression and death, but no cure exists. Moreover, these treatments typically impact brain function. Common complications include mood changes, memory problems, decline in function of daily activities, depression, recurrence of tumor, increased risk of infection, and bleeding (Cleveland Clinic, 2024, p.1) (Thakkar et al., 2024, p. 2). This project proposes a novel fusogenic therapeutic approach, leveraging the SARS-CoV-2 spike protein to trigger cell fusions in GBM. This would stimulate an immune response and directly target GBM cells. Insights from this research could have a significant clinical impact, providing a new strategy for GBM treatment and potentially informing therapies for other cancers.

There will be two main aims to this project; the first being to design an *in vitro* system, optimized to directly kill GBM cells through syncytia formation. This will be done by adjusting

multiplicity of infection (MOI) ratios of the SARS-CoV-2 spike protein and ACE2 receptor. The dynamics of continuous or cyclic protein expression will be investigated, as they can significantly impact the efficacy of therapeutic interventions by influencing factors such as immune response activation, viral persistence, and tumor cell death. Several quantification metrics will be analyzed, including cell viability, syncytia formation (number, size, and percentage), and optimization of the MOI ratio. DAPI-stained images can be taken to analyze the syncytia formation. Another valuable metric will be determining the proportion of SARS-CoV-2 spike proteins and ACE2 receptors involved in syncytia formation relative to the total number of plated cells. These findings could provide valuable insights for personalized treatment strategies to reduce GBM tumor progression. This investigation will yield a doxycycline-inducible system, an established modulator of gene expression, capable of modulating both continuous and cyclic expression of the SARS-CoV-2 spike protein and ACE2 receptor. Quantitative data will compare GBM cell survival, proliferation, syncytia formation, and immunoglobulins via an ELISA assay across the two expression modalities using cell viability assays such as alamarBlue.

The second part of this project will be to develop a predictive model to evaluate the optimal MOI ratio of SARS-CoV-2 spike protein and ACE2 receptor to GBM cells. This will be accomplished by measuring syncytia formation as an indicator of immune response. Currently, no computational model predicts how varying MOI ratios of viruses encoding SARS-CoV-2 spike protein and ACE2 receptor to GBM cells may impact the immune response to GBM cells. Syncytia formation may correlate with immune-stimulating activity *in vivo* because the syncytia are highly inflammatory to the immune system. Developing this model will allow for the determination of optimal conditions for inducing syncytia and triggering an effective immune

response. The output will be a MATLAB computational model designed to predict the relationship between MOI ratios and syncytia formation, aiming for an 85% prediction accuracy. The model will be trained using experimental lab data from the earlier investigation as well as data from existing literature. It is hypothesized that syncytia formation will plateau at a particular MOI ratio, suggesting an effective immune response. Furthermore, excessively high expression levels of the SARS-CoV-2 spike protein are expected to kill GBM cells, potentially reducing the immune response directly. The model will hopefully be able to predict a range of MOI ratios that will induce a targeted immune response and maximize GBM cell death.

STS Research Project

For my STS research project, I will be investigating the COVID-19 vaccine roll out and how people with intellectual or developmental disabilities were treated versus those without disabilities. There is evidence that supports that, "although the COVID-19 response has largely been data-driven, few pandemic surveillance efforts have collected data for people with disabilities, preventing the prioritization of many people with disabilities in the vaccine rollout." (Epstein et al., 2021, p. 1) This was a global issue that did not only affect U.S. citizens. In fact, "59% of COVID-19 deaths in the UK occurred in people with disabilities, despite comprising 17% of the population."(Rotenberg, 2021, p. 3) For this reason, I will be comparing the response in multiple countries to see how different responses may have had different outcomes. Oftentimes we can learn from people who think differently than us to create the best possible overall healthcare experience for everyone.

Nationality is only one structure of power that I will use in my research. I will also be seeing how different races of the people with disabilities and their caretakers impacts vaccination access and rate. One report stated that, "Black and Hispanic direct service providers (DSPs) are more hesitant to receive a COVID-19 vaccine than White DSPs."(Wiggins et al., 2021, p. 2)

Similar to their caretakers, it was found that, “the lowest increase [in overall vaccination rate] over time was observed in adults with disabilities that identified as Black, Non-Hispanic (2.1%, vs. Asian adults: 18%).”(Castro et al., 2023, p. 1) Race is an important lens to address this problem because there has been a history of misuse of African Americans in healthcare, especially with the experimentation of new medical technologies. Two of many examples include Henrietta Lacks’s experience and spirometer misuse (Barla, 2023, p. 1-3). For this reason, it is logical that these populations may be more hesitant to get the latest vaccinations. It is important that we keep this in mind when releasing new treatments. In having strong and transparent communication policies, we can improve interest in new vaccines.

I will also investigate how the socioeconomic status of people with intellectual or developmental disabilities impact access to vaccines. Oftentimes transportation can be an issue in getting to vaccination centers. One source suggests that, “sites need to also consider ‘first-mile, last-mile’ access, as accessible transport, parking, and locations facilitate accessibility, but do not guarantee the entire trip is accessible.”(Rotenberg et al., 2021, p. 3) No one should be denied healthcare because they can not physically access the place of treatment. The design of these buildings and parking lots should be conducive to any patient’s needs regardless of ability level. Additional stress about getting to the vaccination site should not be put on a small minority of the population. Inclusive planning would avoid these concerns. Furthermore, socioeconomic factors impact access to knowledge about vaccines, whether that be through online or mail information. It is not fair for certain groups of people to be left out of the conversation and knowledge distribution. The distribution of knowledge is just as important as the vaccination distribution itself.

Finally, I will be assessing the inclusivity of the vaccination experience. If getting a vaccination is not comfortable for everyone, it will deter certain populations from even attempting to get vaccinated. This includes the space, volume, privacy, and other various factors. One source sums it up best in saying, "accessibility also includes the environment where tests or vaccines are administered. Loud, bright, or otherwise sensory-heavy environments may be inaccessible to people with certain disabilities."(Rotenberg et al., 2021, p. 3) It is critical to consider the needs of everyone when creating environments that are designed to be used by everyone.

In summary, I will analyze how the vaccination roll out was disserving to those with disabilities and how future technology can be better distributed. One source said, "the COVID-19 pandemic exacerbated challenges for those with disabilities who face additional vulnerabilities due to multimorbidity, income disparities, and reliance on formal support systems," which perfectly describes the issue that occurred (Martin et al., 2024, p. 1).

Conclusion

There is much to be learned from the COVID-19 epidemic both for the enrichment of clinical research and the healthcare system as a whole. Investigating how this specific disease could be used to treat other diseases is an important application. The idea that cancer, a disease that thus far has seemed to be untreatable, could be treated with a newer disease is cutting-edge and exciting. Continued efforts and further experimental research could be a game changer for immunotherapy tools. However, we can also look retrospectively at the COVID-19 vaccine distribution disaster to improve inclusivity and fairness in the medical world. We must ensure care and knowledge are accessible across genders, races, nationalities, and abilities. As the next generation of innovators, it is our responsibility to look out for the most vulnerable groups of individuals and consider their needs and not just the needs of the majority of the population.

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