OPTIMIZING GAS AND LIQUID GRADIENT BIOREACTOR TO MIMIC TUMOR MICROENVIRONMENT

EVALUATION OF ADAPTIVE CLINICAL TRIALS FOR ONCOLOGY CLINICALS TRIALS

A Thesis Prospectus
In STS 4500
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Bachelor of Science in Biomedical Engineering

By
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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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Cancer is one of the leading causes of death globally. According to a study of cancer statistics of the United States by Siegel et al (2022), over 609,000 people are projected to die from cancer in 2022, along with 1.9 million new cancer cases projected to occur. Cancer is defined as the uncontrollable growth of mutated cells in the body (NCI, 2007). Cancer treatment is difficult as cancer cell targeting, drug resistance, metastasis, and treatment toxicity are factors that researchers must overcome (Chakraborty & Rahman, 2012). Improvements in cancer treatment, early detection, and lifestyle changes have led to a 32% decrease in cancer mortality rates in 2019 (Siegel et al., 2022). Nevertheless, new treatments and methods are clearly needed in order to further decrease the death toll of cancer.

The technical project and tightly coupled STS research project proposed in this prospectus directly addresses this issue. The objective of the technical project is to design and create a novel bioreactor capable of generating real-time tunable gradients of gas and liquid concentrations simultaneously for cancer cell culturing. By designing such a device, the cellular conditions that cancer cells encounter can be accurately modeled and therefore improve the cellular, *in vitro*, characterization of cancer behavior. Furthermore, the device would expedite cancer drug development by allowing for rapid testing of various cellular conditions and responses from treated cancer cultures to determine drug efficacy and toxicity. However, developing *in vitro* tools for improving drug development is not enough to ensure successful transition to clinical use nor is it sufficient to guarantee safe treatment in cancer patients. Thus, understanding the regulatory pathway for oncology therapeutics is critical to ensure that effective drug development and its clinical transformation, the transition of developing drugs to medical use, is possible.

The paired STS research project will investigate this by examining the development of the recently adopted adaptive clinical trial and their potential risks and benefits in oncological clinical transformations. Both the technical and STS projects will be accomplished during the Fall 2022 and Spring 2023 semesters following the prospective Gantt chart as seen in Figure 1. Dates within the Gantt chart are subject to change with the final product consisting of the completed technical and STS research papers.

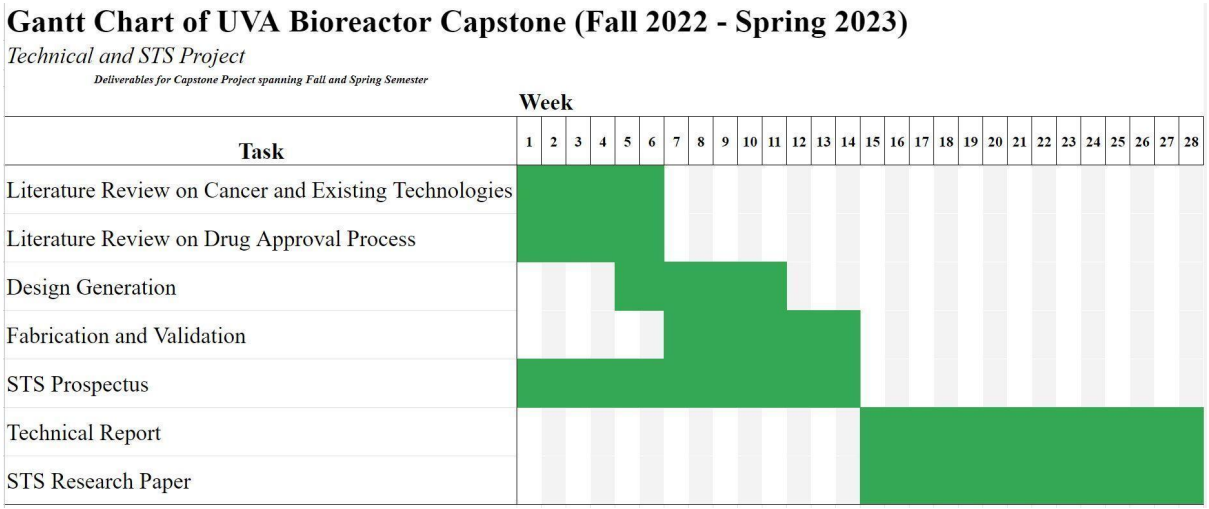

Figure 1. Gantt Chart of UVA Bioreactor Capstone. Prospective timeline and milestones for the Technical and STS projects. (Jian, 2022).

