



Focused Ultrasound and Immunotherapy Workshop

February 11, 2015
New York, NY

WORKSHOP SUMMARY

INTRODUCTION

The field of cancer immunotherapy is progressing rapidly with several new agents approved by the FDA just this year. Most exciting are so-called checkpoint inhibitors that “take the brakes off” the immune response and enable a stronger immune attack against cancer. Despite their demonstrated benefits of tumor regression and increased overall survival, these therapies are effective in only 20-40% of patients. Efficacy may be improved for patients with a baseline immune response prior to treatment, potentially elicited by radiation or other ablative therapies.

Ablative therapies – radiation, radiofrequency, cryoablation, laser, and focused ultrasound – have shown the ability to stimulate an immune response in preclinical and clinical studies.¹⁻⁴ In addition, therapies such as radiation have demonstrated success when used in combination with immunotherapy, by providing the initial immune response that immunotherapy can then enhance.^{5,6}

Focused ultrasound (FUS) could potentially be effective in combination with immunotherapy. Given its non-invasiveness, use of non-ionizing radiation, and ability for conformal and precise ablation with no dose limitations, FUS could be more appealing for this combination therapy than other ablative modalities.⁷

To understand the true potential for FUS immunomodulation in cancer therapy there are many questions that still need to be answered including:

- What are the key biomarkers to demonstrate an enhanced immune response? How can these be measured clinically?
- What has more clinical potential – mechanical or thermal effects of FUS?
- Can FUS alone promote an immune response that is clinically significant?
- Is FUS combined with immunotherapy the most clinically relevant approach? Which therapies? Which patient population(s)?
- What type of clinical evidence is needed to move the field forward? Can we acquire this from immune monitoring of patients within existing FUS clinical trials? Should samples from multiple global sites be assessed by a core lab?
- What technology advancements are needed to further advance this field?

On February 11, 2015, the Focused Ultrasound Foundation partnered with the Cancer Research Institute to convene a one-day meeting of scientists and clinicians to help address these (and other) questions. The group discussed the current status of and future directions for focused ultrasound research as it relates to cancer immunotherapy.

The workshop brought together nearly 30 investigators with expertise in cancer immunology, focused ultrasound, radiation oncology, and clinical immunotherapy, as well as representatives from three non-profit organizations dedicated to bringing patients new cancer therapies.

Participants discussed the state of the field, current challenges, and future research directions for using focused ultrasound alone or in combination with cancer immunotherapies such as checkpoint inhibitors and cancer vaccines -- with an emphasis on how to prioritize future research directions and encourage collaboration to address knowledge gaps in the field.

BACKGROUND

Cancer Immunotherapy

Cancer immunotherapies are agents that harness the power of the immune system to fight cancer. Unlike traditional cancer treatments such as chemotherapy and radiation, which directly kill tumor cells, immunotherapy operates indirectly, through the intermediary of the immune system. Immunotherapies empower the immune system to seek out and destroy cancer cells specifically. Because of the immune system's extraordinary power to selectively target cancer antigens and adapt to a changing landscape of antigens, this approach has the potential to greatly improve cancer treatment, providing durable, long-term responses in many cases.

Some of the most dramatic results have been seen in melanoma, using CTLA-4 and PD-1 blocking antibodies. The agents, called checkpoint inhibitors, are directed to negative regulatory molecules on T cells. By blocking these molecules, they relieve immunosuppression and reanimate suppressed T cells. In a recent trial of ipilimumab (anti-CTLA-4) and nivolumab (anti-PD-1), the 2-year survival rate of patients with advanced melanoma was nearly 90% at the best responding dose. This is in contrast to a 2-year survival rate of 15% for standard chemotherapy. Other indications have shown responsiveness to checkpoint blockade as well, including lung, kidney, and bladder cancer.

