The Effect of Hornerin Knockdown on Tumor Vasculature in Melanoma
(Technical Paper)

The Advancement and Shortcomings of Ethics in Clinical Research
(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

With over 100,000 new melanoma diagnoses each year, the need to elucidate novel therapeutic approaches holds significant weight in the research field (*Melanoma Skin Cancer Statistics*, n.d.) One of the hallmarks of melanoma, and a myriad of other cancers, is the disordered tumor vasculature. In response to this pathological morphology, many current therapeutic approaches aim to target the disordered vessels and normalize their characteristics. As such, characterizing the tumor vessels in comparison to the normal vessels provides crucial insight into therapeutic efficiency. Furthermore, identification of hornerin, a vascular endothelial growth factor independent protein, as part of the angiogenic pathway, presents an avenue for development of a novel therapeutic (Gutknecht et al., 2017). The specific interplay, however, between targeting hornerin, normalizing vessels, and any subsequent direction of T-cell entry, specifically, in melanoma has yet to be entirely understood. Therefore, the technical research study will optimize an existing vessel analysis software to understand the effect of hornerin knockdown on vessel normalization and subsequent impact on T-cell entry into the tumor region.

The field of targeted therapeutics, and more broadly speaking, clinical research, has evolved over time with growing scientific and technological advancements. The closely related study of research ethics has also experienced large amounts of change, especially over the past eighty years. Ethical guidelines in clinical research with human subjects serve to protect patient volunteers and preserve the integrity of the science. Many of the ethical guidelines in place today were established in response to past abuses, lending to influential codes of ethics, such as the Nuremberg Code and Declaration of Helsinki, to name a few. Using these documents as the foundation of conducting research with human subjects, principles such as informed consent and

scientific validity continue to guide the field today (*NIH Clinical Center: Ethics in Clinical Research*, n.d.). As such, the second research study will focus on how the role of ethics in clinical research has evolved over time due to changing cultural and political climates and argue for a longitudinal discussion of the social dimension of science and innovation.

Technical Topic

The human body is an incredibly complex system, which presents a challenge in investigating pathological conditions such as cancer. Cancer is one of the leading causes of death in the United States with approximately two million new diagnoses and 600,000 associated deaths each year (*Cancer Statistics - National Cancer Institute*, 2015). In particular, there are about 100,000 diagnoses and 7,000 deaths each year due to melanoma, a form of skin cancer (*Melanoma Skin Cancer Statistics*, n.d.). Cancer treatments aim to target cancerous cells and related pathways to halt cancer progression. One such pathway involves the coexistence of cancer cells with the surrounding blood and lymphatic vessel network. The generation and maintenance of this vascular network, known as angiogenesis, is essential for tumor growth and favors the spread of cancer, or cancer metastasis. The vessels promote this growth and spread by compensating for the tumor's high oxygen and nutrient demand (Folkman, 2010). Also, the cancer cells secrete high levels of pro-angiogenic factors, which contribute to the creation of a disordered vascular network characterized by immature and permeable blood vessels. The abnormal vascular network then further limits the subsequent immune response, as vessels are one factor that modulate T-cell, a type of immune cell, trafficking. (Viallard & Larrivée, 2017)

As such, tumor blood vessels are a key target for cancer therapeutic management, as anti-angiogenic therapies can normalize tumor vasculature to ultimately restore immune function. Many current therapeutics target vascular endothelial growth factor (VEGF), which is a pro-

angiogenic factor (Melincovici et al., 2018). These current cancer therapeutics have demonstrated effective results, but often yield temporary remission as the possibility of recurrence following treatment is highly likely with most cancers. Therefore, additional pathways that lead to vessel growth are crucial in effectively targeting the vascular network. Previous studies have identified a protein called hornerin, which is located on cells that line blood vessels and is independent of VEGF. Hornerin is part of a compensatory pathway in angiogenesis. Knockdown of the hornerin protein, in studies concerning pancreatic cancer, resulted in normalization of the vessels and reduced tumor burden (Gutknecht et al., 2017). Additionally, hornerin is expressed in psoriatic and wounded skin, elucidating its potential in melanoma treatment (Takaishi et al., 2005).

