



Focused Ultrasound and Cancer Immunotherapy Workshop

White Paper Summary

1-2 September 2021
Virtual Meeting

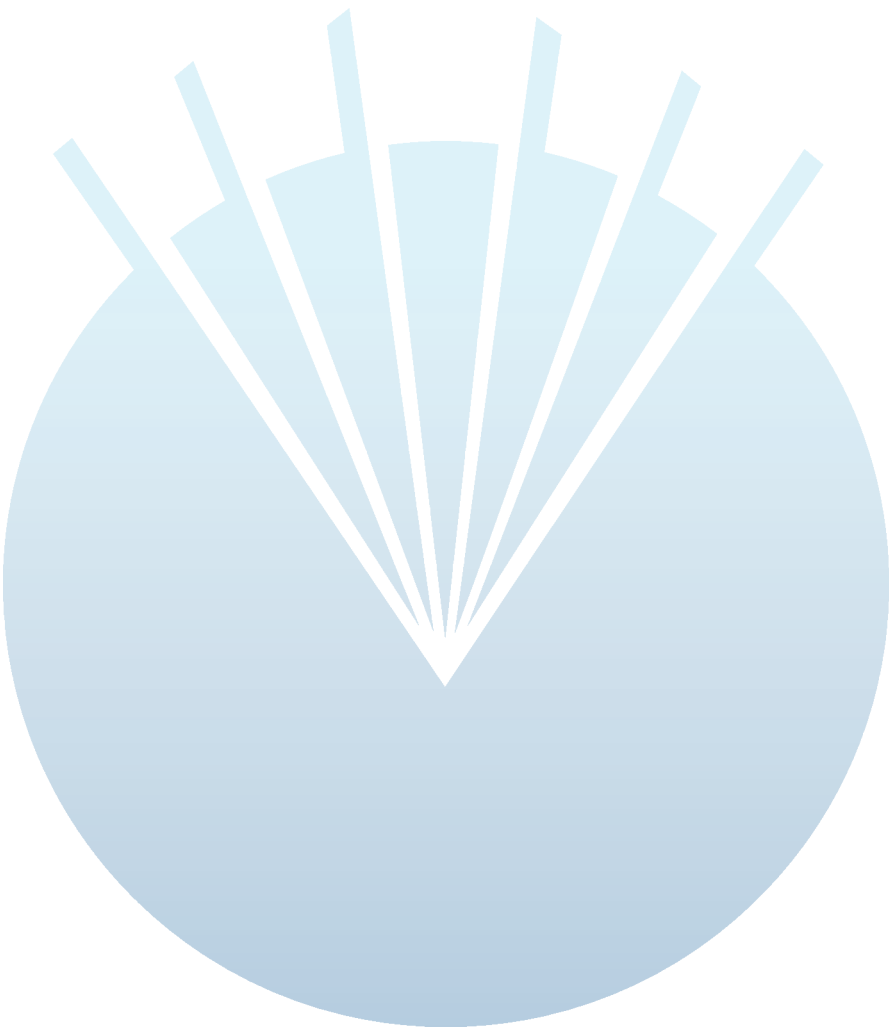
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**Focused Ultrasound
and Cancer Immunotherapy
Workshop**

1-2 September 2021

Executive Summary

The Focused Ultrasound Foundation and the Cancer Research Institute hosted their 4th workshop on focused ultrasound and cancer immunotherapy on September 1–2, 2021 (virtual). The meeting brought together critical stakeholders, including researchers, clinicians, industry, government, and others, to share and combine knowledge to advance the field. Focused ultrasound (FUS) is an early-stage non-invasive therapeutic technology that has the potential to improve the lives of millions of patients with a variety of medical disorders by providing an alternative or complement to existing treatment approaches.

The ultimate goal is to reduce the time it takes for FUS and cancer immunotherapy combination treatment(s) to reach clinical adoption. This workshop was another step towards accomplishing this goal, by critically evaluating the current body of evidence, assessing the value of ongoing work, and creating a roadmap of projects that will address any remaining gaps and “burning questions.” There were several presentations available to view before the meeting to orient participants to the current state of the field, and the bulk of the meeting itself included moderated discussion sessions to develop a roadmap forward.

The primary objectives of the workshop were to:

- 1 Develop a one to two-year action plan of projects that address the “burning questions” for the field and can potentially be supported by the FUS Foundation and/or Cancer Research Institute.;
- 2 Produce a white paper documenting the discussion and results of the meeting.
- 3 Create a collaborative environment to facilitate the achievement of our goals as rapidly as possible.