The most impressive aspect of treatment with cancer immunotherapies is the durability of responses in a small percentage of cases. Some of the earliest melanoma patients to receive ipilimumab treatment are now 10 years out and still in remission. Nonetheless, not everyone responds to these treatments, creating a need to identify ways to improve response rates. There is some evidence that those who respond to checkpoint blockade therapy have tumor-infiltrating immune cells that recognize cancer antigens, and that it is these cells that are re-activated by releasing immunosuppression. Discovering ways to promote T cell infiltration into tumors is an active area of research. FUS may be one way to prime an immune response to tumor antigens.

Several approaches to cancer immunotherapy are actively being explored. In addition to checkpoint blockade, major areas of focus are the use of adoptive cell therapies, including chimeric antigen receptor (CAR) T cell therapies, and cancer vaccines. With all of these therapies, the main complication

is the risk of setting off autoimmune toxicities since immune checkpoints and other regulators exist primarily to prevent tissue damage through autoimmunity.

Focused Ultrasound Immunomodulation

Many preclinical and clinical studies have demonstrated that FUS can elicit an immune response that in laboratory animals has led to enhanced overall survival and protection from growth of new tumors when re-challenged (see Table 1).⁸⁻³³

For example, a randomized study of 48 patients comparing FUS treatment prior to radical mastectomy with surgery alone found that those who had received the focused ultrasound treatment had significantly higher tumor-infiltrating T cells, B cells, and NK cells.² Numerous other preclinical and clinical studies have shown that FUS treatment boosts various parts of the immune system through an increase in CTL cytotoxic activity,^{8,9} activation of dendritic cells,¹⁰ up-regulation of heat shock proteins and ATP,^{11,12} and an increase in circulating CD4+ lymphocytes.¹³

Focused ultrasound is unique among ablative modalities in that it has three distinct “modes” that can be used, each with a different mechanism of action. The first, thermal ablation, is similar to many other technologies in that the tissue at the focal point is heated, causing coagulative necrosis. The second mechanism is mechanical lysis due to acoustic cavitation, rapid expansion and contraction of microbubbles within the targeted tissue. And the third is mild hyperthermia, which will occur in the area adjacent to a thermally ablated region, but can also be induced intentionally for uniform low-level heating of a region of interest.^{14,15} Each of these mechanisms is capable of initiating an immune response by releasing antigens and danger signals from cancer cells (see Figure 1).

Furthermore, taking advantage of the mechanical mechanism of action, new research is looking into using FUS in combination with microbubbles to increase the permeability of cancer and immune cell membranes.¹⁶ These bubbles can also be loaded with tumor antigens, to more efficiently activate immune cells or immune response-encoding genes, and delivered to cancer cells for more sophisticated immune homing.¹⁷ Microbubbles in combination with FUS can also open the blood-brain barrier so that immune cells can target brain tumors.¹⁸

While initial results seem promising, this is still early-stage research, particularly in assessing the impact of FUS on long-term survival.^{10,19} Important future work is needed to more fully understand the different mechanisms for FUS-induced immune response and how to most effectively use them in combination with immunotherapy.

WORKSHOP PRESENTATIONS

Several brief presentations by workshop participants provided an overview of the current state of focused ultrasound immunomodulation research and the use of immunotherapies in combination with radiation.

Betsy Repasky introduced the cancer immunity cycle, and pointed out the various steps in the cycle where focused ultrasound (FUS) could potentially play a role in enhancing the immune response to

cancer. She also encouraged the group to take a holistic view of the process instead of focusing on just one step, since it is possible that FUS will alter several variables within the immune cycle.

Kathy Ferrara presented her work in identifying the baseline immune response in mice to several different sets of focused ultrasound parameters (e.g. mild hyperthermia vs. ablation), and also the use of thermally-sensitive nanoparticles loaded with chemotherapy agents to further enhance these effects. Ablation parameters led to a more pronounced immune response (e.g. significantly more dendritic cells, CD4+/CD8+ T cells in FUS-treated tumors) and nanoparticles enabled more effective delivery of chemotherapy to tumor cells. This combination of FUS with loaded nanoparticles could be an attractive method to treat cancer; it can be repeated many times due to its reduction in systemic toxicity as compared to the systemic delivery of non-bound chemotherapeutics. Adding an immune adjuvant to the combination approach further enhanced the immune response.

