DESIGN AND EVALUATION OF FOCAL THERAPY PARADIGMS FOR BREAST CANCER-DERIVED EXTRACELLULAR VESICLE MODULATION

SOCIOECONOMIC FACTORS AFFECTING BREAST CANCER SCREENING PROCEDURES IN WOMEN

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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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Breast cancer (BrCa) is the leading cause of cancer mortality in women, and the majority of these deaths can be attributed to metastasis (Scully et al., 2012, p.311). As is the case with most cancer types, the disease is often detected too late to respond to less aggressive treatment options, and doctors struggle to control tumor borders. Alarmingly, five-year survival rates for BrCa drop from 100% if diagnosed at stage I to 26% if diagnosed at stage IV (Loh et al., 2019, p.4940). Despite being the second most frequent cause of brain metastasis, a diagnosis associated with reduced life expectancies and overall poor prognosis, late-stage BrCa has been largely underinvestigated and current screening practice for BrCa appear insufficient to prevent late diagnosis. Thus, both the ability to characterize and treat BrCa as it progresses and the development of early detection strategies prove essential in improving overall survival rate. The technical project and tightly coupled STS research paper proposed address each of these solutions respectively, providing insights into both clinical and social approaches.

To address the current difficulty in effectively treating late stage BrCa and preventing metastasis, the technical project aims to design and test combinatorial treatment paradigms involving drug and oncolytic therapies *in vitro*. In leveraging informative biomarkers indicating cancer progression, the objective is to develop a system for targeted drug therapy dependent on metastatic capacity and hormone receptor status which relies upon modulation by focal oncolytic therapies such as focused ultrasound and radiation. Experimentation of an *in vitro* platform for such therapy types and characterization of associated biomarkers in Fall 2022 will be followed by an evaluation of such findings and design of rational treatment paradigms in Spring 2023.

The problem at hand intersects with society though revelation that economic dispariteies may be responsible for the continued lack of proper screening for BrCa. Therefore, in light of Preseident Biden’s new Advanced Research Projects Agency for Health (ARPA-H), the paired

STS research discussed suggests a mandatory battery of testing procedures and educational resources for all women in the United States which may increase early stage detection rates for BrCa and transcend socioeconomic disparities (Advanced Research Projects Agency for Health, n.d.). In providing solutions from a social reform standpoint, this research aims to address the lack of early detection strategies which has contributed to the troublesome BrCa mortality statistics observed for women today. Research regarding the social circumstances surrounding BrCa mortality and development of this prospectus in Fall 2022 proceed writing of the STS research paper in Spring 2023.

DESIGN AND EVALUATION OF FOCAL THERAPY PARADIGMS FOR BREAST CANCER-DERIVED EXTRACELLULAR VESICLE MODULATION

Most cell types, including tumors, release extracellular vesicles (EVs) which play host to proteins, RNA transcripts, DNA, and lipids reflective of the parent cell. Through facilitation of cell signaling, EVs play a critical role in cancer development and alter the tumor microenvironment to allow for growth and metastasis (Clark et al., 2021, p.885). Exosomes comprise a unique subset of EVs ranging from 30-100nm in size that, among their numerous roles, facilitate tumor interactions with the immune system (Prendergast et al., 2018, p.1). In light of this role, tumor-derived exosomes have garnered tremendous interest as key players in the context of oncolytic therapies (OTx) (Sheybani et al., 2020, 7437). One such OTx is focused ultrasound (FUS), a non-invasive, non-ionizing strategy for acoustic energy deposition in tumors. Sheybani et al. (2020), a previous BME Capstone project, demonstrated that FUS hyperthermia augments EV release from glioma cells *in vitro*, thereby enriching the availability of tumor associated biomarkers that may aid in treatment selection, adaptation, and surveillance (p.7436–7447). Extending such work, the described technical project proposes to investigate this relationship in the context of BrCa, where the interaction between FUS and EVs is unknown. In

