Interrogating the Cellular Impact of Sonodynamic Therapy on Glioma Cells

(Technical Project)

Searching for New Life:
Evaluating Modern Healthcare Models Using a Rawlsian Approach
in Pursuit of a More Equitable Solution for Cancer Therapy Accessibility

(STS Project)

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On my honor as a 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

I first met Joe - our house's hardworking, aging handyman – while he was in the midst of an unimaginably difficult time: his wife was battling breast cancer. For the past few months, I have watched as Joe balanced staying by his wife's side for her chemotherapy treatments – and frequent complications – with his need to earn money to fund her treatment, often working long days to cover the bills. His plight is especially eye-opening for me as a cancer researcher. I have spent the past year studying a newly developed therapy strategy – 5-ALA-mediated sonodynamic therapy (SDT) – that promises to contribute significantly to the treatment of glioblastoma, an aggressive and deadly form of brain cancer. This research is now the crux of the technical capstone project described in the following pages. My team will endeavor to improve the tools used to study this therapy in the research lab in the hopes of yielding more reliable experimental results. Additionally, efforts will be made to characterize cell death and the secretome, eventually using these results to optimize treatment parameters. The systematic study of 5-ALA SDT on glioblastoma will contribute to yielding a more optimized and efficacious treatment for patients and lay the groundwork for future augmentation.

Lingering in the background throughout these technical project efforts, though, has been Joe's story. It was simultaneously heartening to see the devotion Joe showed to his wife throughout her continuing cancer treatment and disheartening to witness the economic realities of cancer treatment. The development of new cancer therapies, from a scientific standpoint, only stands to benefit patients. From a practical and sociological standpoint, however, the benefits are less clear. Healthcare costs have consistently outpaced economic growth for the past fifty years, with the cost per capita in the U.S. standing at $10,739 as of 2019 (Laviana et al., 2019). Cancer treatment cost increases have perhaps been the most acute, even as household incomes have barely changed (or

even regressed in some years) (Federal Reserve Bank of St. Louis, 2024). These costs only promise to become more extravagant as medicine rapidly pivots to personalized treatment regimens, where one's own cells and genetic makeup are harnessed to create more successful therapies.

It is not hard to perceive, then, that the U.S. healthcare system, with its high costs, is failing to meet the needs of its constituents. An effective therapy is, after all, only effective if it is attainable by people like Joe and his wife. Thus, in addition to a technical effort, I will also endeavor to research the current economic pitfalls of the U.S. healthcare system, looking at where and how it is failing cancer patients, specifically. With this understanding, the goal of the proposed STS thesis will then be to offer a new healthcare model capable of balancing the economic interests of patients, companies (and institutions) developing treatments, and the U.S. government. Achieving this goal will not be a light task, as the benefit of patients is often seen as contrarian to the interests of cancer treatment developers. To accomplish this goal, interviews with healthcare professionals, healthcare administration officials, and "average Joe" patients will be conducted to garner a firsthand appreciation of the viewpoints of the stakeholders most affected by the current healthcare model and, importantly, any modifications to the status quo. Additionally, the administration and economic outcomes of various healthcare models from around the world will be examined. Based on these interviews and an examination of current healthcare models, successful attributes will be combined into a new model, which will be based on the framework of John Rawl's "veil of ignorance" and its corollary principles of the "original position" and "just savings." This framework is an intriguing one promising to produce a just system balancing all relevant competing interests. To the author's knowledge, no prior work has been conducted in extensively synthesizing such a model; it is hoped that this work will provide the foundation for

future work aimed at reforming the healthcare system, which is vital to ensuring equitable economic access to cancer treatments for patients in the coming decades.