Kullervo Hynynen discussed the ability of FUS in combination with microbubbles to deliver natural killer cells across the blood brain barrier in a mouse model of metastatic breast cancer. It was important to inject the NK-92 cells before FUS application. Different treatment regimens – i.e. number of treatments per week, number or weeks of treatment – resulted in different overall survival; treatment optimization is still needed. Because this method uses focused ultrasound at a much lower power than thermal ablation, it can theoretically be used to target all regions in the brain as well as other organs.

Tanya Khokhlova presented her group's unique focused ultrasound technique called boiling histotripsy. This mechanical mechanism of tumor lysis leaves a "soup" of sub-cellular debris that is not thermally denatured and immunogenic. Using a rat model of renal cell carcinoma, they found an immediate increase in systemic levels of HMGB1, TNF-alpha, and IL-6 after histotripsy treatment.

Feng Wu presented results from a clinical trial of focused ultrasound to treat breast cancer and from several pre-clinical studies. The clinical trial measured the local expression of tumor antigens and found a correlation between HSP-70 and epithelial membrane antigen expression and FUS treatment. The preclinical studies displayed an increase in tumor-infiltrating lymphocytes and a reduction in serum immunosuppressive cytokines, indicating that focused ultrasound may modify tumor immunogenicity.

Mark Hurwitz presented data from his team demonstrating that routine clinical radiotherapy results in increased serum HSP70 levels along with stimulation of pro-inflammatory cytokines and makers of immune response including CD8+ T and NK cells. Laboratory studies confirmed the tumor specificity of this response and enhanced dendritic cell activation. He presented on how hyperthermia is associated with these and additional immune effects. In addition to their proven anti-neoplastic effects apart from immunotherapy, the combination of radiation and hyperthermia may elicit a stronger anti-tumor immune response than either modality alone including abscopal effects.³⁴

Jonathan Schoenfeld provided an introduction to the use of immunotherapies in combination with radiation. This combination approach attempts to address the current challenge: not all patients respond to immunotherapy, and not all who respond do so quickly and uniformly. Radiation leads to immunologic cell death that alters the tumor microenvironment (e.g. stimulates antigen release), and may boost the immune response. Successful studies have been completed using radiation with many different types of immunotherapies ranging from vaccines to immune adjuvants to checkpoint inhibitors.

Chandon Guha presented work using FUS as an in situ tumor vaccine by using low intensity focused ultrasound (LOFU) prior to high intensity focused ultrasound (HIFU) thermal ablation. He found that this combination in this order had a very good response, and proposed that the LOFU caused ER stress and protein misfolding, which increased the immunogenicity of the tumor, enabling the HIFU to be more effective. LOFU was also combined with radiation therapy with good results.

EVIDENCE GAPS DISCUSSION

Following the presentations, a robust discussion focused on three main topics:

- What evidence is still needed to prove there is a FUS-induced immune response?
- How can current and future FUS oncology clinical studies be utilized to gain insight into the FUS-induced immune response?
- There is a need to move towards standardization of some aspects of FUS immunomodulation preclinical studies – i.e. focused ultrasound parameters, tumor models, etc

Overarching questions for this discussion included: Is focused ultrasound an appropriate treatment for cancer? If so, which cancers? Does the mechanism of action include an immune response? If so, what kind of immune response is induced? Is this immune response potent enough or is it necessary for focused ultrasound to be combined with an immunotherapy?

There was a general consensus among the immunotherapy experts that there is not enough evidence to conclude that focused ultrasound induces a clinically-significant immunomodulatory response. Anecdotal evidence suggests there is a general immune response (e.g. expression of tumor antigens), but the specific effects on the tumor microenvironment (i.e. specific biomarkers) have not been sufficiently elucidated. Upon further discussion, it was evident that the field of immunology does not yet support using a single panel of tests (biomarkers) to prove an immune response in all cases; optimal tests may vary based on tumor characteristics, therapy mechanism, etc. It was proposed that mouse models could be used to help identify and optimize the best panels for focused ultrasound studies.

