Engineering Suicide Gene Approaches to Improve Chemotherapeutic Response in Glioblastoma
(Technical Paper)

Consumer Access in the Field of Medicine

(STS Paper)

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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

Prospectus

Introduction:

Cancer is one of the leading causes of deaths in the United States, and it is estimated that approximately 2 million people will be diagnosed with some form of cancer in 2023 (**1**). This is not counting the millions of people who already live with cancer and go through many different expensive treatments that have not progressed in decades for many of the cancers. Currently, the leading treatments for cancers are surgery followed by chemotherapy and radiotherapy. These are all costly methods that a lot of times do not lead to a sure result because there are recurrences after a period of time. For this reason, the technical focus of this project is aimed at finding a novel gene therapy approach to targeted treatment of glioblastoma (a form of brain cancer). The STS research project aims to look into the social factors that affect the cost and accessibility of healthcare.

Technical Topic: Engineering Suicide Gene Approaches to Improve Chemotherapeutic Response in Glioblastoma

I currently work in a lab that works on different cancers to determine new methods of detection and treatment. Over the last two and a half years I have worked on a project that focuses on glioblastoma, which is the most common brain cancer in adults, and it is uniformly lethal (**2**). Existing therapies, such as surgery followed by chemotherapy and radiation, fail to prevent disease recurrence, and new treatment approaches are urgently needed (**3**). Suicide genes are a potentially promising approach for glioblastoma wherein viruses are used to transduce tumor cells to express a foreign protein that converts a prodrug into a toxic product, resulting in cell death. The focus of my project is to engineer a suicide gene that cooperates with temozolomide chemotherapy in glioblastoma. Our design is based on herpes simplex virus thymidine kinase (HSVtk), which converts the prodrug ganciclovir to a toxic phosphorylated

form. We are creating versions of HSVtk fused to PEST sequences (domains rich in proline, glutamate, serine, and threonine that control protein turnover) that we predict will be stabilized by p38, a kinase activated by the stress of temozolomide-mediated DNA damage. The ability of these next-generation suicide genes to augment the effects of chemotherapy and the molecular mechanism involved was studied in glioblastoma cells using immunoblotting, RT-qPCR, immunofluorescence microscopy, and flow cytometry. The results of this work will allow for post-translational control of a suicide gene with the combination of chemotherapy drugs.

The previous suicide gene design developed by our lab utilized a fusion of HSVtk to the PEST domain from FRA1, a phosphorylation target of ERK. PEST domains generally regulate protein stability through post-translational modification (**4**). Our new designs have PEST domains which are predicted to be phosphorylated by p38, a kinase activated in response to cellular stress, which can be induced by treatment with the chemotherapy drug temozolomide. The goal of this approach is to show efficacy of combined chemotherapy-suicide gene therapy. In Figure 1, I have provided schematics to better understand the signaling pathways that are used.

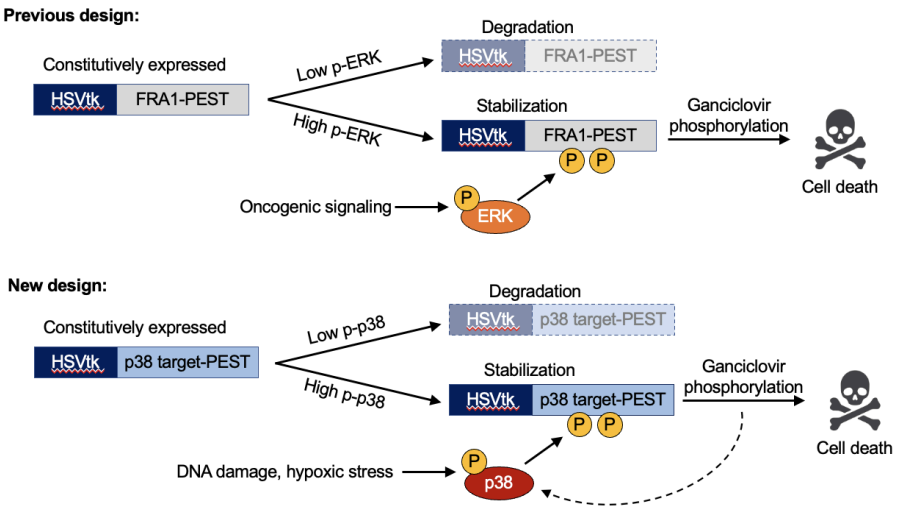

Figure 1. Schematic to represent the pathways that are being targeted by the designs.