The community agreed on several major goals moving forward. The Focused Ultrasound Foundation (FUSF) in collaboration with CRI and PICI will work to develop central data analysis and storage hubs to promote cross-study comparisons and communication within the community. There was also consensus around the idea of hypothesis-driven study design on both the preclinical and clinical level. Careful consideration of the preclinical model or clinical disease target as well as the proposed FUS modality is necessary to ensure a positive outcome. Suggested preclinical projects included probing the effects of FUS on the tumor vasculature (with or without antiangiogenic therapy) and examining the outcomes of partial versus total tumor ablation. Phase 1 and window of opportunity trials were recommended to evaluate the effects of FUS in the clinical setting. A detailed roadmap and list of action items is provided at the end of this document, and the presentation and discussion sessions are available for viewing on [YouTube](#). The attendees are encouraged to reach out to either FUSF or CRI with additional ideas.

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Virtual Workshop Presentations and Discussion

Immune Effects of FUS

Presentation

Innate and Adaptive Responses

Eli Vlaisavljevich, PhD, briefly described the different FUS modalities. FUS is a non-invasive method for tissue ablation or modulation achieved with sound waves applied by an external transducer. Volumetric treatment of tumors is guided by real-time imaging. Bioeffects range from complete cell death (ablation) to reversible tissue modulation and improved drug delivery. FUS encompasses a broad range of thermal and non-thermal (mechanical) effects. Types of FUS therapies include:

- Thermal FUS
 - High-intensity focused ultrasound (HIFU), hyperthermia
- Non-thermal FUS ablation
 - Intrinsic threshold histotripsy, shock-scattering histotripsy, boiling histotripsy
- Non-ablative FUS therapies
 - Low-intensity focused ultrasound (LIFU), vascular disruption (blood-brain-barrier (BBB) opening & drug delivery)

FUS hyperthermia occurs at moderate or low-intensity FUS. The tissue at the focus absorbs energy at sub-lethal temperatures (40–45°C).

Histotripsy is a non-thermal and non-invasive FUS ablation method to generate a cavitation bubble cloud that results in the mechanical ablation of tissue into acellular debris with very high precision.^{1,2} Histotripsy can be used for tissue selective ablation for high-risk tumors as differences in tissue mechanical strength allows for the preservation of critical structures such as blood vessels, bile ducts, and nerves.

LIFU uses low amplitude pulsed FUS (pFUS) to induce a wide range of bioeffects. Mechanical effects of LIFU include causing inflammation, tissue disruption, enhanced permeability, neuromodulation, or drug delivery. LIFU can be used to selectively ablate cancer cells in vitro.³ LIFU can enhance drug delivery directly, or when used in combination with artificial agents such as microbubbles or nanoparticles.⁴ LIFU can be combined with acoustic droplet vaporization to non-invasively generate gas emboli within the vasculature surrounding the tumor, which may potentially be used to reduce tumor volume and can be combined with other therapies.⁵

Nanoparticle-mediated histotripsy uses acoustically active nanoparticles for the targeted histotripsy ablation of multi-focal and diffuse tumors.⁶

Moderated Open Discussion

Immune Effects by FUS Modality & Roadmap

Kelsie Timbie, PhD | Moderator

Attendees participated in an open discussion on the pre-recorded material.

A question was asked about the increased expression of IFN γ and PD-1 following histotripsy and whether this was specific to histotripsy or whether it was applicable to all FUS modalities. Increased IFN γ occurs after other non-thermal ablation modalities, such as irreversible electroporation, but it does not occur with thermal modalities. In some tumor types (pancreatic cancer (Pan02)), there is increased PD-L1 following histotripsy, but it is not observed in other tumor types (breast cancer (4T1)). There was agreement that this effect was more tumor-dependent than modality-dependent. In a kidney cancer model, IFN γ increases were delayed following histotripsy while IFN γ increases immediately in a glioblastoma (GBM) model. The timing of IFN γ increases following histotripsy (immediately or delayed) and the responses of specific kinds of cancer remains to be identified. There was a comment that mechanical HIFU enhanced expression of PD-L1 also occurs in myeloid cells (e.g., macrophages), and that more work is needed to assess how FUS affects specific immune cells.