comparing radiotherapy and FUS hyperthermia, the project aims to establish a functional intersection of OTx with BrCa-derived exosome release and profile, which may be informative for cancer management in reflecting tumor response to treatment. Thus, working under the guidance of Dr. Natasha Sheybani, the assistance of PhD candidates Zehra Demir and Sarah Hernandez, and in conjunction with fellow student Nini Tran, the overall goal is to (1) dissect the impact of both radiotherapy and focused ultrasound (FUS) on the release and profile of EVs and (2) to leverage these insights to design rational paradigms for BrCa therapy. To accomplish these goals, the impacts of both OTx on three BrCa cell lines will first be determined, which requires design of an *in vitro* set-up and parameter optimization to enable targeted treatment.

To establish proper modeling, choice of BrCa cell lines for this project must reflect the significance of hormone receptor status, as it may vary between stages. A late stage diagnosis typically describes triple-negative breast cancer (TNBC), indicating the downregulation of three hormone receptors which dictate metastatic capctiy and aggression level: Human epidermal growth receptor (HER-2), estrogen receptor (ER), and progesterone receptor (PR). Triple-negative breast cancer, resulting in a high incidence of brain metastasis, does not respond to typical hormonal therapy options and presents further treatment challenges due to restriction of the blood-brain barrier (Loh et al., 2019, p.4942). Therefore, 4T1, E0771, and BRPKP100 cells, each distinct in their hormone receptor status, metastatic capacity, and immune composition *in vivo*, will be cultured in acoustically transparent cell culture vessels and subjected to either FUS hyperthermia, radiation, or sham treatment. Comparison of thermal and absorbd radiation dose will be performed according to published methods, and a breakdown of the treatment groups for experimentation with targeted therapy design in Fall 2022 may be found in Figure 1 on page 4 (Schlesigneret al., 2017, p.10-11).

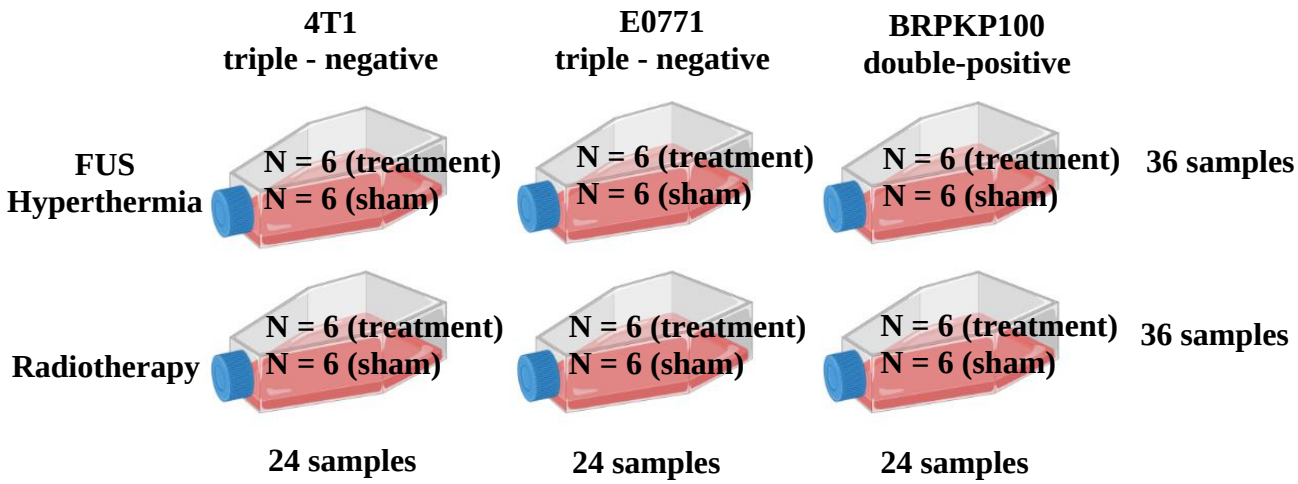

Figure 1: Sample sizes for each treatment group. The visual shows sample sizes for each experimental condition as well as their sham counterparts. Sham treatments are control mechanisms which subject cells to identical conditions but omit actual treatment. Experiments will be carried out for each of the three cell lines outlined in this project, 4T1 cells being the most aggressive form of BrCa and BRPKP100 being the least aggressive form. (Created by Imbarlina (2022) with BioRender.com)

