Evaluation of Focused Ultrasound for the Induction of Immunogenic Cell Death
(Technical Paper)

Factors Influencing Patient Treatment Decisions in Breast Cancer
(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

Introduction: Cancer is a complex disease to treat because improvements in life expectancy from other medical innovations increases the risk that a person will eventually develop cancer. The complexity and severity of cancer have led to a great deal of time and money being devoted to cancer research, resulting in an ever-increasing number of available treatments. In combination with the growing expectation that patients should have the final say in their treatment to preserve their rights, the decision of what treatment technology to use has fallen more heavily on the patient in recent years. However, many cancer patients lack extensive medical knowledge and thus may have difficulty in selecting the treatment that is medically best for them (Waljee, Rogers, & Alderman, 2007). In breast cancer, for example, patients often have a plethora of options including hormone therapies, chemotherapy, radiation, immunotherapy, and experimental therapies that are in clinical trials such as focused ultrasound (FUS). In addition to simply producing treatment options, however, it is important to understand how patients decide which treatment options are best for them and what factors have the greatest influence on their decisions to improve the ability to educate them about their options and ensure that they select the optimal therapy. This understanding may be leveraged to educate patients on emerging technologies that may benefit them such as FUS. FUS is currently being investigated for its capacity to treat primary tumors directly through thermal ablation (F. Wu et al., 2003). FUS may also have the ability to sensitize the cancer to a potent immune response and enhance the efficacy of concomitantly delivered immunotherapies but this is a newer area of study (Feng Wu, 2013). This project will be composed of two primary topics: a technical topic focusing on the use of FUS as an immune-priming agent, and the factors that influence breast cancer patients to opt for certain therapies. Through this research, I seek to determine whether FUS treatment makes cancer cells more immunogenic and susceptible to immunotherapies, and what factors influence patients to select certain therapies.

Technical Topic: Every year, 170,000 women worldwide are diagnosed with triple negative breast cancer (TNBC("A review of triple-negative breast cancer. - PubMed—NCBI," n.d.)) (Ismail-Khan & Bui, 2010). TNBC presents numerous clinical problems for treatment, particularly aggressive metastases and an immunosuppressive tumor microenvironment (TME). Most deaths from TNBC come from the metastatic burden rather than from the primary tumor as it quickly metastasizes to the lungs and brain (Ismail-Khan & Bui, 2010). These metastatic locations present several major complications including the difficulty treating those tumors without damaging the surrounding tissue and the impaired function of the native tissue due to cancer invasion. Furthermore, TNBC has the capacity to evade native immune response due to normal cancer immune escape mechanisms as well as large populations of myeloid derived suppressor cells (MDSCs) that reside in the TME. These MDSCs inhibit antigen presenting cell (APC) recruitment and activation to the tumor bed which in turn prevents the development of a lymphocyte driven anti-tumor immune response. However, new research suggests that it may be possible to overcome these barriers to APC activation when cancer cells undergo immunogenic cell death (ICD) (Gebremeskel et al., 2017).

Historically, the field of immunology has considered apoptotic cell death to be non-immunogenic and necrotic cell death to be immunogenic (Thompson, 1995). Recently, however, there has been an accumulation of evidence that ICD is a distinct mode of cell death that stimulates immune response against the antigens released by dead cells, and can be elicited in both apoptotic and necrotic cells (Inoue & Tani, 2014; Obeid et al., 2007). ICD involves a unique expression of damage associated molecular patterns (DAMPs) that recruit and stimulate the maturation of APCs (Kroemer, Galluzzi, Kepp, & Zitvogel, 2013). The first DAMP expressed during ICD following a stress on the cell is the exposure of calreticulin (CRT) on the extracellular surface of the cell

membrane without a breakdown of the membrane. In ICD, CRT is often exposed concomitantly with other proteins of the endoplasmic reticulum (ER) such as heat shock proteins (HSPs) - which also serve as DAMPs. CRT is detected by CD91+ APCs (primarily macrophages and dendritic cells), and appears to lead to the priming of a Th17 response which increases the activity of macrophages and neutrophils at the site of the tumor (Pawaria & Binder, 2011). The next major marker of ICD is the release of ATP in blebs from the cell. ATP is detected by P2Y purinergic receptors which activate macrophages and lead to the increased recruitment of dendritic cells (Elliott et al., 2009). The third indicator of ICD is the presence of high mobility group box 1 (HMGB1) protein, which is bound by TLR2/4 on phagocytic cells. Dying cells induce an antigen specific immune response when HMGB1 is released, but in the absence of HMGB1, they induce tolerance (Kazama et al., 2008). These DAMPs recruit and mobilize APCs which are important for priming and activating CD8+ (cytotoxic) and CD4+ (helper) effector T-cells, which are the key actors in tumor eradication.