Considering that sub-ablative radiotherapy is typically given prior to immunotherapy, there was a question on if this is relevant to the timing of FUS in combination with immunotherapy. One difference is that FUS is a single treatment and radiation therapy is usually multiple treatments over a period of time. In the neoadjuvant early-stage non-small cell lung cancer (NSCLC) setting, low-dose non-ablative radiation combined with immunotherapy increased pathological response in the combination group.⁷ There is supporting evidence to study FUS in combination with checkpoint inhibitors in the neoadjuvant setting, but the optimal FUS modality remains to be determined (ablative versus non-ablative). The way that a specific FUS modality interacts with the immune system should also be considered when designing future trials.

A question was asked on the effects of histotripsy on the tumor-draining lymph nodes. Participants agreed that this is very important to study, but there is little research in this area. Radio-labeled large proteins, such as albumin, tracked via PET suggests that FUS increases drainage of albumin from the ablated tumor. Using PET imaging of radio-labeled proteins is a promising method to study the movement of proteins out of the tumor.

There was a question on how much inflammation is considered beneficial following histotripsy or other FUS modalities. In some cases, cytokine production may increase tumor size. Participants discussed the treatment envelope for optimizing treatment response. This is an area of active research but remains unknown at this time. The early cytokine response following FUS was discussed, and whether the response is dependent on tumor type or FUS modality. Pattern recognition receptors play an important role through the damage-associated molecular patterns that are produced following treatment. These patterns drive the immune response, and the type of cell death is an important factor. The cytokines released following FUS depend on the types of cells that remain after treatment. For example, macrophage tumor cells produce IL-1 β and epithelial tumor cells produce IL-18. Another consideration is the timing and duration of cytokine release; prolonged cytokine exposure can be harmful to immune cells.

The wound healing immune response that follows FUS thermal ablation could suppress the effects of immunotherapy. A suggestion was made to use imaging, in place of flow cytometry, to track the long-term myeloid response to various FUS regimens. There is also the opportunity to use precision medicine tools for selecting patients that might respond better to FUS.

Participants were asked to comment on recommendations for future projects:

- Different cell lines influence the immune responses to FUS. Preclinical models, such as 4T1 (breast cancer), have limitations. It might be best to look at a tumor type that is very immunologically responsive to perform a ‘window of opportunity study’ in a subset of human patients. This could provide information on kinetics, timing, and rational therapeutic combinations that might accelerate FUS treatment for patients.
- Additionally, consider the baseline immune profile of the tumor to select a tumor type that will respond to combination treatment.
- A suggestion was made for a database to allow researchers greater access to and capability to share new publications and findings.
- Further discussion on a way to compare parameters of each treatment FUS modality. Particularly for successes, define the FUS treatment parameters along with the immune profile results.
- From a clinical standpoint, it might be interesting to take the success story from the PACIFIC trial and add FUS to the same trial design.
- Patient selection for the combination approach will ultimately involve personalized medicine. Biomarkers for patient selection (immune status and tumor type) need to be developed.
- There was a suggestion to consider if there is enough preclinical evidence for efficacy in a cancer type where checkpoint inhibitors do not typically achieve a response. If a cancer type could be identified, the next step would be to add FUS to an ongoing clinical trial to allow deep analysis of the immune response. Participants mentioned that early phase safety studies of FUS in combination with immune therapy are planned or recruiting patients.

Moderated Open Discussion

Immune Effects by Tumor Types & Roadmap

Frédéric Padilla, PhD | Moderator

- There was a suggestion to separate the brain tumor from the tumor microenvironment (TME). For example, instead of studying preclinical models of GBM, look at a metastatic melanoma model. This would allow comparison of FUS treatment applied both outside the brain and to the brain TME.
 - Some patients and cancers are not responsive to immunotherapy because of primary or adaptive resistance. It is important to consider prior therapies and the TME. Both responders and non-responders should be further studied. In order to use FUS to improve treatment, the specific goal (e.g. increasing CD4+ T cells in the tumor) needs to be identified.