Optimizing Gas and Liquid Gradient Bioreactor to Mimic Tumor Microenvironment

A major barrier in cancer research is that preclinical findings may not translate to clinical results. According to a statistical study by Wong et al. (2019), the total overall success rate for all therapeutic categories of drugs to pass clinical trials is 13.8%. Success rates for drugs to pass clinical trials do vary per year, but the success rate for oncology drugs to pass clinical trials is consistently lower, around 3.4%. Common causes for cancer drug failure in clinical trials are a lack of efficacy and off-target toxicity (Lin et al., 2019).

These failures of oncology drugs can be partially attributed to inaccuracies found during preclinical research. Preclinical research relies on *in vitro* followed by *in vivo*, testing with living subjects, studies to evaluate safety and efficacy of drugs before transitioning to clinical trials (FDA, 2019). Oncological preclinical drug studies are often poorly reproducible as these studies rely on poorly characterized tumor cell lines, do not accurately model the tumor environment or development, and rely on problematic procedures (Begley & Ellis, 2012). In order to improve the development of cancer drugs, *in vitro* studies must be improved by increasing the accuracy and reproducibility of these studies.

Cancer can appear in any part of the body, where the primary cause of cancer death is by metastasis (Sleeboom et al., 2018). Metastasis is the spread of secondary tumors in surrounding tissues and distant organs. The tumor microenvironment (TME), which is composed of the dynamic cellular and noncellular components surrounding the tumor, plays a critical role in the development of metastasis (Wang et al., 2017). Oxygen is an especially critical component of the TME as tumor hypoxia, low levels of oxygen, contributes to tumor resistance towards standard cancer treatments and metastasis (Byrne et al., 2014; Muz, B. et al., 2015). Traditional 2D tissue culturing methods, which subject cells to static conditions, are therefore unsuitable for accurately modeling the dynamic TME and cancer cell development. One method of accurately modeling the TME would be through the use of the 3D cell culturing technique known as organ-on-chips (OoCs). OoC culturing uses microfluidics to accurately mimic the physiological and cellular conditions of organs (Low et al., 2021). Furthermore, OoCs have been successfully used in drug development and discovery applications (Low et al., 2021). By developing a high throughput OoC that can mimic the TME, the characterization of anti-cancer therapeutics will significantly improve allowing for better translation to clinical applications.

The ultimate goal of this project is to design and validate a novel bioreactor that will accurately model the dynamic TME. As seen in Figure 2, this will be accomplished by combining two techniques of generating gas and liquid gradients such that simultaneous delivery of varying concentrations of gas and liquids to cultured cells will occur. The liquid gradient will be generated by pumping two different concentrations of culture medium and other liquids through the two liquid gradient inlets. The gas gradient will be generated using tanks of varying mixtures of oxygen, nitrogen, and carbon dioxide at each gas gradient inlet. As the delivery of both gradients relies on pumping, real-time adjustments of liquid flow rate and gas pressure are possible. The specific aims of this project are (a) to design and create a bioreactor capable of subjecting cell cultures simultaneously to liquid and gas gradients; (b) validate the gas and liquid gradients generated; (c) characterize cancer cell behavior through culturing within the bioreactor.