Technical Project:

Interrogating the Cellular Impact of Sonodynamic Therapy on Glioma Cells

Glioblastoma (GBM) is one of the most prevalent and deadly forms of brain cancer. Despite modern advances in chemotherapy, radiotherapy, and surgery, it continues to have an abysmal 5-year survival rate of only 5.8%, leaving a need for more efficacious novel treatment options (Tan et al., 2020). One such emerging therapeutic strategy is sonodynamic therapy (SDT), which uses therapeutic focused ultrasound (FUS) in conjunction with the sonosensitizer 5-aminolevulinic acid (5-ALA) to treat tumors. While 5-ALA is normally metabolized into the fluorescent intermediate product protoporphyrin IX (PpIX) – which eventually becomes processed into heme – this metabolic pathway is disrupted in tumor cells due to reduced ferrochelatase activity, leading to preferential accumulation of PpIX (Bilmin et al., 2019; Ishizuka et al., 2011). SDT can leverage this accumulation of PpIX and its fluorescent properties to selectively exert cellular damage and death upon tumor cells exposed to the low-intensity ultrasound waves precisely deposited by FUS (see Fig. 1) (Shono et al., 2021).

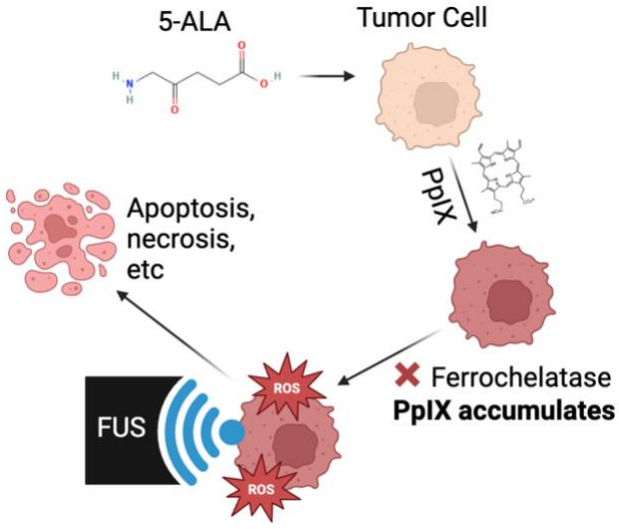

Figure 1: Mechanism of sonodynamic therapy

Advantageously, 5-ALA SDT is already being explored in clinical trials (including at the University of Virginia) and has shown much promise as a non-invasive, non-ionizing therapy with clear advantages

over traditional treatments. However, to date, no study has systematically explored the cellular response of glioma cells to this therapeutic strategy. Many studies in the literature even lack adequate reporting of treatment parameters, hampering development progress (Choi et al., 2020). Such a systematic study, then, is necessary to optimize the treatment regimen for patients and investigate methods for curbing treatment resistance. This in vitro project seeks to fill this knowledge chasm and will do so through several avenues.

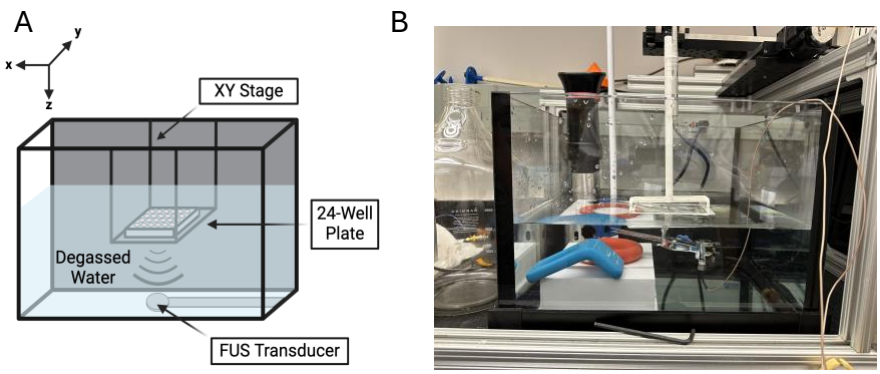

Figure 2: Schematic (A) & actual build (B) of sonication platform for in vitro SDT