In continuation of efforts towards EV modulation and characterization for an *in vitro* BrCa platform, EVs will be isolated from all three cell lines via ultracentrifugation, the current golden standard for purification. To evaluate the modulatory effects of OTx, a nanoparticle tracking instrument will characterize isolated EVs by size and concentration, By the end of the Fall semester, investigators will isolate RNA from BrCa-derived EVs and develop transcriptomic profiles based on previous optimizations of such practice (Prendergast et al., 2018, p.4). In the Spring semester, the project will be continued through visualization of transcript expression, specifically in the realm of small non-coding microRNAs, which may allow for tumor cells to influence the immune system and support the tumormicroenvironment (Long et al., 2018, p.91) . In this way, investigators will identify key differentially regulated transcripts that are therapeutically targetable, identify 1-2 drugs relevant to these targets, and design rational treatment paradigms involving select drug and oncolytic therapy combinations. An overview of the design process for this technical project, including key experimental procedures essential to its completion, is outlined outlined in Figure 2 on page 5.

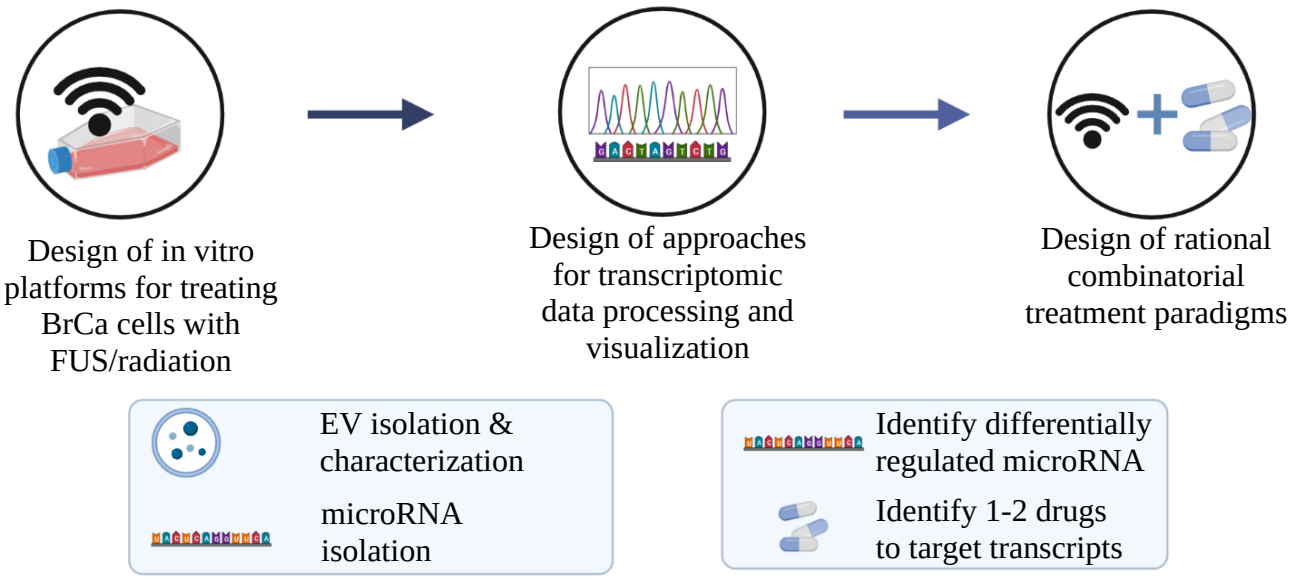

Figure 2: Design process with experimental elements. This figure depicts each step in the design process for the technical project at hand, including collection of data for both EV modulation by OTx and differentially regulated transcripts for each of the three BrCa cell lines in use. The box beneath each arrow represent experimental procedures which must be carried out in order to proceed to the next phase in development of a combinatorial treatment for BrCa. (Created by Imbarlina (2022) with BioRender.com)