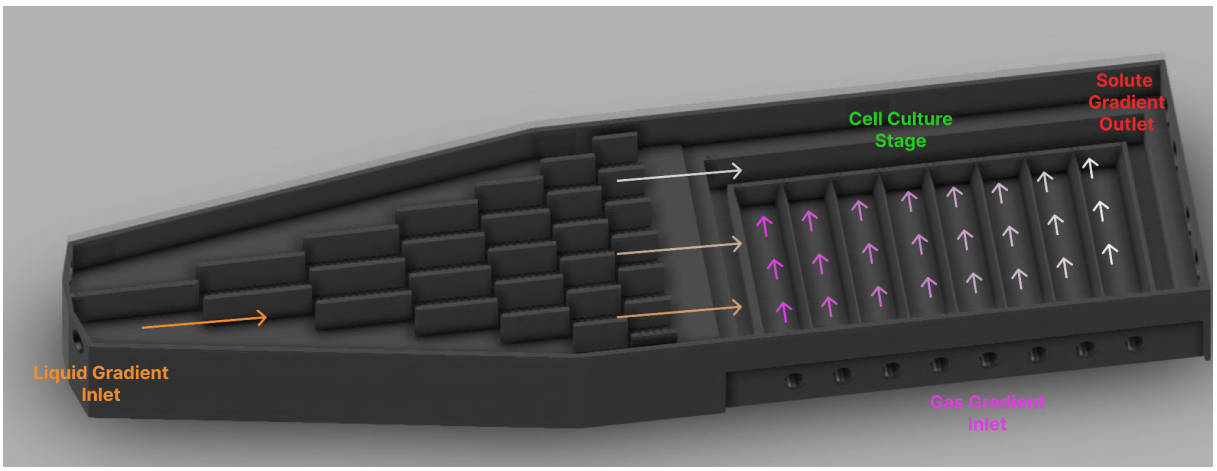

Figure 2. Functional Diagram of Dual Liquid Gas Gradient Bioreactor. Design subject to change. The resulting stage will contain a layer of adhered cancer cells subject to liquid and gas gradients simultaneously. (Jian, 2022).

This technical project is an extension on a previous technical project by Evan Clark, Emma Lunn, and Elizabeth Wood in 2021 (Clark et al., 2022). They were able to successfully create a prototype of a dual gradient bioreactor, perform validation testing on the generation of gas and liquid gradient, and perform cell culturing. Thus the previous designs by the 2021 team

will be further iterated on to achieve the goals of the project. Members of the 2022 team include Samantha Pugh, who is also a fourth-year undergraduate student studying Biomedical Engineering at the University of Virginia School of Engineering and Applied Science. The bioreactor will be designed, built, and tested under the guidance of Thomas Gennetta, an assistant professor in the Department of Radiation Oncology at the University of Virginia School of Medicine. The expected product of this project will be a fully functional dual liquid and gas gradient generating bioreactor for cancer cell culturing. The project will be documented in an academic paper.

Evaluation of Adaptive Clinical Trials for Oncology Clinical Trials

The probability of a successful translation of drugs to clinical applications is around 13.8%, however without factoring in oncology drugs this overall probability jumps to 20.9% (Wong et al., 2019). Cancer drugs have been known to have lower rates of successfully passing clinical trials, about 3.4%. Part of the reason why this drop in success rate occurs is that many drugs that enter clinical trials are developed with poorly reproducible studies, are misreported, or are falsified. On December 7th, 2021, the Reproducibility Project: Cancer Biology (RP:CB) sought to replicate 50 select experiments from high-impact articles. According to RP:CB, only 46% of the reported effects from these select experiments were successfully replicated (Errington et al. 2021). This is not a new phenomenon, as in a study by Begley & Ellis (2012), of 53 replicated landmark cancer studies only six were successfully replicated. In this regard, the importance of clinical trials is demonstrated in preventing these prospective drugs from successful clinical transformation.

In 2010, a new regulatory pathway was accepted within the FDA to accelerate clinical trials (Wang, 2013). Known as adaptive design or adaptive clinical trials, these clinical trials allow for preplanned modifications to trial procedures and use of statistical analysis to generate information on drug safety and efficacy (*FDA*, 2019). Modifications can include, adjusting ratios of volunteers treated with and without the drug, dropping treatment formulations and groups, modifying criteria of evaluating drug effectiveness, relying on simulations to predict number of planned modifications, and many others. According to Catherine Montgomery (2017), a Chancellor's Fellow of science, technology, and society at the University of Edinburgh, the concept of adaptive trials began in the 1950s out of motivations to reduce the impact that randomized controlled trials could have on participants' well-being. There was an initial focus on decreasing the number of volunteers and amount of treatments applied to participants so that the harmful effects of drug testing could be minimized. Through the sacrifice of the few, the safety of a drug could be determined and save the many. This motivation eventually shifted towards the future which brought anticipatory concerns to the front. Now, there is a shift in focus on predicting the effects of drugs on patients to develop safer drugs and saving the pharmaceutical industry from the high costs of drug development. In order to evaluate the potential benefits and impacts of adaptive clinical trials for oncology drug development, this STS research seeks to examine the development of adaptive trials and potential problems and benefits that may arise with current adoption of adaptive design.