First, the project will attempt to design and construct a novel mechanical staging device to facilitate sonication of treatment well plates. The existing device at UVA is used to move the ultrasound transducer to desired locations under the well plate (see "XY Stage" in Fig. 2), but it employs manual movement and is prone to variations in ultrasound transducer positioning and frequent malfunctions, thereby reducing replicability of experimental results. This new 3D-printed staging mechanism under design will seek to address these problems by increasing ease of use while simultaneously allowing more precise positioning of the transducer. The second aim of the project will be to characterize cell death inflicted by SDT on GL261 murine glioma cells under different parameter sets. Parameters to be explored include cell seeding density, 5-ALA dose, incubation period, sonication duration, sonication power, and duty cycle (the fraction of time within a given period in which the transducer is actively sonicating the sample). Systematically

and precisely tuning these parameters to elicit maximized cell death will be immensely useful for optimizing clinical treatment times and minimizing unintentional side effects.

Finally, the third aim will be analyzing the secretome following treatment. Many cytotoxic and immunologically relevant compounds will be released by the treated cells, and it is vital to characterize the types and extent of these secreted compounds. PARP-1, caspase-3, caspase-8, caspase-9, and TUNEL assays will be used to characterize the extent of apoptotic cell death. Reactive oxygen species (ROS) production will be measured as well since it is theorized that ROS is the main driver behind SDT-induced cytotoxicity (Nowak et al., 2022). The immunosuppressive chemokines TGF-β and IL-10 will be examined to determine the immunogenicity of treatment. Lastly, extracellular vesicles (EVs) will be isolated and interrogated. EVs, potent carriers of signaling compounds, will be characterized to better understand how their size, concentration, payload, and surface markers change due to treatment.

Achieving these aims will contribute substantially to moving forward the field of research surrounding 5-ALA SDT. With the current difficulty treating GBM, 5-ALA SDT promises, as a non-invasive precision therapy, to be revolutionary for GBM treatment. However, a long road remains ahead for this therapy to reach its full potential, and one of the most pressing issues today is the need for a more thorough understanding of the therapy's downstream effects. With the knowledge gained from this project, researchers will be able to more effectively achieve tumor reduction with lower treatment times and doses of 5-ALA, thereby limiting side effects. The results will lay the groundwork for further expansion and augmentation of the therapy and contribute to a future where the memory of glioblastoma as a deadly cancerous disease with be just that – a distant memory.

STS Research Project:

Evaluating Modern Healthcare Models Using a Rawlsian Approach

in Pursuit of a More Equitable Solution for Cancer Therapy Accessibility

Cancer is doubtlessly a ravaging disease. While the American public can hardly remember a time when once deadly and debilitating diseases like polio, smallpox, influenza, and tuberculosis frequently swept through the populace, cancer remains a disease ever present and uncured. It can strike old and young alike and often with unsuspecting speed. While much progress has been made within the past hundred years – with several cancers like melanoma and thyroid cancers now having five-year survival rates over 90% – many cancers like glioblastoma remain quite deadly (Miller et al., 2022). Cancer remains the second leading cause of death with over 600,000 Americans dying from it each year (CDC/National Center for Health Statistics, 2024).

Yet, even for cancers for which effective treatments have been developed, one issue remains which science cannot solve: cost. Tens of billions of dollars have been rightfully poured into research across the private, government, and academic sectors seeking to understand cancer and develop therapies. In the U.S. alone, $14 billion was spent on cancer research between 2016 and 2020; subsequently, the state of cancer treatment today has massively benefited, with treatment options unimaginable even decades ago now readily available (McIntosh et al., 2023). The aforementioned technical project is just one example of the fruits of the massive cancer research effort. But, what does all this research mean if the average patient cannot afford to access treatment?