The discussion then transitioned to identifying opportunities for short-term gains with the tools currently available, specifically how to gain clinical evidence of the focused ultrasound induced immune response. A potential early opportunity is bone metastases, the only FDA approved oncology indication for focused ultrasound. However, there was some concern about the difficulty of performing various immunologic stains on bone tissue, as well as a lack of immunology research specific to bone cancer. There was consensus that current and future clinical trials for other oncological indications – e.g. prostate cancer and sarcoma – may be better opportunities to accumulate data on the immune effects of focused ultrasound. FUSF could propose additions to the protocols of these trials to include collection of tissue and blood samples, both pre- and post-treatment, that could be either assessed prospectively and/or stored for retrospective testing. Important considerations include: (1) immune assessment expertise and capabilities at the institutions leading the clinical trials, (2) whether a core laboratory for assessment is necessary, (3) expected evolution of the specific panel(s) for testing (i.e. having tissue available for future testing is a good idea).

The pathway towards clinical investigation of the potential benefits of focused ultrasound in combination with immunotherapies has not yet been defined. A potential model for this combination approach, including the sequence of treatments and immune assessment protocol, is the upcoming breast cancer clinical trial at the University of Virginia. This trial will investigate the use of focused ultrasound and an IDO (indoleamine 2,3-dioxygenase) inhibitor for treatment of metastatic breast cancer. Early talks with the FDA have determined that the regulatory pathway for this combination drug-device treatment should flow through the Center for Drug Evaluation Research rather than through the Center for Devices and Radiological Health; an IND will be submitted. The protocol details – specific biomarkers to monitor, clinical outcome measures – are still being defined, but it will likely include assessing T cell infiltration in the primary tumor (and metastases) and systemic cytokines. Once the details are finalized, the group will gladly share their proposed immune assessment protocol.

A word of caution was raised in regards to moving straight to clinical measurements of the FUS-induced immune response without first performing additional and more robust preclinical work. This work should be done to inform clinical protocols – to give the best chance at success and to provide context for failures. Several considerations for future preclinical work were discussed:

- Genetically modified mouse lines may be more realistic tumor models than transplantable tumors, but it is reasonable to use transplantable tumors to determine the initial feasibility of protocols (e.g. specific ultrasound parameters, drug type) before testing in a genetically modified mouse line.
- Pancreatic tumors and gliomas – both with dense stroma – would be good models to test the mechanical effects of focused ultrasound to enable penetration of the stroma for a more effective immune response.
- Breast and prostate tumors may be good models to test the thermal effects of focused ultrasound since there are several FUS systems in clinical trials for these conditions.
- Using defined antigen models to prove an antigen-specific immune response would be much easier and cheaper to test in animal models than in patients.
- A study investigating the efficacy of focused ultrasound in nude mice would indicate the importance of the immune system in FUS's mechanism of action.
- Several groups should use the same tumor model to enable better comparisons and eliminate some redundancy.

A major takeaway from the discussion was the need for better standardization among the field in order to better understand and compare results. Because of the different mechanisms of focused ultrasound to induce an immune response – thermal (hyperthermia or ablation) and mechanical – and also the variability of the parameters within each of these categories, it is difficult to compare the results from two separate studies. One proposed solution for comparing studies using FUS in a thermal mode (hyperthermia or ablation) is to more fully investigate the thermal dose response. Because many FUS systems are MR-compatible, the thermal measurement capability of MR can be used to correlate thermal dose with the immune effects. Cavitation was mentioned as a way to compare studies using FUS in a mechanical mode, but current technology is unable to quantify the amount of cavitation, only its presence or absence.

To date, there has been a lack of consistency in protocols used to assess focused ultrasound's effects on the immune response. As the field moves forward, it will be necessary to receive guidance from immunologists to develop more consistent protocols. However, as was evident from the discussion, there can also be a great deal of variability among results obtained by different labs performing the same tests. For future preclinical and clinical work, focused ultrasound groups are strongly encouraged to collaborate with immunology experts during their study design and assessment of results. These experts should design immune assessment protocols appropriate for the specific tumor and proposed mechanisms of treatments. If and when a consensus can be reached on the best testing panel(s) to use for a wide range of studies, it may be beneficial to identify a core lab that can process samples from multiple sites to reduce variability within the results.