In order to accomplish this task, the frameworks of the Handoff model and Actor Network Theory (ANT) will be applied to analyze the transformation of drugs to clinical applications and the actors, as well as their interactions, within adaptive clinical trials. Baritaud and Carlson's (2009) theoretical science, technology, and society framework II, the Handoff

model, details the steps involved with the diffusion of technology, ideas, or artifacts. Between each handoff, the risks and tradeoffs, of both economic and societal, that each party encountered are detailed to showcase how an idea or technology comes to fruition in society. In the case of drug development, the Handoff model is an appropriate model given the regulatory pathway for clinical transformation shown in Figure 3. Drugs are discovered by scientists and pharmaceutical companies who invest time and money to characterize the effects of the drug as well as its efficacy, safety, and formulation. During this time questions like, what disease could be treated with this drug and how will the drug be delivered within the body are asked. The drug enters pending approval and then clinical trials where participants are needed to further evaluate the drug's efficacy and safety in people. Questions regarding patient demographics, compensation, and safety are some of the tradeoffs determined at this stage. The FDA then examines the data obtained by the trial and decides whether the drug has been sufficiently characterized and safe for clinical use. Patients purchase the newly approved drug to treat their disease. Each of these above steps contains a multitude of risks and requirements that need to be addressed and accomplished before complete progression of the Handoff model occurs.

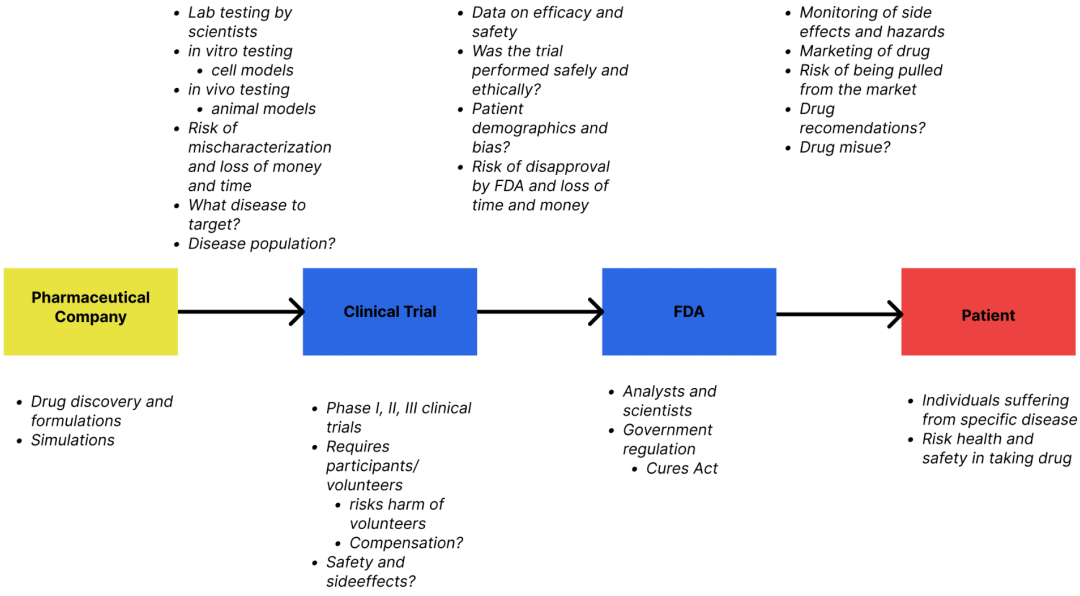

Figure 3. Handoff Model for Drug Clinical Transformation. Potential risks and tradeoffs between each handoff are listed. Adapted by Jian (2022) from Baritaud & Carlson (2009).

Another mode of analysis for the STS research project will be an ANT map of the interactions within adaptive clinical trials shown in Figure 4. First developed by Callon, Latour, and Law in the early 1980s, ANT maps the material, societal, and symbolic elements of a network to examine the relationships between them (Law & Callon, 1988). The main actors involved in this map are the pharmaceutical companies who develop and market drugs, the FDA who reviews clinical trials and monitors drug safety, and the patient who provides patient data and receives the drug for treatment.