- A participant mentioned that the field of immunotherapy has many gaps. For example, the reason that some melanoma patients respond to checkpoint inhibitors has yet to be defined. A list that identifies specific tumor types that do not respond to checkpoint therapies could be useful for identifying opportunities for FUS.
- Therapeutic cell trafficking is important and this idea should be explored further to see if FUS could support cytotoxic cell activity.
 - Preliminary evidence suggests that CD8+ T cell infiltration is not predictive of success for checkpoint inhibitors, but rather it is overall immune fitness. Several factors matter such as dendritic cells, antigen-experienced T cells, state of exhaustion, and the cytokine profile. The overall immune profile at baseline is a key factor in understanding how to optimize treatment.
 - There are not good biomarkers for immunotherapy, which makes applying a new technology like FUS difficult. An area that should be explored is immune cell infiltration in the TME (not just cell type but spatial assessment). The next logical step is to apply a safe method of FUS in combination with immunotherapy and perform analytics on the tumor tissue with repeat biopsies.
- Pancreatic cancer is an interesting target for FUS given preclinical data. However, pancreatic cancer does not have a high degree of inflammation. It is also difficult to deliver therapeutic agents to the pancreas in general.
- There was a discussion on preclinical models of breast cancer that could be used to study FUS combinations. The issue is that some models have immunosuppressive environments, such as 4T1, but 4T1 model is not representative of typical patient immunosuppression as it is mostly driven by MDSC. While other models, like EO771, are responsive to checkpoint inhibitors unlike most patients, though consistent with breast cancer patients in that this model has a lot of M2 macrophages.
- Another approach could be to compare each FUS modality and the effects on the TME. Specific FUS parameters would also likely have an impact on TME.
- Development of a set of immune parameters that would be predictive of outcome, which would allow all FUS investigators to use the same parameters to compare outcomes in clinical trials.
- Another approach that should be studied further is priming the immune system prior to thermal ablation.

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Clinical Disease Targets

Presentation

Focused Ultrasound and Immunotherapy for Liver Cancer

Joan Vidal-Jove, MD, PhD, first discussed brief specifics on liver immunobiology. The liver is an important contributor to innate and adaptive immunity involvement.^{8,9} In hepatocellular carcinoma, activity of CD4+ T cells is suppressed and Treg cells are increased, which allows cancer cells to evade the adaptive immune system and proliferate.¹⁰

Preclinical research demonstrated that HIFU ablation increased cytotoxic T cells and cytokine secretion.^{11,12} Histotripsy increases cellular and systemic immunity and decreases pro-tumor-immune cells.¹³ Histotripsy also significantly alters immune cell populations systemically and in the TME.¹³ Preclinical research demonstrated that histotripsy had a number of effects that promoted local intratumoral innate and adaptive immune responses.¹⁴ These included mediating stronger intratumoral CD8+ T cell infiltration, releasing immunogenic tumor neoantigens, inhibiting the development of distant metastases, and augmenting checkpoint inhibitors.¹⁴

The THERESA liver study enrolled 8 patients with multi-focal liver malignancy. All patients met the primary endpoint, which was defined as acute technical success and creation of an ablation zone per the planned ablation volume as assessed by MRI 1-day post-procedure. Average histotripsy time was 23 minutes and treatment was well tolerated with no patient discomfort or pain. Over time, involution of the ablation area was observed and there was a 95% resorption of the treated area at 3 months. Some abscopal effects were observed in non-targeted lesions along with a sustained effect on reduction of tumor markers systemically.

Presentation

HIFU for Pancreatic Cancer: Challenges and Future Directions

Keaton Jones, MD discussed the role of HIFU in pancreatic cancer in relation to immunotherapy. Pancreatic cancer has a paucity of antigens/neoantigens, dysfunctional dendritic cells, dense stroma (>50% mass) preventing T cell trafficking, and an abundance of immunosuppressive myeloid cells. The ablative effect of HIFU can release tumor antigens, promote chemokine-driven recruitment of T cells, and polarization of myeloid cells to an inflammatory phenotype. Many clinical trials have demonstrated the efficacy of HIFU for pain relief in pancreatic cancer, but there is little evidence for HIFU extending overall survival.¹⁵ There is little clinical and preclinical data on the combination of HIFU with immunotherapy in pancreatic cancer.

A small phase I trial looking at safety of HIFU for pancreatic cancer and other endpoints such as ablation, pain control, and immune priming is underway. Patient recruitment has been a challenge because of additional organ involvement and patients moving to chemotherapy very quickly before HIFU. Lessons from the trial in progress include pancreatic tumors are more favorable to HIFU treatment, workflow from screening to treatment needs to be rapid, histological material for secondary endpoints is challenging, and patients are interested in HIFU. Additional trials of HIFU for pancreatic cancer are ongoing and recruiting. Recently, a preclinical mouse study using pFUS reported increased survival and recruitment of effector T cells in the TME.¹⁶

Presentation

A Clinical Trial of Focused Ultrasound with Low-dose Gemcitabine to Augment Immune Control of Early-Stage Breast Cancer

Patrick Dillon, MD, described the use of FUS for the treatment of breast cancer. Prior research suggested that checkpoint inhibitors alone do not work in estrogen receptor (ER)+ breast cancer. Preclinical work in a mouse model (4T1) with FUS ablative treatment increased dendritic cells expressing the costimulatory molecule CD86, and granulocytic myeloid-derived suppressor cells (MDSCs). Another preclinical study that combined FUS and gemcitabine increased overall survival in an immunocompetent preclinical model, but not in an immune-compromised model (RAG1 knockout).