One technique that has shown an increase in the activity of effector T-cells in the breast cancer TME is focused ultrasound (FUS) (Lu et al., 2009). FUS is a safe, clinically available technique for non-invasive, non-ionizing tumor destruction that has demonstrated efficacy in the treatment of breast cancer (Furusawa et al., 2006; Gianfelice, Khiat, Amara, Belblidia, & Boulanger, 2003; Kennedy, 2005; Merckel et al., 2013; Schmitz, Gianfelice, Daniel, Mali, & van den Bosch, 2008; F. Wu et al., 2003; Feng Wu et al., 2006, 2007). FUS produces thermal and mechanical bioeffects in tumor cells by focusing sound waves into a small, targeted volume. FUS is capable of nearly instantaneously heating tissues with a sub-mm precision to produce stresses on cells. FUS can be used at high intensity (HIFU) in a continuous wave regimen to induce thermal ablation that results in coagulative necrosis at the focal zone. Around the focal zone, hyperthermic temperatures are

achieved which may not induce necrotic cell death but do result in heat-mediated damage to cells. Additionally, FUS can be used at low intensity (LOFU) to induce an immediate, sub-lethal rise in temperature at the focal zone.

Thermally-based FUS treatments have been shown to produce the markers of ICD in individual studies across multiple solid tumor models (Hu et al., 2005). Studies using HIFU to thermally ablate murine solid tumors (C1300 neuroblastoma, MC38 colon cancer, H22 liver cancer) have also shown resistance to re-challenge with the same tumor model subsequent to FUS exposure, suggesting that there is an immunologically driven abscopal effect imparted by FUS treatment (Hu et al., 2005; Yang et al., 1992; Zhang, Deng, Feng, & Wu, 2010)(Hu et al., 2005; Yang et al., 1992; Zhang, Deng, Feng, & Wu, 2010)(Hu et al., 2005; Yang et al., 1992; Zhang, Deng, Feng, & Wu, 2010). Furthermore, it has been shown that priming a mouse immune system with dendritic cells pulsed with HIFU-treated antigen imparts a more robust cytotoxic T-cell response against that tumor model than dendritic cells pulsed with untreated tumor lysate (Deng, Zhang, Feng, & Wu, 2010; Zhang et al., 2010). HMGB1 release in a neu deletion breast cancer model has been shown *in vitro* to be increased with increasing temperature in a water bath (Silvestrini et al., 2017). However, no comprehensive investigation has yet been reported to rigorously characterize the ability of FUS to induce ICD based upon the above-mentioned markers of ICD.

In this project, I aim to characterize the capacity of FUS thermal regimens to induce ICD in 4T1, a murine mammary carcinoma model of TNBC, as well as to determine whether FUS treatment augments the immune response against treated cells. Using a custom, in-house FUS system, 4T1 cells will be treated with FUS hyperthermia and thermal ablation regimens. CRT translocation, ATP release, and HMGB1 secretion into the supernatant will be recorded for these FUS regimens in order to optimize FUS parameters for the expression of ICD-related DAMPs.

4T1 cells treated with FUS *in vitro* will also be used to prime the immune systems of appropriate, wild-type, syngeneic mice in a vaccination study. This study will investigate whether FUS regimens cause 4T1 cells to elicit a robust, anti-tumor immune response that is comparable to or greater than alternative methods of immune sensitization such as chemotherapeutic inducers of ICD.