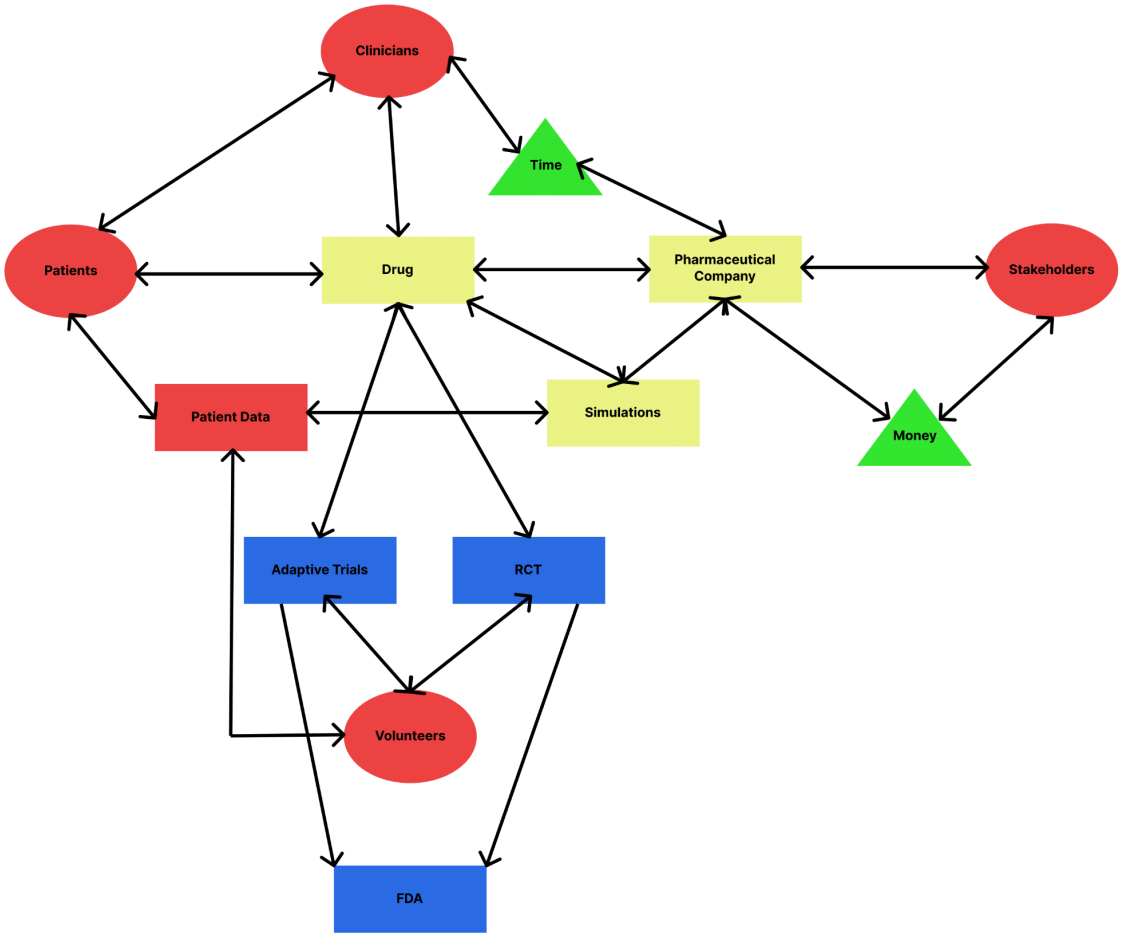

Figure 4. Actor Network Theory Map of Drug Clinical Transformation. Map subject to change. Analysis will reveal potential gaps and interactions between actors that adaptive trials may need to resolve in order for safe and widespread adoption. (Jian, 2022)

Through ANT analysis and further research, potential ethical and regulatory concerns will be determined between the different elements of the ANT network. By determining these concerns, it will be possible to recommend regulatory and procedural changes for affected elements within the network to ensure patient well-being with safe therapeutics from cancer drug development.

Improving Cancer Drug Development

The current methods of cancer drug development often suffer from inaccurate and irreproducible methods to characterize anti-cancer drugs. These drugs, justifiably, do not pass clinical trials due to toxicity and efficacy concerns. In order to further decrease the mortality rate of cancer, new and improved treatment methodologies and drugs need to be developed. To resolve this issue, pharmaceutical companies are attempting to accelerate drug development by using adaptive design trials which may not be sufficient to characterize drug safety and efficacy. Furthermore, the existing regulatory pipeline for drug development may be insufficient in regulating pharmaceutical companies from ethically developing safe and effective cancer-therapeutics. To answer these questions, this project seeks to improve the accuracy of cancer drug development and examine its regulation to ensure patients can obtain safe and highly effective anti-cancer therapeutics such that the mortality rate of cancer is further decreased. The two components of this research will employ different methodologies but will ultimately seek to improve the development of anti-cancer therapeutics to improve the wellbeing of cancer patients.

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