The Theraclion echopulse device provides HIFU ablation guided by ultrasound imaging and is being tested in clinical studies. This trial will have 3 arms: gemcitabine, FUS ablation, and gemcitabine combined with FUS. Inclusion criteria were histologically confirmed, newly diagnosed breast cancer any stage 1 to 3. Specimen collection includes sentinel node biopsy for immunohistochemistry and RNAseq analysis of transcriptional alterations, and blood collection to understand impact on circulating immune cells.

Presentation

Potential of Focused Ultrasound as an Immunotherapy Tool for Treatment of Melanoma

Lynn Dengel, MD, discussed the potential of FUS for melanoma treatment. Patients with more tumor-infiltrating lymphocytes (TILs) have a better chance for survival. The effect of this finding has fueled immunotherapy research for melanoma. However, only 10-50% of patients respond to immunotherapies, and responses are not always durable. Tumors continue to escape the immune system and limit the success of immunotherapy. FUS has the potential to boost the immune response to immunotherapy. Preliminary research suggests that FUS can increase tumor antigen release and antigen capture leading to greater antigen presentation at the lymph nodes, which increases circulating T cells and T cell recruitment at the tumor site.¹⁷ FUS can also lead to increased permeability at the tumor via increasing cytokine expression and adhesion molecules.¹⁷ FUS ablation of breast cancer demonstrated increased infiltration of TILs.¹⁸ An ongoing melanoma-focused trial of FUS (NCT04021420) aims to open the BBB to improve drug delivery.¹⁹

The trial described here uses FUS to bolster the immune response in patients with advanced solid tumors. FUS partial ablation is administered alone or in combination with immunomodulatory adjuvants to provide additional stimuli with the aim of overcoming tolerance and inducing greater dendritic cell and T cell activation. The protocol is designed to optimize the immune-stimulatory effect as opposed to total tumor ablation, with a treatment volume of 33% of estimated tumor volume. Primary endpoints are safety of FUS (alone or in combination) and immunologic effect (CD8+ T cell infiltration). Three patients have been enrolled with metastatic thyroid and colon cancer. Recruitment has been challenging due to the COVID-19 pandemic and requirements to travel for treatment.

Presentation

FUS and Immunotherapy for Glioblastoma

Michael Lim, MD, presented on FUS and immunotherapy for glioblastoma (GBM). Despite major advancements immunotherapy has brought to cancer treatment, low response rates, toxicities, and resistance to immunotherapy remain a challenge. Preclinical work with checkpoint inhibitors for GBM suggested improved survival. However, in large phase 3 trials, there were no improvements in progression-free survival (PFS) or overall survival in human patients with newly diagnosed or recurrent GBM.

Focused radiation can activate T cells,²⁰ which often take on an exhausted phenotype in the tumor. FUS-induced BBB opening can induce an immune response.²¹ Preclinical work with FUS and IL-12 suggested that immune cells can infiltrate the tumor and prolong overall survival.²² Myeloid cells may play an important role in ‘cold’ cancer and may be responsible for sustained immunosuppression in tumors. Tumor macrophage density correlates with overall survival.²³ Myeloid cell targeting continues to gain interest as a cancer treatment.^{24,25} Preclinical work has demonstrated that FUS can reprogram myeloid cells, particularly macrophages, and improve overall survival.²⁶ In summary, focal therapies may act as kindling for the immune system. However, there are many questions that remain to be answered for the use of FUS including frequency, sequence, dose, and the optimal immunotherapy agent for combination.

Q&A

Trial Design

Theresa LaVallee, PhD | Moderator

LaVallee summarized that the presentations gave some good suggestion for how to combine FUS with immunotherapy in solid tumors. A key theme was that the clinical hypothesis should be science-driven when selecting the tumor type and optimizing treatment. Another important consideration is the appropriate immune readout to be able to detect if immune modulation has taken place. Secondary endpoints with immune endpoints are challenging in terms of defining success in the statistical analysis plan. Another theme was trial design and patient population in terms of recruitment.

LaVallee asked participants to comment on trial design and the key criteria in terms of time to treatment, and stage of disease.