I have currently designed four different suicide genes. The four designs tested included peptide sequences from the MAPK targets CHOP, MEF2A, p21, and FRA1. Schematics of each design are shown in Figure 2, with the predicted PEST domains and relative position of serine residues demonstrated in the literature to be phosphorylated by p38 (yellow circles). Sequences shown in blue were taken from proteins primarily phosphorylated by p38, while domains in gray were taken from the original FRA1-PEST design. The ERK recognition sequence was removed from these designs to improve specificity toward p38.

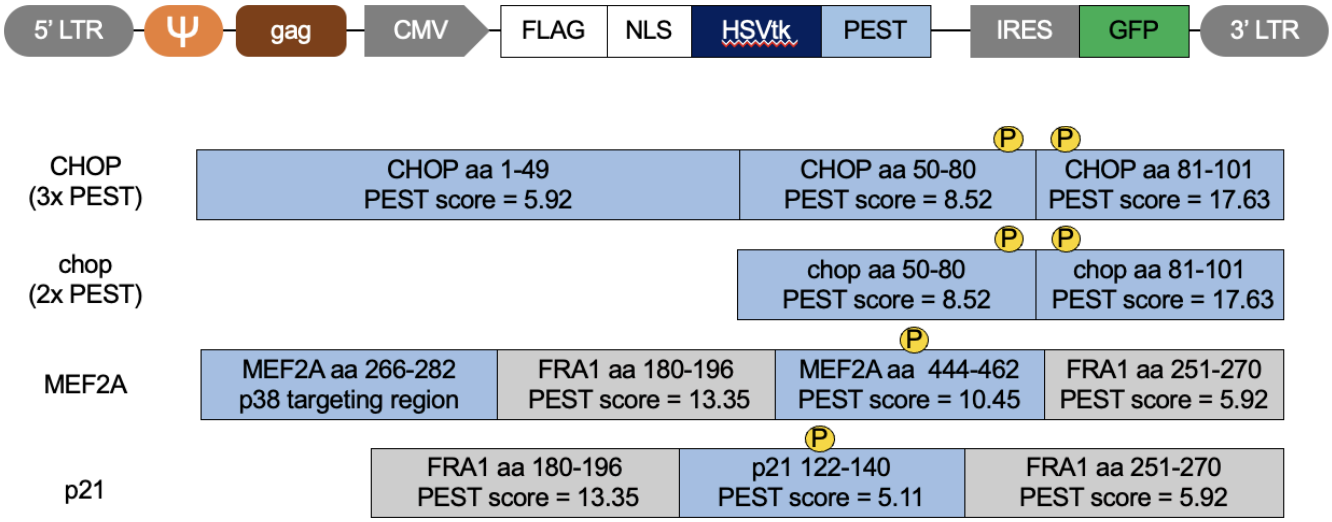

Figure 2. Schematics to show the suicide gene design as well as specific PEST domains.

Next steps for this are to determine the effectiveness with combination therapy. We have passed the first hurdle of design, but next is to test and screen whether the gene works in the fashion it has been designed to work in. This is an independent project, so I will work on this through different testing methods with the aim of validating our gene being a stress induced suicide gene which can help with targeted therapy of glioblastoma.

<u>STS Topic:</u> Consumer Access in the Field of Medicine

Introduction

Consumer access to healthcare is a topic of much interest especially in the United States. Many say that current treatments for different diseases are priced too highly to be able to be accessible to all that need it. The main focus of this STS research paper is to look into consumer access in the field of medicine from the perspective of the sociology of scientific knowledge STS framework. The sociology of scientific knowledge (SSK) framework focuses on "science as a social activity" specifically the social implications of scientific growth (**5**). This is an appropriate framework to use because with new treatments and therapeutic approaches to treat certain diseases there is the concern for the social effects on this. In 2021, 27.2 million people in the United States did not have health insurance, which is necessary to help with the costs of medical care (**6**). For the thesis my plan is to understand the cost and profit ratios for the pharmaceutical companies as well as the consumer costs with or without insurance. SSK advocates would say that with the growth of scientific knowledge and with advancements in the field it is important to consider the social implications and those that are affected on a personal level such as the consumers of the treatments.

