Engineering Immunogenic Focused Ultrasound (FUS) Paradigms for Breast Cancer through Incorporation of Adenosine Receptor Blockade
(Technical Paper)

The Psychological Response of Breast Cancer Patients to Treatment and Its Impact on Outcomes
(STS Paper)

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By
Beyzanur Ak

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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.

ADVISORS

Prof. Pedro Augusto P. Francisco, Department of Engineering and Society

Dr. Natasha D. Sheybani, Department of Biomedical Engineering

Introduction

Breast cancer (BrCa) stands as the foremost contributor to cancer-related fatalities in women, with metastasis being the primary culprit behind the majority of these tragic outcomes. Triple negative breast cancer (TNBC) is considered a highly metastatic subtype of BrCa, and it has been reported that distant metastasis occurs in nearly half (46%) of TNBC patients (Lin et al., 2008). The therapeutic options for TNBC patients is limited, illuminating a clear need for alternative treatment approaches that address the metastatic burden prevalent in this disease (Crown et al., 2012). Therefore, there is a growing and urgent demand for innovative treatment approaches that combine various methods to effectively address both primary and widespread lesions, all while prioritizing the comfort and quality of life for BrCa patients.

Focused ultrasound (FUS) is a non-ionizing, non-invasive technology that concentrates sound waves into a precisely defined ellipsoidal volume. Within this focal zone, it enables the targeted damage or destruction of tumor tissue with submillimeter precision, all while safeguarding the integrity of adjacent healthy tissues (Sheybani et al., 2020). Previous study has shown that FUS-treated cancer cells release endogenous danger signals, such as ATP, due to mechanical and/or thermal disruption (Hu et al., 2005). When cells undergo apoptosis, they release higher amounts of ATP, leading to increased levels of extracellular adenosine, also called as ecto-adenosine. Elevated levels of extracellular adenosine have been found to reduce anti-tumor activity and enhance mechanism of immunosuppression within the microenvironment of solid tumors (Hammami et al., 2019). Adenosine plays a critical role by activating four G protein-coupled receptors, with A2A and A2B being particularly known for their immunosuppressive effect. The main goal of technical part of the project is to interrogate this

phenomenon in the context of FUS ablation regimens and interrogate combination FUS approaches leveraging adenosine receptor blockers as a more favorable approach for fully unleashing the immunogenicity of FUS.

Anxiety is the most common and challenging psychological outcome of breast cancer. Elevated stress level is found negatively correlated with patients' quality of life and coping with the disease (Baqutayan, 2012). Stress level varies depending on phase of the disease and treatment. Other reasons, such as concerns of potential damage from treatments (scars from the surgery, weight loss, alopecia, etc.), that cause stress and lowers quality of life among the breast cancer patients. Negative psychological response is found related with development of the disease, immune cell response and survival rates (Shapiro et al., 2001). Science, technology and society (STS) perspective of the project will focus on breast cancer patients's psychological response to the treatment and its effect on treatment's outcomes. Analyzing the breast cancer from two perspectives will help us to improve the treatment options which can lead to improved physical and psychological outcomes of the breast cancer.

Focused Ultrasound and Adenosine Receptor Blockade

FUS is the non-ionizing, non-invasive concentration of sound waves into a localized ellipsoid volume. FUS can be precisely directed with millimeter accuracy using MRI or ultrasound assistance. This precision enables the precise application of heat to damage and eliminate tumor tissue while protecting the healthy surrounding tissues in between and on the periphery (Sheybani et al., 2020). FUS is considered to be the best available treatment because of non-invasive procedure, high flexibility over treated area and control of delivered dose (Gianfelice et al., 2003). The clinical research conducted on 12 breast cancer patients with single

invasive tumor smaller than 3.5 mm in diameter showed no long-term complications or chronic pain has been reported (Gianfelice et al., 2003).

In this project first we will compare the impact of two different high intensity FUS; thermal ablation (T-FUS) and boiling histotripsy (BH) (a.k.a mechanical ablation). When high-intensity ultrasound travels through tissues, it is consistently absorbed by the tissue and converted into thermal energy. As a result, the temperature of the tissue rapidly increases to a range of 65–100 °C at the focal point, leading to the necrosis, thermal ablation, of cells within the tissue (Figure 1) (Chen et al., 2022). Unlike thermal ablation, mechanical ablation typically employs short pulses, which restrict the overall heat production during the procedure. Histotripsy is a treatment that employs short pulses at pressures that exceed the tissue's inherent cavitation threshold (Peng et al., 2019). In this project, we will systematically dissect the impact of high intensity FUS (i.e thermal and mechanical ablation) in the release of ATP.