- From the field of GBM, standard-of-care treatments (steroids, surgery, and temozolomide) may suppress the immune system making it difficult to use immunotherapy in this setting.
- Preliminary data from patients treated with chimeric antigen receptor (CAR) T cells suggests post-infusion expansion of T cells in patients with GBM. A recent study used FUS in combination with CAR T cells and reported that FUS may be able to prime the immune system prior to CAR T therapy.²⁷
- There is also some preliminary evidence that a PD-1 inhibitor in combination with gemcitabine/abraxane chemotherapy may extend survival in patients with pancreatic cancer.
- In the clinical trials for histotripsy in patients with liver cancer there were no clinically relevant signals from the immune system

Moderated Open Discussion

Clinical Disease Targets & Roadmap

Jessica Foley, PhD | Moderator

Foley suggested that participants should discuss ways to move the field forward and ask questions regarding the day's presentations. Participants were reminded that the FUSF developed [guidelines](#) in consultation with the Foundation's Cancer Immunotherapy Scientific Advisory Board. These guidelines are meant to serve as a minimum set of parameters for immune assessment.

- A suggestion was made to create a database with published work on the transcriptome or genomic profile by FUS modality and immune cell changes across tumor types over time.
 - There are several preclinical studies that have reported on this, but the data are not collected in a single place.
 - Another issue can be the accuracy of the mouse model. Preliminary findings from a study that compared proteomic, genomic, and growth profiles in the same mouse strain obtained from 3 different vendors following tumor implantation in response to mechanical FUS and found wide variation with mice obtained from different vendors.
- What kind of data are needed in order to match FUS modality to patients?
 - More preliminary clinical and preclinical data are needed. More information on ablation and partial ablation with histotripsy is needed to draw early conclusions.
 - In breast cancer, FUS trials are still in the early phases of analysis.
- There was a comment that the field generally believes that immunotherapy should be given early in the treatment process prior to multiple lines of chemotherapy. However, the US Food and Drug Administration (FDA) and regulatory bodies often want to see data in refractory patients that have been heavily pretreated when testing new treatment modalities. A current challenge is in working with the FDA to design the kinds of trials that will provide useful data for immunotherapy.
 - There was a comment that one area of opportunity for FUS and immunotherapy is patients that are waiting for liver transplants, as these patients are generally not offered treatment. This would also allow tissue analysis of the liver following transplantation.
- What should be the sampling time points for biomarkers?
 - A comment was made that timing might differ by cancer type. Some cancers are easier to biopsy than others. Blood sampling has not yielded useful data so far and tumor tissue is likely the most useful.
 - Although tumor tissue is very important, core needle biopsies are only a small portion of the overall tumor. Recent advances in blood analysis and multiplex proteomic panels allow for longitudinal analysis. Multidisciplinary teams may allow for a greater depth of analysis in the immune system and other factors.
 - Bioinformatics provides a lot of opportunity, but many academic researchers do not have access to these resources. This could be an opportunity for FUS to create access to data analytics tools for very large data sets.
 - The clinical trials described during the meeting will provide a great deal of information, and researchers were urged to take the time to analyze all the data to provide as much information as possible.

- What is the next step for FUS and immunotherapy?
 - For the GBM field, greater cross-disciplinary collaboration between FUS researchers outside the GBM field, but also working in the brain could be useful. Particularly those working in Alzheimer's disease.
 - A similar comment was made for the field of pancreatic cancer. It would be useful to have a FUSF-facilitated network of researchers working on clinical trials of FUS for pancreatic cancer.

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Optimization of FUS for Immunomodulation