For analysis of therapeutics, such as those that result in hornerin knockdown, comparing the characteristics of tumor vasculature to normal vasculature can provide an understanding of the drug efficacy. The Rapid Analysis Vessel Element (RAVE) software is a vessel analysis software that was introduced almost a decade ago (Seaman et al., 2011). RAVE performs analysis of vascular environment images with a few variations introduced. An image is uploaded to the software, which performs several operations to compile and output vessel parameters such as vessel volume fraction, vessel length density, vessel radius and fractal dimension. Although operational, RAVE only has the ability to analyze one image at a time, limiting the efficacy of large-scale analysis due to the time-consuming nature of the program. Additionally, the software lacks a single data output function as well as the capacity to copy and paste. Therefore, by the end of the academic year, the capstone team will deliver an optimized RAVE software. The deliverable will include characteristics such as batch processing and a more user-friendly output, such as a single, exportable file containing all the vessel parameter data. Further, the technical

research project will upgrade the existing RAVE software by incorporating an algorithm to automatically set appropriate thresholds for individual images. This algorithm will reduce the processing time for multiple images and reduce potential variations produced by manual threshold entry.

The proposed research project will offer an optimized therapeutic screening software that presents an effective way to screen new therapeutics directly targeting the tumor vasculature. The software will specifically be used to test the effect of a hornerin targeting therapeutic in melanoma; however, it has the potential to serve as a tool for vascular network analysis in a wide range of cancers.

STS Topic

Scientific research, whether concerned with individual cells or human bodies as a whole, presents a range of ethical guidelines to consider. For hundreds of years, clinical breakthroughs have altered the face of science, though many times without the human subjects' interests in mind. Developed in the aftermath of World War II, in response to the abuse of human subjects in non-therapeutic research by Nazi physicians, the Nuremberg Code was the first international code of ethics for research on human subjects to be adopted (Resnik, n.d.)**.** The code intended to guide future human experimentation; however, unethical experiments continued to take place at major academic institutions in the United States in the years after World War II (Lefor, 2005). Over the coming years, additional codes of ethics were formulated to supplement the Nuremberg Code, each one in response to unique cultural and political climates to ultimately shape how researchers conduct their studies today.

As stated, the Nuremberg Code was the first international code of ethics for research on human subjects. The Code was formulated around 70 years ago, in August 1947, in Nuremberg,

Germany, by American judges sitting in judgment of Nazi doctors accused of conducting murderous and torturous human experiments in the concentration camps (Ghooi, 2011). The ten basic principles enumerated in the Code yielded a blueprint for today's principles to ensure the rights of subjects in clinical research. Of the ten principles, the most crucial asserted the necessity of informed and voluntary consent (Shuster, 1997).

Twenty years later, the Declaration of Helsinki (DoH) provided a slightly reformed understanding of research ethics in relation to its predecessor, the Nuremberg Code. Originally published in 1964 by the World Medical Association, the DoH represented a subtle shift in balance between the responsibilities of the research to individual research participants and that of public health. In particular, though the requirement to obtain the informed consent of participants was absolute in the Nuremberg Code, the DoH softened such requirements to allow for research on children, especially for vaccine development (*WHO The Declaration of Helsinki and Public Health*, n.d.) Regardless of this shift, the basis of the DoH was to safeguard the interests of human subjects.

With the Nuremberg Code and Declaration of Helsinki redefining the research ethics field, society continues to see the documents' legacy in the conduct of research today. Using these sources, and several others, seven ethical guidelines guide its conduct. These include social and clinical value, scientific validity, fair subject selection, favorable risk-benefit ratio, independent review, informed consent, and respect for potential and enrolled subjects (*Seven Requirements for Ethical Clinical Research*, 2013).

Though the ethical guidelines in research ethics have evolved over the years, the focus of these principles has centered on the human subjects. However, several other actors and artifacts, such as physicians, researchers, and the ethical codes themselves, have shaped our current

conduct of research. The actor-network theory (ANT) thus provides a framework that analyzes the interplay between these human and non-human actors. The actor-network theory is a methodological approach to analyze how social effects are generated as a result of associations between different actors in a network (Cresswell et al., 2010). Though it offers a broad analysis of actors, critiques of the theory have arisen. One such criticism is that the ANT may imply that all actors are of equal importance in the network. This may limit the influence of one actor over another, skewing or falsely attributing one's true and accurate impact. Additionally, as the actors and artifacts are defined by the researcher themselves, the ANT requires judgement calls from the researcher as to which actors within a network are important. This decision on behalf of the researchers may limit the analysis to only a few actors or, again, incorrectly attribute an actor's impact on a network as more significant than it is in reality (*Criticism of Actor-Network Theory*, 2010). Despite these intrinsic limitations, the ANT provides for a network analysis that accounts for interplays between human and non-human actors in the social shaping of society.

