Developing EGFR-Targeted Nanoliposomal Therapeutics in Head and Neck Squamous Cell Carcinoma

(Technical Paper)

Assessing How to Best Approach Patient Gene Therapy Education Through a Case Study of Vaccines

(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

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Introduction:

Since 2000, nearly twenty "new chemical entities" on average are discovered or developed each year by pharmaceutical companies across the globe (Daemmrich, 2009). In addition to these "new drugs," hundreds of potential therapies are also discovered and researched, taking advantage of novel applications of existing drugs or combining multiple existing drugs to create synergistic treatments and drug cocktails. Discovering and producing effective pharmacological for the patients that need them has never been the major barrier; it is the effective delivery of these agents *in vivo* that poses the biggest challenge for physicians, researchers, and engineers alike. Many drugs show success in laboratory cell culture models, but, when tested in humans, are filtered out of the bloodstream too quickly, accumulate in the liver instead of reaching their desired target, or, even worse, have unintended and dangerous side effects (Chung et al., 2008). Even if the challenges of drug discovery and delivery are surpassed, effectively educating patients and their families of the safety, potential risks, and proper administration of these treatments, especially with respect to vaccines, and in the near future, gene therapies, remains a roadblock. Through all of the many technical and social challenges, the overarching question arises: how can the engineering design and public perception of different drug delivery mechanisms impact their effectiveness and widespread use?

Technical Research Project:

With approximately 600,000 new cases diagnosed each year, Head and Neck Squamous Cell Carcinoma (HNSCC) is the seventh most common form of cancer worldwide. Patients diagnosed with HNSCC exhibit a 61% 5-year survival rate, which drops to 50% at the 10-year mark (Mao, Hong, & Papadimitrakopoulou, 2004). Despite the large volume of cases and poor outcomes for patients, only one targeted therapy has been developed specifically for patients

with HNSCC. The only major molecular target that has been identified in HNSCC is the Epidermal Growth Factor Receptor (EGFR). Cetuximab (Erbitux), the only FDA-approved therapy for HNSCC patients, is an antibody that inhibits EGFR's ability to bind Epidermal Growth Factors (EGF), blocking the receptor's cell proliferation signal. Cetuximab alone has shown some success in treating HNSCC; however, drug resistance has been common and its efficacy has been inconsistent (Stanam, Gibson-Corley, Love-Homan, Ihejirika, & Simons, 2016). The shortcomings of Cetuximab highlight the need to develop new targeted therapies for patients with HNSCC.

The Kester Lab at the University of Virginia has developed a therapeutic called the Ceramide Nanoliposome (CNL), consisting of the death-inducing lipid C6-ceramide encapsulated inside a spherical, lipid-bilayer delivery vehicle, which is currently in clinical trials as a single agent for treating several different cancers (Stover & Kester, 2003). The CNL alone has shown strong efficacy in treating HNSCC *in vitro*, and prior studies have demonstrated that the CNL can sensitize cancer cells to other standard chemotherapeutics when added in combination (Adiseshaiah et al., 2013; Jiang et al., 2011; Myrick et al., 1999; Sok et al., 2006). Under this rationale, this capstone team has discovered that the CNL can sensitize HNSCC cells to two EGFR inhibitors, Erlotinib and Gefitinib, that had both previously failed HNSCC clinical trials as single agents (Perez et al., 2012; Soulieres et al., 2004). The combination of CNL and each of these EGFR inhibitors demonstrates a synergistic cell death response that is greater than the sum of the solo effects of both drugs in the combination. This discovery provides a strong rationale that the combination of the CNL with Cetuximab may produce a similar effect, as Cetuximab is also an EGFR inhibitor. This project aims to exploit the above findings to prepare

three novel drug combinations for use in HNSCC mouse models: CNL + Erlotinib, CNL + Gefitinib, and CNL + Cetuximab.

Both Erlotinib and Gefitinib will be encapsulated individually in similar delivery vehicles as the CNL. Encapsulation will improve the drug's retention time in an organism's circulatory system, shield the drug from host immune system detection and destruction, and facilitate the targeted delivery of the drug specifically to a HNSCC tumor (Lu et al., 2006; Ranson & Wardell, 2004). A different delivery system will be constructed for Cetuximab, chemically attaching the drug to the surface of the CNL. As Cetuximab binds very specifically to EGFR, which is located on the surface of cells, its attachment will help discriminate between HNSCC cells and healthy cells, as HNSCC cells have higher surface quantities of EGFR. As an antibody, Cetuximab will also mobilize the host's immune system against the cancerous tissue upon binding EGFR (Pozzi et al., 2016). The combinations of CNL + encapsulated Erlotinib, CNL + encapsulated Gefitinib, and CNL with Cetuximab attached to its surface will all be tested in HNSCC mouse models. By the conclusion of this project, the team aims to have laid the groundwork on producing novel drug delivery systems and treatments for patients diagnosed with HNSCC.