Overall, anticipated outcomes for this project include a difference in the concentration of EVs between control samples and those treated with OTx and a difference in transcript expression between BrCa cell types which may be leveraged to design combinatorial treatment paradigms. The University of Virginia Biomedical Engineering Department, specifically the Sheybani Lab, provides all resources and accommodations for this project. A portion of the funding derives from the Office of Undergraduate Research, as this technical project emerged from prior research which received the Harrison Undergraduate Research Award in April 2022. A final solution to the issues discussed with current late-stage BrCa treatment measures will be outlined in a scholarly article to be submitted to the Biomedical Engineering Department and a formal presentation for the department's Capstone Symposium towards the end of the Spring 2023 semester.

SOCIOECONOMIC FACTORS AFFECTING BREAST CANCER SCREENING PROCEDURES IN WOMEN

The introduction of the Affordable Care Act (ACA) in 2010 expanded Medicaid to cover all adults below the Federal Poverty Line and allow a national mandate instructing all Americans to purchase some form of health insurance. A landmark Supreme Court decision in 2012 offered further assistance to achieve this goal, and 32 states opted to further expand Medicaid. Figure 3 color codes expansion and non-expansion states to illustrate this divide. Seizing the unique opportunity to determine governmental influence on rate and scope of BrCa screening as a result of such insurance expansion, Toyoda et al. (2020) found that states which accepted the Supreme Court's offer saw higher rates of BrCa mammogram screening than the 19 states that did not adopt the new policies (p.780).

Figure 3: United States map of 2012 ACA Medicaid expansion. In 2012, only 32 States (including Washington, D.C.) opted to expand public insurance coverage benefits, allowing for healthcare disparities between these states and the 19 states which did not. (Study: Without Medicaid expansion, poor forgo medical care, n.d.)

However, this cancer type is still the leading cause of cancer mortality in women, which prompts examination of further mechanisms for early detection. Sprague et al. (2011) suggest that economic disparities are possibly responsible for the continued lack of proper screening, as

survival rates among women diagnosed with BrCa are lower for those with a lower socioeconomic status (SES) (p.1543). In addition to identifying a positive trend in screening due to Medicaid expansion, Toyoda et al. find ample evidence that racial and economic disparities negatively impact access to care (2020, p.781). Specifically, health insurance seems to play a major role in access to proper screening procedures, accounting for approximately half of the observed disaprities in BrCa diagnosis (Ko et al., 2020, p.386). Though a lack of overall health insurance coverage may certainly contribute to a lack of access, Medicaid expansion and the differences between public and private health insurance offer an additional perspective. Notably, individuals with private insurance demonstrate lower rates of stage IV diagnosis while uninsured individuals and those with Medicaid demonstrate comparably higher rates of late detection (Ko et al., 2020, p.388). As ACA Medicaid expansion lowered premiums in the privte marketplace of non-expansion states, increasing access to care for those with more favorable SES, it follows that the lack of such coverage among lower SES groups exacerbates barriers to care which potentiates a lack of adequate BrCa screening, associated late detection, and poor patient outcomes (Toyoda et al., 2020, p.781).