Though much of the intricacies of clinical research is hidden from the public eye, the developments from these investigations often have profound implications in society as a whole. Without the participation of human subjects, the validity of clinical research would fall flat. As such, considering how clinical research is ethically conducted, considering the subject's interest is of utmost importance. Recognizing the historical contours of how clinical research is conducted today further highlights how research ethics has been, and continues to be, in constant flux.

Methodologies

Research Question: How has the role of research ethics evolved over time due to unique cultural and political climates?

To address the research question, I will utilize documentary research methods, historical case studies, and conclude with a network analysis to provide a comprehensive discussion of the material. Beginning with background information, I will use primarily documentary research methods to provide the necessary context for the conduct of clinical research today. The documentary research methods will also aid in creating a larger narrative of research ethics through the study of multiple documents surrounding various events and individuals (Elder, n.d.). Specifically, I will first describe how ethics, if at all, shaped research prior to the publication of the Nuremberg Code. Then, I will chronologically continue to look at the changes in the ethical dimensions of clinical research by analyzing documents such as the Nuremberg Code and the Declaration of Helsinki. In the case of the Declaration of Helsinki, I will review previous versions and outline present forms of the text (Carlson et al., 2004). The Nuremberg Code and Declaration of Helsinki will be two of the primary sources constituting the historical case studies from different time periods. These two documents will also create the three time periods of interest in the research study: post-Nuremberg Code, post-Declaration of Helsinki in 1964, and present-day clinical research and research ethics.

Across the aforementioned time periods, the scope of my research will be narrowed down with the following keywords: research ethics, human subjects, ethical guidelines, and informed consent. These keywords will allow the research paper to form an underlying thread to compare the similarities and differences between how and what research ethics as a field presented as across the varying time periods. In addition to the mentioned historical case studies, I will also utilize a specific case study that portrays a more specific and real example of the implications of research ethics. The case study is the story of Henrietta Lacks, a 30-year-old African American woman who was diagnosed with an aggressive form of cervical cancer at Johns Hopkins

Hospital in 1951. Her tissue samples were taken during her treatment, and portions were passed along to researchers without her knowledge or permission, introducing an immortal cell line - labeled “HeLa” - that drastically altered the scientific field (Beskow, 2016). However, the ethical considerations, or lack thereof, in her case continue to have implications on how research is conducted today. Therefore, using the primary documents of Nuremberg Code and Declaration of Helsinki, the research paper will look at the story of Lacks through lenses across three time periods and how her story may have been approached differently during each of these times.

Lastly, I will utilize network analysis to identify the human and non-human agents involved in ethical decisions surrounding clinical research (*Network Analysis ScienceDirect Topics*, n.d.). Together, these research methods will support the basis of the research question in showing the unique evolution of the field of research ethics.

Conclusion

With many cancer treatments ending in recurrence of disease, novel therapeutics are constantly being developed. One valuable target in the tumor microenvironment is the vasculature, using anti-angiogenic therapies. The recent identification of hornerin as a protein involved in tumor growth presents promising clinical applications. As such, characterization and comparison between tumor and normal vasculature warrants a software such capability. The Rapid Analysis Vessel Software, optimized for batch processing with a user-friendly output, will allow researchers to screen novel drugs to test their efficacy in a broad spectrum of cancers.

On a broader note, given the importance of clinical research from understanding diseases to developing novel therapeutics, the human subjects and their ethical rights must always stand at the forefront. Though these rights have not always been present in the research field, researchers must incorporate such considerations to uphold the clinical research field and promote

innovation in an ethical manner. The ethical guidelines in place today have evolved from various documents of ethics, with the Nuremberg Code and Declaration of Helsinki being two of the most prominent in the field. How researchers conduct studies with human subjects today has been shaped by changing cultural and political climates, and this evolution can only be addressed with a longitudinal discussion of the research ethics field.

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