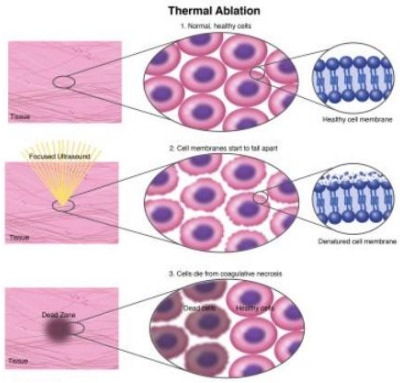

Figure 1. Thermal ablation

Clinically, adenosine receptor antagonists have surfaced as a potential target for anti-tumor response and a mechanism for inhibiting metastatic formation to distal tissues (Cekic et al., 2012). Adenosine plays a crucial role in the activation of four G protein-coupled receptors, with A2AR and A2BR notably exhibiting immunosuppressive effects. Previous work has shown that cells release elevated levels of ATP during apoptosis, in turn yielding high levels of ecto-adenosine that can promote anti-inflammatory effects upon engagement with adenosine receptors. Specifically, it has been shown that enrichment in ecto-adenosine levels results in a reduction in anti-tumor activity and elevation in mechanisms of immunosuppression in solid

tumor microenvironments (Cekic et al., 2014). In this project, we interrogate this phenomenon in the context of focused ultrasound (FUS) ablation regimens, and interrogate combination FUS approaches leveraging adenosine receptor blockers as a more favorable approach for fully unleashing the immunogenicity of FUS. Thus, in the second part, we will assess the effects of four distinct adenosine receptor blockers (comprising two A2B blockers, one A2A blocker, and one non-selective blocker) on tumor growth and survival rates in a murine model. According to the results will choose the most effective adenosine blocker to combine with customized FUS parameter.

In conclusion, we will compare thermally and mechanically ablative forms of FUS for rational combination with A2AR and/or A2BR blockers in murine TNBC. This will first require us to customize our ultrasound-guided FUS system for this application and design and optimize acoustic parameters in our model system. Since we will be using a luciferase-expressing mouse model, we will use bioluminescence imaging to track primary and distal lesion progression. We will then design rational treatment paradigms involving adenosine receptor blockade and FUS, wherein efficacy will be evaluated with tumor outgrowth and survival data.

Psychological Response of Breast Cancer Patients on Treatments

The experience of breast cancer, from diagnosis to post-treatment, is marked by considerable stress. Alongside the physical challenges, patients also deal with the psychological effects of the disease and treatment which leads to lower quality of life and response to the treatment. One of the main reason that patients' level of distress and anxiety is related to the low body image. Patients often find current surgical treatments unfavorable due to the scarring that occurs in surgical areas. Patients who had mastectomy or invasive breast surgery show higher body image distress compared to patients who received non-invasive treatment (Chen et al.,

2012). Previous study showed lower body image, physical attractiveness, and femininity among breast cancer survivors compared to pre-surgery/treatment. Data collected from different treatment recipients showed that almost 40% of the patients experienced stress in looking at their naked body. Those results also stated that there is significant relationship between body image and stress, which leads to lower quality of life (Begovic-Juhant et al., 2012). The increased stress among the breast cancer patients doesn't merely affect their quality of life and mental health but also exerts an impact on their response to treatment. It is known that severe chronic stress negatively affects immune cell response in breast cancer patients. Elevated stress has a negative correlation with natural killer (NK) cell lysis and ability of NK cells. Patients with low NK cells have higher risk of infection, and prolonged disease (Andersen et al., 1998). Decreased immune response leads to lower survival rate among the breast cancer patients. In the socio-technological aspect of the research, our focus will be on stress related to body image, as well as the evaluation of psychological responses to both invasive and non-invasive treatments among breast cancer patients. Given the limited number of participants in similar studies, unlike the technical part of the research that focuses on TNBC patients, we will gather data from various types of breast cancer patients. Participants will be selected from different treatment groups. To assess the responses, we will utilize the Impact of Event Scale (IES) and a 10-item Body Image Scale (BIS).

Conclusion

In this research we will integrate FUS with adenosine blockers. Our objective is to amplify the FUS effect by inhibiting the activation of G-coupled protein receptors, particularly A2A and/or A2B, as their activation has been demonstrated to induce immunosuppression within the tumor microenvironment. To achieve this, first we will measure ATP release in 4T1-LUC

cells that go under FUS-induced apoptosis. In this step we will compare two different FUS regiments (mechanical and thermal) and optimize the parameters of the FUS system. Then by using the insights from that, we will determine the FUS regiments compatibility and efficiency through corporation with adenosine receptor blockers.

Beyond the physical implications of breast cancer, patients' mental well-being and their response to treatments are profoundly influenced by their stress levels. A significant proportion of breast cancer patients experience elevated stress levels during diagnosis and post-treatment phases. The primary contributor to this increased anxiety among breast cancer patients is a negative body image, which can lead to concerns such as reduced femininity and attractiveness. Increased stress levels have a negative impact on overall quality of life and treatment response, ultimately leading to decreased survival rates among breast cancer patients. From a socio-technological perspective, we aim to investigate the psychological response of breast cancer patients to invasive versus non-invasive treatments. We anticipate that patients who receive non-invasive treatments, such as FUS, will exhibit lower stress levels associated with body image concerns.