Cost then becomes a major consideration that must be solved if cancer is to become truly cured in the future. The current U.S. healthcare system is designed as a for-profit system (Kantarjian & Rajkumar, 2015). Drug and therapy prices are for the most part unregulated, with

therapy developers having free rein to determine prices. The reasoning goes that the free market will regulate prices, with time ultimately introducing competition and lowering costs. As Kantarjian notes, though, this paradigm simply is not the case with modern pharmaceutical companies having established oligopolies that limit competition and raise prices. For example, the tyrosine kinase inhibitor imatinib was approved by the FDA in 2001 and was revolutionary in treating chronic myeloid leukemia, which at that point did not have an effective therapy (Henkes et al., 2008). Yet, for insured patients (yet alone uninsured patients), average monthly copayments rose from $2798 in 2002 to $4892 in 2011, and patients with higher co-payments were found to be 70% more likely to discontinue treatment within six months (Dusetzina et al., 2014). This statistic is alarming, especially considering that imatinib was designed to be used indefinitely or else risk cancer recurrence.

With these stakes in consideration, this thesis sets out to propose a new healthcare model using John Rawls' "Theory of Justice" as its foundation. According to Rawls, in order for justice to be fair for all citizens in a democracy, two conditions must be satisfied. First, every citizen must have equal rights to a system in which equal rights and liberties are applied to *all* participants in this system. Rawls also makes a second condition, one which makes it distinct from other political systems like socialism. According to this second criterion, social and economic inequalities can exist; however, they must be leveraged to the greatest degree in favor of the least privileged in society. Additionally, where power is concentrated in political positions, all citizens must have equal opportunity to reach these positions (Rawls, 1991, p. 148).

It should be noted that the goal of this novel healthcare model will be to provide equitable – and not necessarily equal – treatment for patients. To ensure equitable treatment, it must sometimes be necessary to reallocate resources to those who need it most. As Rawls explains in *A*

Theory of Justice in elaboration of the second condition, justice cannot be maintained if benefits are taken from a few for the benefit of the many (Rawls, 1999, p. 13). This situation could very likely occur in a system pursuing equal treatment as the collection of resources and the distribution of benefits do not have uniform impact on each individual. The benefit of the greater good could very well mean the detriment of the few. Thus, equal treatment would promote unfairness. However, the opposite of equal treatment can be true and just: namely, providing better treatments for the least advantaged is justified if it results in improved outcomes for the group in question even if it does not result in better outcomes for all in the aggregate (Rawls, 1999, p. 13). In this way, greater equality in the sense of "fairness" is reached through this utilitarian framework even though equality through equal treatment is not itself the goal.

Basing a system from this framework offers the advantage that one can grant increased resources according to need and build hypothetical frameworks where true equality can be reached. In practical terms, a healthcare model based on Rawls' theory will be capable of ensuring economic access for all while also protecting the rights and interests of all involved parties. The following research will attempt to turn this hypothetical framework into a workable system. To do so, interviews will be conducted with healthcare administration officials, doctors, patients, and biotechnology company executives. Their perspectives and interests will be vital to shaping this proposed model. Other healthcare models will be examined as well to determine if useful aspects have already been developed and are compatible with Rawls' theory.

Looking to the literature, one prior proposal to reform healthcare was briefly outlined by Zoe Fritz and Caitríona Cox in 2019. Fritz and Cox proposed that Rawls' "veil of ignorance" could be an effective guide to reforming socialized healthcare systems. Behind this veil, everyone was stripped of his or her status. Race, gender, wealth, religious and political views – none of it

mattered anymore. Everyone was moved back into the "original position." Since members of a group would no longer be knowledgeable of their positions in society, acting by consensus in their own individual interests would by virtue of this veil mean that a just and equitable solution would be reached. For example, behind this theoretical veil, one could not reasonably impose laws that would disadvantage Muslim women, for the law's author would not know whether in this new social system, the author would be a woman or an adherent of Islam. Thus, behind this veil, a discriminatory law targeting gender or religion would not be passed. This system pivots upon the understanding that one will reasonably always pursue what would be considered valuable in his or her life (Rawls, 1991, p. 153)