OUTCOMES AND NEXT STEPS

At the conclusion of the workshop, a high level list of next steps was determined to include:

- Preclinical work comparing the immune responses induced by different “modes” of focused ultrasound including immune effects elicited by various thermal dose profiles; should include standardization of focused ultrasound parameters that are labeled HIFU, LOFU, histotripsy, hyperthermia. See Appendix for more detail.
- Preclinical work to assess focused ultrasound in combination with immunotherapies (e.g. checkpoint inhibitors, vaccines, immune modulators).
- Use existing FUS clinical oncology trials to assess focused ultrasound induced immune response

Additionally, new multi-disciplinary research collaborations were fostered. The Focused Ultrasound Foundation and the Cancer Research Institute encourage applications for funding collaborative projects in this field. FUSF will also lead efforts to promote further awareness of this field with other potential funding organizations and seek co-funding opportunities to support collaborative projects. FUSF also intends to coordinate smaller group meetings, with the inclusion of clinical experts, to further define roadmaps for specific clinical indications. A working group of investigators in the field will be established to enable more effective communication and collaboration on study design as the field progresses.

WORKSHOP PARTICIPANTS

Focused Ultrasound and/or Immunotherapy Experts

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Kathy Ferrara – University of California, Davis
Larry Fong – University of San Francisco
Tim Greten – National Cancer Institute
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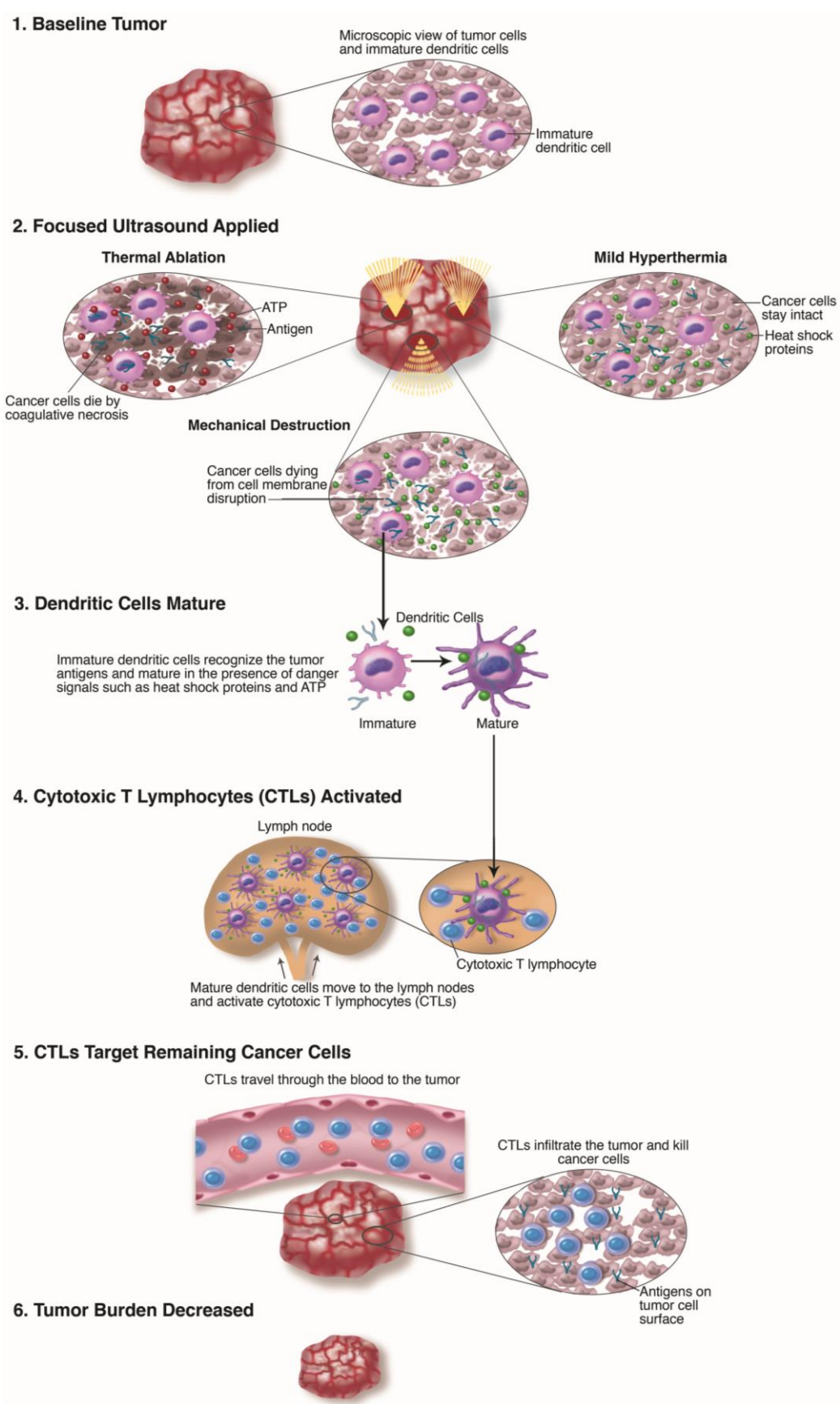