References

Andersen, B. L., Farrar, W. B., Golden-Kreutz, D., Kutz, L. A., MacCallum, R., Courtney, M. E., & Glaser, R. (1998). Stress and immune responses after surgical treatment for regional breast cancer. *JNCI: Journal of the National Cancer Institute*, *90*(1), 30–36. https://doi.org/10.1093/jnci/90.1.30

Baqutayan, S. M. (2012). The effect of anxiety on breast cancer patients. *Indian Journal of Psychological Medicine*, *34*(2), 119–123. https://doi.org/10.4103/0253-7176.101774

Begovic-Juhant, A., Chmielewski, A., Iwuagwu, S., & Chapman, L. A. (2012). Impact of body image on depression and quality of life among women with breast cancer. *Journal of Psychosocial Oncology*, 30(4), 446–460. https://doi.org/10.1080/07347332.2012.684856

Cekic, C., Sag, D., Li, Y., Theodorescu, D., Strieter, R. M., & Linden, J. (2012). Adenosine A2B receptor blockade slows growth of bladder and breast tumors. *The Journal of Immunology*, 188(1), 198–205. https://doi.org/10.4049/jimmunol.1101845

Cekic, C., Day, Y.-J., Sag, D., & Linden, J. (2014). Myeloid expression of adenosine A2A receptor suppresses T and NK cell responses in the solid tumor microenvironment. *Cancer Research*, 74(24), 7250–7259. https://doi.org/10.1158/0008-5472.can-13-3583

Chen, Z., Cheng, L., Zhang, W., & He, W. (2022). Ultrasound-guided thermal ablation for hyperparathyroidism: Current status and prospects. *International Journal of Hyperthermia*, *39*(1), 466–474. https://doi.org/10.1080/02656736.2022.2028907

Chen, C.-L., Liao, M.-N., Chen, S.-C., Chan, P.-L., & Chen, S.-C. (2012). Body image and its predictors in breast cancer patients receiving surgery. *Cancer Nursing*, 35(5). https://doi.org/10.1097/ncc.0b013e3182336f8b

Crown, J., O'Shaughnessy, J., & Gullo, G. (2012). Emerging targeted therapies in triple-negative breast cancer. *Annals of Oncology*, *23*, vi56–vi65. https://doi.org/10.1093/annonc/mds196

Gianfelice, D., Khiat, A., Amara, M., Belblidia, A., & Boulanger, Y. (2003). MR imaging–guided focused US ablation of breast cancer: Histopathologic assessment of effectiveness—Initial Experience. *Radiology*, 227(3), 849–855. https://doi.org/10.1148/radiol.2281012163

Hammami, A., Allard, D., Allard, B., & Stagg, J. (2019). Targeting the adenosine pathway for cancer immunotherapy. *Seminars in Immunology*, *42*, 101304. https://doi.org/10.1016/j.smim.2019.101304

Hu, Z., Yang, X. Y., Liu, Y., Morse, M. A., Lyerly, H. K., Clay, T. M., & Zhong, P. (2005). Release of endogenous danger signals from hifu-treated tumor cells and their stimulatory effects on apcs. *Biochemical and Biophysical Research Communications*, *335*(1), 124–131. https://doi.org/10.1016/j.bbrc.2005.07.071

Lin, N. U., Claus, E., Sohl, J., Razzak, A. R., Arnaout, A., & Winer, E. P. (2008). Sites of distant recurrence and clinical outcomes in patients with metastatic triple-negative breast cancer. *Cancer*, *113*(10), 2638–2645. https://doi.org/10.1002/cncr.23930

Peng, C., Sun, T., Vykhodtseva, N., Power, C., Zhang, Y., Mcdannold, N., & Porter, T. (2019). Intracranial Non-thermal Ablation Mediated by Transcranial Focused Ultrasound and Phase-Shift Nanoemulsions. *Ultrasound in medicine & biology*, *45*(8), 2104–2117. https://doi.org/10.1016/j.ultrasmedbio.2019.04.010

Shapiro, S. L., Lopez, A. M., Schwartz, G. E., Bootzin, R., Figueredo, A. J., Braden, C. J., & Kurker, S. F. (2001). Quality of life and breast cancer: Relationship to psychosocial variables. *Journal of Clinical Psychology*, *57*(4), 501–519. https://doi.org/10.1002/jclp.1026

Sheybani, N. D., Witter, A. R., Thim, E. A., Yagita, H., Bullock, T. N., & Price, R. J. (2020). Combination of thermally ablative focused ultrasound with gemcitabine controls breast cancer via adaptive immunity. *Journal for ImmunoTherapy of Cancer*, *8*(2). https://doi.org/10.1136/jitc-2020-001008