STS Research Project:

Gene therapies have shown tremendous promise in treating a variety of diseases, mainly those that are hereditary. The majority of gene therapies take advantage of a virus's ability to target and inject its viral genome into specific cells, reprogramming its malicious intent to inject healthy copies of a gene and restore the normal function of a diseased tissue. In 2017, the FDA approved Kymriah, better known as CAR T-cell therapy, as one of the first gene therapies available to patients. Kymriah involves first extracting a subset of a patient's white blood cells called T-cells, then using a virus to inject genes into the T-cells that allow them to recognize

cancer cell-surface markers, after which the modified T-cells are injected back into the patient to treat leukemia (Miliotou & Papadopoulou, 2018). Kymriah represents only one example of how gene therapies can change how diseases are treated, and at this very moment, thousands more gene therapies are progressing through clinical trials.

With their potential to target diseases that traditional medicine can never fully reach, the public attitude towards gene therapies has been nothing but optimistic. However, in light of this optimism, it is easy to gloss over one simple, but important fact (which forms the basis for vaccine development): viruses are fundamentally dangerous. Nearly all genetic diseases impact a patient throughout their entire life, and gene therapies will likely be expected to do the same (Verma & Somia, 1997). Yet, the technology to virally transfer genes inside an organism has only existed for 30 years, and FDA-approved gene therapies have existed for two years, so how are scientists to know the long-term impact of artificially produced and inserted viruses and genes on the human body? In order to reprogram viruses, scientists edit out all of the malicious genes and replace them with therapeutic genes (Verma, 2013). But in a society that still believes that vaccines cause diseases rather than prevent them, how can it be expected that patients and their families not show even more skepticism and pushback to willingly injecting live viruses into their body? Even more worrisome, despite the risks associated with gene therapies, the FDA has recently removed regulations and cut red tape to loosen restrictions on gene therapies because of their therapeutic efficacy in an attempt to allow more to reach patients faster (Patel, 2018). While gene therapies have the potential to change the way medical professionals approach diseases, the many questions and unknowns still surrounding their use, most of which are scary from a patient's point of view, prompts the following question: how can both the risks and rewards of gene therapies be most effectively communicated to patients as to ensure they receive

these life-saving treatments?

STS Research Methods:

To answer the above question, a case study of vaccines will be conducted to determine how best to approach educating patients about the medical treatments they are receiving. Vaccines serve as an excellent case study because, similarly to gene therapies, vaccines were once the revolutionary medical treatment, helping eradicate diseases such as smallpox, measles, and polio from the United States during the 1900's (Burton, 2002; Praderio, 2019). However, despite their success, fraudulent research reports, public mistrust of "Big Pharma," among other factors tainted the relationship between vaccines and patients. Preliminary research has determined that the propensity for a patient to agree to receive a medical treatment is strongly correlated to their level of trust in the treatment (Dudley et al., 2018). In order to assess what factors are involved in gaining a patient's trust, a study and analysis of vaccine education will be used to identify relevant social groups that influence a patient's trust in the vaccine they are receiving. Preliminary research has identified many social groups that do this, including clinicians, pharmaceutical companies, biomedical researchers, and insurance companies. Further research and analysis through intensive literature review will be conducted to determine how each one of the social groups interacts with the others and how that can impact the patient's trust in a vaccine, culminating in an actor-network model describing patient trust. The social groups relevant to vaccines will be compared with the gene therapy social groups and actors identified in the section above to assess how well they overlap. The actor-network model for patient trust in vaccines will then be evaluated for how well it can describe the network of factors that impact a patient's trust in gene therapies, while also considering the technical similarities and differences between vaccines and gene therapies. Ultimately, the goal of this research will be to develop a

model to describe the factors that impact a patient's trust in gene therapies as to suggest strategies to gain that trust and properly educate patients about the gene therapies they would receive.

Conclusions:

The technical arm of this project aims to develop and test targeted drug delivery systems in head and neck squamous cell carcinoma (HNSCC) models. This technical project has the potential to establish the foundation for a new generation of HNSCC-targeted therapies and give more treatment options to a patient population that currently has few. It is anticipated that all drug combinations and delivery vehicles outlined above will be developed and tested successfully *in vitro*, but the outcome of the *in vivo* studies is much more difficult to predict. The STS arm of this project aims to investigate how to best promote patient gene therapy education through the lens of a vaccine case study. Patient trust is incredibly valuable, and by using vaccines as a model, patient education strategies can be employed to optimize that trust and ultimately get patients the treatments they need. With all of the obvious similarities between gene therapies and vaccines, it is anticipated that the actor-network model developed from the vaccine case study will be an accurate representation of that for gene therapies; however key differences between vaccines and gene therapies, including the recipient patient population for each, as well as the treatment regimen of each, will serve to differentiate the two models. Overall, this study in its entirety attempts to further analyze how engineering design and patient trust of different drug delivery mechanisms can impact their effectiveness and widespread use.

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