Furthermore, under Rawl's "just savings principle," people have a duty to create a system that would preserve a just basic structure for future generations (Fritz & Cox, 2019). As Rawls explains, each generation would benefit from the infrastructure and societal contributions (the "savings") of the prior generation and pass those savings on to the next generation (Rawls, 1999, p. 254). With this consideration in mind, Fritz & Cox go on to argue that biomedical research is necessary for the stability of a modern healthcare system. Fully cutting research funding is not an option as it robs the next generation of the resulting scientific and medical benefits; however, equally undesirable is dedicating too much funding into research and draining patients of the ability to access resources.

Using Rawl's principles, one could make several proposals for reforming the healthcare system to optimize on lowering costs. While it might push the boundaries of Rawl's just system, I propose a free market term-limited pricing model could be appropriate, for example. In this system, a pharmaceutical company would have a set number of years to keep a therapy on the free market before having it come under price caps. During this "free reign" period, the company would,

furthermore, be required to provide cost assistance to low-income patients. Thus, developmental research is encouraged as the profit incentive remains intact, and satisfying Rawl's principles, those without the monetary means can afford the therapy during the free reign period, while future generations (regardless of ability to pay) can access the therapy, creating the basis for a stable long-term system. This system is equitable to all regardless of wealth level in the long-run, but in the short-term, focuses on lower-income patients, thereby ensuring that it is even equitable during a high price period. This view is consistent with the interpretation of Rawl's principles as outlined by Bommier and Stecklov in their 2002 paper comparing Rawl's theory to social welfare theory. There, they argued that the government (although a private company could also be substituted in place of the government) had a duty to target health interventions from a cost-perspective only when income and health outcomes positively correlate (Bommier & Stecklov, 2002).

This proposed part of a larger system is just one application of Rawl's theory to remodel healthcare. This is but a model fully based on my own understanding of Rawlsian theory, and it shall be continued to be developed based on added research concerning prior and current systems. Further proposals to complement and build this model will additionally be made, and it is worth exploring other frameworks in contrast to Rawl's veil as well as the opinions of relevant parties. More research must be conducted to provide a thorough exploration and development of a novel, more equitable healthcare system. Ultimately, this endeavor – combined with interviews – will be the principal work of the proposed thesis.

Conclusion

Upon completion of the technical project, it is expected that a more user-friendly and reliable staging mechanism for sonication will be developed. It is further expected that using this

new staging mechanism, a thorough study of the effects of different parameter sets on cell viability will be documented in addition to characterization of EVs, ROS, and chemokines released under ideal conditions. The information garnered from this study will yield a better mechanistic understanding of the therapy which will in turn benefit patient outcomes in the clinic.

Additionally, in addressing the affordability problem of cancer therapy development and marketing, it is expected that the results of the thesis will provide a thorough understanding of the current economic drawbacks of the current U.S. healthcare system for patients and the hope of a novel, more equitable model. Furthermore, the results of this analysis will influence a proposal for a new healthcare system operating under Rawls' *A Theory of Justice* that aims to create a more equitable system, especially for lower-income patients. It is a primary goal of this system under development to balance the interests of healthcare professionals, patients, and therapy developers. A mutually beneficial solution must exist, and it will be found. To do so, interviews with relevant parties will be conducted, and a comparative analysis of multiple current healthcare models will be done. While it is not expected that this research will miraculously change the existing status quo, it is hoped that the scholarship produced will help lay the groundwork for reformations in the near future, especially as the cost of cancer treatment reaches an inflection point. In doing so, this project will contribute to ensuring that effective research leads to effective cancer therapies which, with widespread accessibility from a cost perspective, will ultimately lead to better treatment outcomes for *all* patients suffering under the burden of cancer.

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