Moderated Open Discussion

Optimization of FUS for Immunomodulation & Roadmap

Kelsie Timbie, PhD | Moderator

- Currently, it is challenging to integrate FUS into clinical trials because of a lack of interest from the oncology field.
- Participants discussed ways to translate preclinical data to human clinical trials for ablative trials.
 - Early clinical trials with longitudinal sampling are ongoing. Understanding how thermal ablation affects the TME and the draining lymph nodes is an important step.
 - For histotripsy, early trials are performing partial ablation in order to collect tissue over time. Future trials will also perform full ablation of the tumor. Future trials for histotripsy should compare partial versus complete ablation. Additionally, patients often prefer complete ablation to partial ablation. The metastatic setting may be a better place to look for systemic effects of FUS because not all of the tumors will be resected.
 - Patients often withhold consent for repeat biopsies that do not contribute to treatment. There was a suggestion to give more careful consideration to blood-based markers because of the difficulty with obtaining tumor tissue samples following FUS treatment.
 - A comment was made that a FUS trial could be designed similarly to some immunotherapy trials for prostate cancer where the immunotherapy is given prior to resection, and then the resected tissue is used to measure immune function.
- A participant asked about the FDA perspective on partial versus full ablation.
 - In others' experience, FDA was concerned about time to resection and does not want to impede the access of the patient to surgery and was also concerned that FUS would make the surgery more challenging.
- There was a suggestion to make a list that would include each FUS modality, the hypothesis, and data to support the hypothesis. For example, in treating liver cancer the hypothesis is that FUS would allow CD8+ T cells to infiltrate the tumor. Is there evidence to support the immune modality that is put forward; either trying to get T cell infiltration and invoke a new T cell response versus reinvigorating an antigen-experienced exhausted T cell.
 - The FDA will consider any human data, even case studies.
 - Additional preclinical studies could be done to support the rationale for the human clinical trials, such as administering immunotherapy prior to chemotherapy particularly for those cancers that result in exhausted T cells following standard-of-care treatment.

- Another option is to add FUS to standard-of-care treatment, as a way to enhance treatment.
- Another key consideration is the duration of observations. In a window of opportunity trial prior to resection, immune parameters may only be measured over a few days. However, preclinical evidence suggests that, in some cases, it may take weeks to see changes after immunotherapy. Examining immune function over time in patients is a key consideration for future trial design.
- Participants were asked to comment on what data was needed for the role of FUS combined with immunotherapy.
 - Early data suggests that FUS may initiate or augment adaptive immunity, but the mechanism behind this is unknown. It is also unknown how myeloid cells and the lymphatic system are each affected by FUS. Chemokine responses to FUS are not understood.
 - There was a recommendation to focus on hard-to-treat cancers, pancreatic cancer or GBM. Following those trials, consider whether there is a role for FUS to further improve on the results. Preclinical studies could inform trial design by looking at different FUS modalities and other parameters.
- A question was asked on whether histotripsy could completely ablate a pancreatic tumor.
 - In an animal model with pigs, large tumors have been ablated with histotripsy. The head of the pancreas is easier to treat because the gastrointestinal (GI) tract can be filled with dietary modifications.
 - In human clinical trials with the liver, histotripsy can completely ablate the tumor. However, the acoustic window may not be sufficient in the pancreas or liver to create the high pressure necessary for histotripsy treatment using an external device. An endoscopic device should be able to overcome this limitation.

Presentation

Engineering Remotely Controllable CAR T Cells for Cancer Immunotherapy

Peter Wang, PhD, presented on the topic of CAR T cells, which have become increasingly popular as cancer treatments. In short, patient T cells extracted from blood are genetically modified to express chimeric antigen receptors (CARs) on their surface. This method has been successful for the treatment of blood cancer, but not as successful for solid tumors. There are different methods of engineering CAR T cells, including sensitization to FUS activated heat-shock proteins.²⁸ Using short-pulsed FUS was sufficient for gene activation in vitro. Preclinical research showed that magnetic resonance guided FUS (MRgFUS) could activate gene expression in specific local regions in a mouse model. Preclinical studies also showed that MRgFUS-inducible CAR T cells slowed tumor growth over time in lymphoma and prostate cancer. These CAR T cells have less off-tumor toxicity compared with standard CAR T cells.²⁷ Next steps include the development of a jacket with wearable transducers controlled by wireless networks (i.e. cell phones) for increased control of treatment.

Moderated Open Discussion

Role for FUS in Therapeutic Delivery

Natasha Sheybani, PhD | Moderator

- While CAR T has been successful for lymphomas, they have not worked as well in solid tumors because they do not have ‘homing’ capabilities for cancer cells. FUS could be used to help deliver CAR T with heat-shock protein promoters, but this remains to be determined. Additionally, preclinical testing with mechanoreceptor-promoters suggested that the efficiency was lost without the addition of microbubbles, making this method less robust. However, refinements to this approach are ongoing.
- The endothelium is dysfunctional in many tumors and will prevent T cells from entering the tumor. FUS could be used to modulate the endothelium to be more receptive of treatments such as CAR T.
 - There was agreement that this was an interesting suggestion and that reprogramming the vasculature network was worth investigating. FUS alone may not be sufficient, genetic reprogramming could be used in combination with FUS to manipulate the tumor vasculature.
- FUS may be able to facilitate homing, but timing of treatments is critical and. Early phase clinical trials (phase 0 or 1) could be used to look at the effect of FUS alone on endothelial cells. This could also be done systematically with different FUS modalities.
- Checkpoint inhibitors seem primed for combination with FUS. Participants were encouraged to consider the types of monitoring tools (e.g., PET) that could be used for these combination trials.