Figure 1. Proposed mechanisms of focused ultrasound induced immunomodulation. Thermal ablation, mild hyperthermia, and mechanical destruction may all have different effects on the tumor microenvironment that lead to a unique type of immune response.

Paper	Year	Clinical or Preclinical	Indication (# of Patients)	Immunologic Effect
Yang et al. ²⁰	1992	Preclinical	Neuroblastoma	Resistance to tumor re-challenge
Rosberger et al. ²¹	1994	Clinical	Choroidal Melanoma (5)	2/3 patients reverted CD4+/CD8+ ratio from abnormal levels
Madersbacher et al. ²²	1998	Clinical	Prostate Cancer (5), Bladder Cancer (4)	Increase in HSP27 expression. Up-regulation strongest 2-3hr after ablation, but still demonstrable after 5-8 days
Wang and Sun ²³	2002	Clinical	Pancreatic Cancer (15)	Increased CD3+ & CD4+ cells and CD4+/CD8+ ratio in 10 patients, not significant. NK cell activity was significantly enhanced
Kramer et al. ²⁴	2004	Clinical	Prostate Cancer (6)	Up-regulated expression of HSP72, HSP73, GRP75 and GRP78. Increased release of IL-2, IFN-gamma, and TNF-alpha. Decreased release of IL-4, -IL-5, and IL-10
Wu et al. ¹³	2004	Clinical	Osteosarcoma (6), Hepatocellular Carcinoma (5), Renal Cell Carcinoma (5)	Increased CD4+ T cells and CD4+/CD8+ ratio
Hu et al. ¹¹	2005	Preclinical	Colon Adenocarcinoma	ATP and HSP60 is released from tumor cells. Activation of DCs and macrophages. Enhanced IL-12 and TNF-alpha secretion. Mechanical activation of APCs is stronger than thermal
Hu et al. ⁸	2007	Preclinical	Colon Adenocarcinoma	Mechanical FUS better at activating DCs than Thermal FUS. Resistance to tumor re-challenge. Increased CTL activity and tumor-specific IFN-gamma-secreting cells
Hundt et al. ¹²	2007	Preclinical	Melanoma, Fibroma, Squamous Cell Carcinoma	HSP70 expression can be induced at lower temperatures than heat stress alone
Wu et al. ²⁵	2007	Clinical	Breast Cancer (23)	Epithelial membrane antigen and HSP70 had 100% expression in tumor debris. Other proteins found were CA15-3 (52%), TGF-beta1 (57%), TGF-beta2 (70%), IL-6 (48%), IL-10 (61%), and VEGF (30%)
Zhou et al. ²⁶	2007	Preclinical	Hepatocellular Carcinoma	Increased CD4+ levels and CD4+/CD8+ ratio. Decreased CD8+ levels. Resistance to re-challenge
Zhou et al. ²⁷	2007	Clinical	Liver Cancer (13), Sarcoma (2)	Decreased serum VEGF, TGF-beta1, and TGF-beta2 levels. Patients without metastases had significantly lower immunosuppressive cytokine levels
Kruse et al. ²⁸	2008	Preclinical	[Transgenic Reporter Mice]	Peak HSP70 expression at 6-48 hours after treatment. Significant activity up to 96 hours after treatment
Xing et al. ²⁹	2008	Preclinical	Melanoma	Increased CTL cytotoxicity. No increased risk of metastasis after HIFU treatment
Deng et al. ¹⁰	2009	Preclinical	Hepatocellular Carcinoma	Increased CTL cytotoxicity, DC activation, IFN-gamma and TNF-alpha secretion