STS Topic: Roughly 1 in 8 women in the developed world will be diagnosed with breast cancer in her lifetime ("U.S. Breast Cancer Statistics | Breastcancer.org," n.d.). Due to the prevalence of this disease and the variability of the characteristics of cancer between people, there are often multiple treatment options available to a patient with breast cancer. Patients are frequently provided with such options as hormone therapy, chemotherapy, radiation, surgery, immunotherapy, and others depending on the type and severity of the cancer. However, none of these treatments are without risks, not all options will work for every patient, and it is impossible to know whether a treatment will be successful. Despite these uncertainties, patients are ultimately expected to be able to make a potentially life-altering treatment decision and advocate for themselves, often without any formal medical training. With these immense stakes, I aim to understand what factors impact a breast cancer patient's treatment decision.

In order to understand this problem, one must examine the stakeholders and relevant artifacts. Perhaps the most obvious stakeholders and technological artifacts involved in a patient's medical decision is the patient themselves and the treatments selected (Sepucha, Ozanne, Silvia, Partridge, & Mulley, 2007). Other major stakeholders involved in this process include the doctor, caregiver, companies involved in the production of the treatment, research organizations that develop new treatments, and regulatory and advocacy groups. The doctor must provide medical advice which can influence a patient's decision and administer the chosen treatment (Brédart, Bouleuc, &

Dolbeault, 2005). The caregiver, if separate from the doctor, must also be considered as they assist her with everyday living and different treatment options may result in disparate expectations for the caregiver. Companies that produce therapies may play a role in the process of treatment selection by providing information to doctors about their available technologies (Forum, Services, & Medicine, 2013). Other technological artifacts to consider in the process are support groups and sources of information about cancer and available medical interventions (Attai et al., 2015).

Patient treatment decisions can be better understood within the context of the co-production model. Co-production, as described by Sheila Jasanoff, is best defined as the process of the "natural and social orders being produced together" (Jasanoff, 2004). While co-production is often used in STS research, it has several shortcomings. The idea of science and society modifying each other as they are produced gives some actors involved in the co-production of technology an excuse to shirk their responsibility for technology (Taco Brandsen, Trui Steen, & Bram Verschuere, 2018). When a group involved in the creation of a technology can claim that it was the result of "society," it becomes far easier to avoid taking responsibility for any negative results of the technology. However, research on the topic of patient decisions requires some acknowledgement of co-production as patient decisions are both informed by available technologies and shape what technologies become available to them.

This model provides a useful framework for the analysis of the chosen research question. In order to understand how patients make their decisions, researchers must understand how a patient's options came to exist. New cancer therapies can shape the medical field by offering new treatment options, especially when they are tailored for patients that otherwise lack options. The needs of patients and the medical community also shape available technologies through the research that

they inspire. It is in this manner that technology helps to shape society while society shapes technology within the context of breast cancer therapies.

Research Question and Methods: The STS research question to be explored is: "What are the factors that impact a woman's decision on which treatment to receive for breast cancer?" The question seeks to understand what elements affect the cancer therapy that a breast cancer patient chooses to receive, and how those elements can be shaped by technology. This question will be answered using a combination of documentary research and discourse analysis. Documentary research will be used to combine existing, peer-reviewed scientific literature describing breast cancer patient decisions and verified elements affecting them, including but not limited to: the doctor-patient relationship; the impact of increased information on patient decisions; and knowledge of patient-specific genetic or environmental risks of cancer. Discourse analysis will be used to investigate non-peer-reviewed sources in order to develop an understanding of how decisions are made from the patient's perspective. These sources will include online breast cancer support blog, online sources describing patient experiences, and resources providing information about breast cancer and treatment options. The use of documentary research enables the research question to be answered using empirical data to compare the relative significance of factors and to understand how treatment choices correspond to medical outcomes. Discourse analysis provides a method to understand what resources patients access, what they value, and what factors consciously affected their decisions.

Conclusion: Through a combination of research into the ability of FUS to augment an immune response and the ways in which patients choose therapies, this project aims to help patients select the best treatment for their disease. The technical deliverable is a vaccination strategy leveraging FUS to sensitize TNBC. This deliverable will open new potential treatment strategies for breast

cancer that may be beneficial for patients that do not benefit from existing treatments. The STS deliverable will be a description of the factors impacting patient treatment decisions and a qualitative comparison of those factors. Once completed, the STS deliverable can inform the marketing of emerging therapeutic technologies to increase the odds that it becomes accepted by patients as a potential treatment. With these deliverables, it may be possible to reduce the burden of TNBC on patients.

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