# Quantitative Characterization of Anti-Tumor Immune Response in Metastatic Breast Cancer Induced by Focused Ultrasound

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**Background:** Despite significant advances in breast cancer screening and therapy, the challenges associated with metastasis, at initial diagnosis or with disease recurrence, remain largely unaddressed. With 5-year survival rates less than 25%, metastatic breast cancer presents the most significant barrier to reducing the mortality associated with breast cancer. Recently, cancer therapy research has shifted from generalized treatments that kill rapidly growing cells (e.g. cancer cells, hair follicles, etc.), like chemotherapy and radiation, to a more durable therapy intended to prevent the development of a cancer resistant to treatment. A promising method to accomplish this individualized approach is cancer immunotherapy, which activates the patient's own immune system against their tumor cells. While the immune system has the ability to kill tumor cells, breast tumors and their metastases have proven particularly adept at evading the immune system. As such, immunotherapy seeks to overcome the protective barriers that the tumor creates, so that immune cells can identify and eradicate the tumor. Previous research has demonstrated that creating acute inflammation in the tumor, often by application of heat or localized radiotherapy, may be an effective method to trigger a therapeutic immune response. Although several means of inducing acute inflammation in tissue exist, there is one easily accessible, advantageous, and non-invasive option that warrants further exploration: ultrasound. Similar to the ultrasound method used for imaging, focused ultrasound (FUS) concentrates ultrasound waves, like light through a magnifying glass, to noninvasively create a focal point of acoustic energy in either breast tumors or their metastases. The body responds to the FUS treatment like a wound and sends immune cells to the tumor, thus effectively overcoming the natural masking defenses of the tumor cells. FUS is applied externally, is nonionizing (unlike radiation therapy), is FDA-approved for other applications, and exists as a clinical instrument. Specifically, mechanical FUS (mFUS) will be used to induce cell disruption without heat deposition into the tissue. We also seek to combine mFUS with an immunostimulant (CpG), which is known to create desirable anti-tumor immunity. Our approach of activating the immune system with mFUS combined with a known immunostimulant could be a reliable method to unlock the immune system's natural cancer-fighting capabilities to **eliminate the mortality associated with metastatic breast cancer**.

**Our approach:** Our approach centers on a mouse model that develops breast cancer and metastases with disease progression similar to human breast cancer. mFUS will be applied to a single mouse tumor, either alone or in combination with CpG, at a low intensity to avoid direct death of the tumor cells. Tumor size, number, and location will be monitored after treatment to track disease progression. These measures of progression and outcome are critically necessary to support our existing, successful studies in creating a robust, high-impact NIH R01 application. Mice will be euthanized at a humane endpoint determined by tumor burden, and further analysis of collected tissues will allow for more meaningful evaluation of the induced immune response. The immune cell populations in the tumors will be measured to assess distribution and quantity of specific immune cell types as recruitment of specific cells to the tumor is a useful predictor of an anti-cancer immune response. This immune response will be evaluated by specific measurements after mFUS alone or with CpG treatment. We will use engineering-based measurements to connect the mFUS treatment to anti-cancer immunity through a number of intermediate steps. Our work is based, in part, on the often-repeated observation that there is 'no long term remission without immune involvement', even for conventional chemotherapies and radiation therapy. FUS has demonstrated potential to induce systemic anti-tumor immunity for lasting disease-free remission. **This will be the first attempt to generate durable, systemic anti-tumor immunity *in vivo* using mFUS and CpG for localized treatment of metastatic breast cancer.** We intend to examine the impacts of this novel immunotherapy in generating effective anti-cancer immune involvement in ways that could inform the development of next-generation therapeutic approaches for metastatic breast cancer.

**Impact:** The overall goal of this study is to effectively treat metastatic breast cancer for improved long-term survival and quality of life, using treatments that are less toxic and have fewer side effects than current therapies. With no current cure for metastatic breast cancer and such a challenging survival outlook when compared to the localized form of the disease, this approach has immense potential for clinical impact.