Xu et al. ³⁰	2009	Clinical	Breast Cancer (23)	Increase in local infiltration and activation of DCs and macrophages. Greater DC and macrophage expression of HLA-DR, CD80, and CD86 in the HIFU group
Lu et al. ³¹	2009	Clinical	Breast Cancer (23)	Increased tumor-infiltrating B lymphocytes, NK cells, and CD3, CD4+, and CD8+ T cells. Increased CD4+/CD8+ ratio and FasL+, granzyme+, and perforin+ TILs
Liu et al. ³²	2010	Preclinical	Melanoma	Increased DC infiltration and maturation. Sparse-scan mode more effective than dense-scan at enhancing DC infiltration and maturation
Zhang et al. ¹⁹	2010	Preclinical	Hepatocellular Carcinoma	Increased CTL cytotoxicity and DC activation. Resistance to tumor re-challenge
Xia et al. ⁹	2012	Preclinical	Hepatocellular Carcinoma	Increased CTL cytotoxicity, IFN-gamma secretion, TNF-alpha secretion, and number of tumor-specific CTLs
Huang et al. ³³	2012	Preclinical	Prostate Cancer	Resistance to tumor re-challenge. Down-regulation of STAT3. Increased CTLs and decreased Tregs in the spleen and tumor draining lymph nodes
Liu et al. ¹⁶	2012	Preclinical	Colorectal Carcinoma	FUS + microbubbles increased TILs, infiltration of CTLs, and the CD8+/Treg ratio
Alkins et al. ¹⁸	2013	Preclinical	Metastatic Brain Tumor	FUS + microbubbles enables NK cells to cross the blood-brain barrier to target brain tumors

Table 1. Immunological Effects of Focused Ultrasound. All currently published clinical and preclinical studies that report immunologic effects of focused ultrasound treatment are listed. Only results pertaining to the immune system were included, although the studies may have other important results.

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APPENDIX

Preclinical Framework

A. Release of antigen.

There are several protocols where US can release antigen.

- i. Low intensity mechanical
- ii. High intensity mechanical (histotripsy)
- iii. High intensity thermal (HIFU)
- iv. Low/moderate intensity thermal (hyperthermia)

These could be compared with some additional downstream assay. Phantoms and CEA-transfected cell line could be a good fit. There is preliminary data comparing these but further investigation is needed.

B. Effects on tumor physiology

C. Effects on cytokine milieu, both local and systemic (inflammatory vs reparative)

D. Hypothesized and demonstrated direct effects on immune cells

Probably low and high intensity thermal and mechanical could all be tested.

E. Other unique things that US can do that could produce a profound change. These will be more repeatable but require a combination treatment.

- i. Opening the BBB or endothelium to let in cells and large molecules.
- ii. Enhancing the accumulation of drugs/adjuvants in tumors by other mechanisms (direct release).
- iii. Permeabilization of the tumor vasculature and/or stromal matrix by cavitation (e.g. pancreatic tumors) to enhance the lymphocyte infiltration of the tumor