Cost and Profitability of Pharmaceutical Companies

As discussed throughout this research paper one of the leading diseases in the United States is the many different types of cancers. The leading therapy methods for treatment consist of the use of chemotherapy drugs as a first line treatment method. The current cost on average for a single treatment of a chemotherapy drug is approximately $10,000 (**8**). It has been shown that annual treatments can cost above $100,000 and have led people to declare bankruptcy just because they need the treatment that may be able to save their lives. These costs are for already approved FDA treatments, but interestingly there is a very miniscule difference between in-class

drugs, approved and in market drugs, versus novel drugs, recently FDA approved drugs (**7**). The prices for these drugs have actually increased substantially over the past several years. Imatinib, a leukemia chemotherapy drug, which has helped increase the 10 year survival rate from 20% to 85%. However, the price increase over the past few years had been $30,000 in 2001 but increased to $92,000 in 2012. As quoted by the Journal of Clinical Oncology, "a prime example of a drug being worth as much as it can be sold for" because there has not been much change in the drug itself however they have been able to markup the price by so much more (**8**). Using the SSK ideas, they would talk about how this is negatively affecting society because there is no advancement in scientific technology, however, there is still an increase in cost.

Most profit margins in non pharmaceutical companies averages around 7.7%, however, for pharmaceutical companies it is an average of 13.8% (**8**). Pfizer, which is a major pharmaceutical company, actually has a profit margin of approximately 42% (**9**). These huge profit margins is something that is an isolated issue to the United States. In the United Kingdom there was an uproar from the public when pharmaceutical companies were going to have an increase in profit margins from 4% to 8%, however the treatments used in the United Kingdom are the same as the ones used in the United States. It is interesting to think about why the cost for the same drugs is so much more in the United States compared to internationally. Some SSK theorists would claim that it may be due to the great amount of innovation and discovery that occurs in the United States compared to internationally which is why they need the costs to be higher to help fund the next innovations. However, the social impacts of the higher costs is something that seems to not be considered for the American public. There are approaches that can be taken to improve the issue of price gouging in the United States. One method could be to have the government get involved and put caps on the profitability of medical treatments and

care. There may also be a method to make sure prices begin to decrease if a treatment has been in the market for a certain amount of time. This would be to promote the increase in pushing for new scientific discoveries.

Accessibility to Affordable Health Care

Most healthcare in the United States is through private insurance companies which allows for them to have high rates for care on top of the monthly payments that must be made. There is also government funded Medicare and Medicaid to allow for affordable healthcare, however there is still a huge amount of out of pocket spending that occurs in this option. Approximately 20% of the monthly income of retired Americans is actually used on healthcare even though it is government funded (**10**). It is also an approximate of 27.2 million American citizens that are uninsured and this causes them to not be able to afford basic medical treatments. In contrast, internationally countries such as the United Kingdom have universal healthcare for all which allows for everyone to have equal access to healthcare. It is also interesting that many Americans live in constant fear of not being able to afford treatment even with healthcare, because there are so many fine prints that would lead them to not pay for the treatments.

Another important aspect of healthcare is the fact that Medicare will actually pay for all cancer treatment 100%. This can be good for those who are covered to be able to get treatment, however, this can lead to pharmaceutical companies to increase their price of treatments knowing they will still get paid. This is a disadvantage for those who are either uninsured or have private insurance that will not cover the treatments, because these folks are not able to afford the treatments that have these new increased costs. There is also a lack of regulation of price gouging in the United States which allows for uncontrollable increase in therapy prices with no one to make them decrease them. The SSK theorist would say that without proper regulation

allowing companies to increase prices affects the society negatively without any new research being done to find new treatments.

Research Methods:

For the technical portion of this paper I will be working with Dr. Matthew Lazzara to continue to test and change my suicide gene designs to hopefully create a gene that is able to be used for targeted glioblastoma treatment. I will also work hand in hand with a UVA neurosurgeon to understand current treatment methods for cancer and how I could use them in my favor when designing my genes. I will be helped by graduate student William Hart to assist with techniques I am not trained to conduct due to additional training needed.

For the STS portion of this paper I will refer to studies that have been referred to earlier in the paper that outline the discrepancies in profit margins for pharmaceutical companies. I will also be using the *Consumer Access to Health Care: Basic Right 21st Century Challenge* to understand healthcare and insurance in the United States. I will be able to use this literature to further my understanding of why insurance costs are so high and cause healthcare to still be so unaffordable for many Americans.

Conclusion:

The main goal of the technical portion of this paper is to find a treatment method for glioblastoma cancer. However, a long term goal would be able to find other cancers that have similar pathways that can be targeted and the same suicide gene can be effectively used for them as well. The main goal for the STS component is to fully understand the issues in healthcare accessibility for Americans through the lens of sociology of scientific knowledge framework and the ethics around the current healthcare system.

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