Clearly, ACA Medicaid expansion, while certainly offering hope for government policy as a viable means of progress, did not address concerning BrCa statistics in full. In light of the high mortality and morbidity rates for the disease, it appears that current legal action to improve access proves insufficient, regardless of its intentions to protect lower income Americans. Thus, this research paper investigates the degree to which President Biden's new Advanced Research Projects Agency for Health (ARPA-H) can combat SES disparities and provide a mandatory battery of testing procedures for all women as well as educational support for increased BrCa screening rates in the United States (Advanced Research Projects Agency for Health, n.d.). In

investigating current insurance limitations of testing, costs, time commitments associated with regular surveillance, and successful health management methods adopted for other diseases, the scholarly article written will thoroughly evaluate the possibility for a universal diagnostic plan to improve overall patient outcomes through three focus points: nationwide Medicaid expansion, universal practice between public and private health insurance companies, and an emphasis on education and self-health management. Keeping in mind the potential for success presented by the 2012 ACA Medicaid expansion, a more widespread adoption of such policy may prove necessary for continued improvement of BrCa early detection. Nationwide expansion then sets the stage for a universal battery of testing procedures, which has potential to minimize the gap in access to care between those with public and private insurance plans. Lastly, the insufficiency of public insurance expansion as it stands also presents an argument for supplemental social measures to support government policy and ensure early detection among women of all backgrounds. Quaife et al. demonstrate a tendency of lower SES groups towards negative attitudes and a general lack of hopefulness when presented with treatment options, as these groups tend to experience inferior access to healthcare due to the aforementioned inequities between public and private insurance (2015, p.256). This pessimistic belief system may partially explain continued late stage diagnoses in these groups and, as less faith in medical intervention logically imbues hesitancy to seek help from medical professionals. Given these findings, it may be necessary to supplement federal policy with social reform efforts to provide a reasonable starting point in addressing the lack of early detection among women with BrCa. To this point, success in self-health management via technological development suggests a viable alternative for promoting increased BrCa screening and combating negative beliefs held by lower SES groups, given modern demonstrations of such practice as particularly motivating for hormonal

health (Ford et al., 2021, p.50-52). Integrating education into the constantly advancing climate of technology-driven society holds potential to overcome the less political and economic limitations to early detection.

In light of the relationships between each of the proposed focus points, a linear Actor Network Theory (ANT) most accurately describes the current relationships between relevant groups which perpetuates poor patient outcomes for women with BrCa, as each "actor," whether social or technical, interacts with the next to ultimately result in high mortality and morbidity rates for women diagnosed (Law & Callon, 1988, p.295). Figure 4 depicts such a model, clearly representing the way in which partial expansion of ACA Medicaid presents several barriers to improve overall prognosis in women of lower SES groups. In summary, government expansion of ACA Medicaid in only some states allows for lowered premiums for private insurance companies in non-expansion states, leading to an overall gap between private and public health insurances that leaves Medicaid insufficient for proper healthcare access. This lesser care causes women of lower SES status who cannot afford private insurance to hold false negative beliefs regarding BrCa prognosis and associated treatment options, thus this group tends to avoid proper

Figure 4: ANT model for lower SES women diagnosed with BrCa. ACA Medicaid introduction by the government intended to increase access to care but acted on other social forces to create poor patient outcomes in women with BrCa via a handoff model. (Adapted by Imbarlina (2022) from Carlson, 2009)

screening procedures and medical intervention. Lastly, lower SES women avoiding diagnosis tend to seek help after their BrCa has progressed to a later stage, leaving doctors to face cancers which are much harder to treat. Ultimately, this process contributes to the high BrCa mortality and morbidity rates observed in US women.

An envisioned linear ANT model, rendered in Figure 5, demonstrates the overall effect of nationwide ACA Medicaid expansion on improved BrCa outcomes, illustrating how a re-evalulation of the model's first three social factors may collectively and sequentially shape diagnosis and treatment to reduce mortality and morbidity rates. In this new model, nationwide expansion of ACA Medicaid presents an opportunity for universal screening procedures to be implemented by ARPA-H policy, therefore closing the gap between private insurance offerings and the health insurance options available to women of lower SES. Likewise, this universal coverage in regards to BrCa screening provides an outlet for educational resources to become a major contributor to awareness of early detection, perhaps even taking advantage of the modern technological age towards this purpose. In altering the first three handoffs involved in the original linear ANT model presented, doctors may experience an influx of earlier stage BrCa

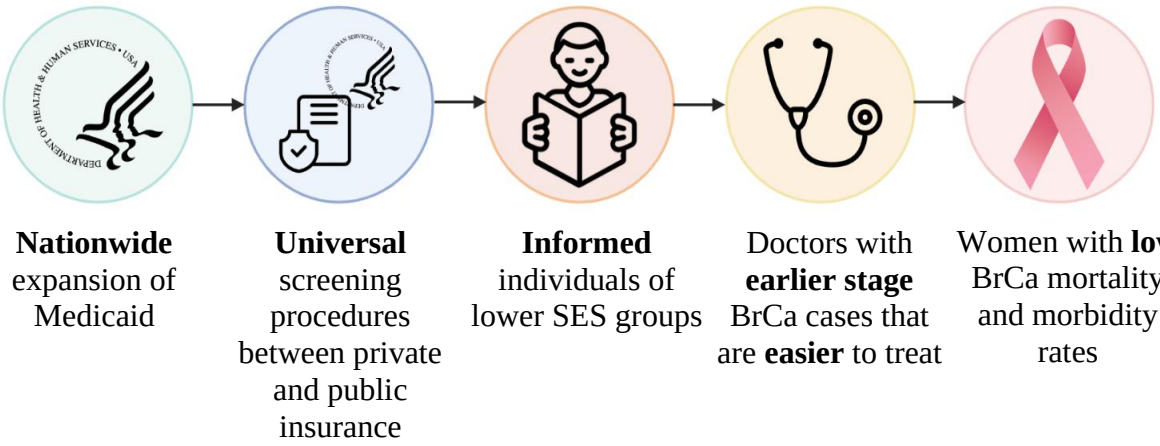

Figure 5: Envisioned linear ANT model for lower SES women diagnosed with BrCa. Social and political factors act to change the climate of BrCa patient outcomes, beginning with a nationwide expansion of ACA Medicaid. (Adapted by Imbarlina (2022) from Carlson, 2009)

diagnoses which prove easier to treat and manage, thereby reducing overall mortality and morbidity rates for US women.

AN ALLIANCE BETWEEN TECHNOLOGY AND SOCIETY

Current gaps in prognostication and late-stage treatment of BrCa have lead to not only reduced survival rates among women, but also poor quality of life in those diagnosed with the disease. Therefore, it is important to identify solutions for these gaps and develop strategies for both early detection and treatment of late-stage BrCa types. The technical project and tightly coupled STS research topic proposed provide insight into options for both via *in vitro* studies revolving around EVs as potential biomarkers and the prospect of a mandated set of screening procedures and educational resources which transcend SES disparities. Exosomes are a rich and unique repository of cancer biomarkers, and identifying biomarkers that reflect how tumors are responding to treatment can be vastly informative for cancer management. OTx mechanisms for non-invasive treatment of cancers already exist clinically, and the prospect of its enhanced ability in controlling tumor borders and preventing metastasis is certainly promising for a future of universally effective cancer treatment and erradication. As for political and social obstacles which hinder early detection of BrCa, a comprehensive view of the factors negatively impacting lower SES individuals holds potential to attack the problem from all angles and ensure that women seek and receive help sooner.

Clearly, the troublesome statistics which headline discussion of BrCa in women are held captive to both underlying biological and societal complications. Though the sheer number of barriers to proper care seems overwhelming, hope resides in the acknowledgment that both social and technical factors intertwine to create both problem and solution. In this way, the separate

projects outlined are indeed paired in that the overall goal of reduction in mortality and morbidity rates for women with breast cancer relies on both approaches for success.

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