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Metrics to Predict Clinical Success

Presentation

Guidelines for Immune Analysis of Focused Ultrasound Treatment

Frédéric Padilla, PhD discussed the development of guidelines for immune analysis following FUS. After the previous FUS and cancer immunotherapy workshop, [guidelines](#) for immune analysis following FUS treatment were developed by the FUSF Cancer Immunotherapy Scientific Advisory Board. The guidelines are meant to serve as suggestions for analyses routes, assays, and timepoints for monitoring the characteristics and temporal evolution of the immune response to provide key information needed to maximize the effectiveness of FUS treatments.^{29,30} The primary focus of studies should be to:

- 1 Analyze changes for the development of more immunologically favorable/less immunosuppressive microenvironment.
- 2 Establish a rationale for combined treatment regimens including FUS and agents with immunostimulatory effects.
- 3 Identify predictive biomarkers.
 - a Static biomarkers that are present at baseline and can inform patients and treatment selection.
 - b Prognostic biomarkers that are generated upon treatment initiation and can be used to monitor the anti-tumor immune response.

Other general considerations include the storage of samples, which should allow for assays/analysis on fresh samples with the rest stored for later analysis. The follow-up analysis should be informed by follow-on questions, patient outcomes, clinical data, etc. The analysis will greatly depend on the drug used and should be optimized accordingly. For example, some drugs only target a specific immune cell subset. The use of flow cytometry is imperative for the analyses of immune cells. If the facilities/equipment to run flow cytometry are not available, researchers should establish contracts or collaborations with other academic centers or private companies prior to the start of the trial.

For the design of analyses for clinical trials, an emphasis on the biologic primary endpoint is preferred over overall survival or PFS.³⁰ Trials should aim to address fundamental questions, such as whether FUS is associated with changes in the composition of immune infiltrate, spatial distribution of immune cells, or T cell status. The importance of window of opportunity trials was also suggested with an emphasis on neoadjuvant FUS/immunotherapy combination approaches. Specific suggestions for analysis routes for clinical trials were detailed in the presentation. The guidelines also provide recommendations on study design for preclinical studies.²⁹ Studies should be hypothesis driven and not only look at survival, but also immune response involvement.

These guidelines will be reviewed on an ongoing basis to include evolving technologies. The FUSF is willing to support immune monitoring in FUS clinical studies. The next steps are to explore the opportunity to create a bioinformatics infrastructure for data analysis. A data storage and data sharing platform has been created in collaboration with the Open Science Foundation. A central facility for clinical sample analyses and shared expertise is also in development.

Presentation

Introduction to Imaging Applications in Immuno-Oncology

Natasha Sheybani, PhD presented an introduction to imaging applications in immuno-oncology. In the coming years, spatial resolution, reproducibility, and system sensitivity are expected to improve, and other factors such as examination time and radiation exposure are expected to decrease although system costs are expected to increase. There are several strategies for immune-imaging strategies. These include targeted probes for endogenous biomarkers, direct cell labeling, and indirect cell labeling, with each having advantages and disadvantages.

Sheybani discussed examples for these strategies.³¹ Cell-labeling methods were able to label CAR T cells with bioluminescence.^{31,32} Indirect cell labeling with superparamagnetic iron oxide nanoparticles showed accumulation of labeled cells after FUS.³³ Images themselves can be used as data, such as radiomics that extracts computerized sub-visual features from radiologic imaging.³⁴ Imaging tools and advanced analytical tools can also lead to pseudo-progression, a radiological response pattern where tumor size increases or a new lesion appears, which is followed by tumor regression. This phenomenon is typically associated with an inflammatory response and diagnosed retrospectively.^{35,36} Continued advancement of imaging will facilitate precision immuno-oncology. In conclusion, with more sensitive/specific imaging techniques and more data will come opportunities to advance robust approaches in artificial intelligence for immunotherapy deployment and monitoring.

Presentation

Multi-Omic Biomarker Analysis for Tumor and Immune Learning in Clinical Studies

Theresa LaVallee, PhD discussed how to use multi-omic biomarker analysis to interpret the results from a clinical trial. The PRINCE pancreatic cancer trial was used as an example.³⁷ In this trial CD40 was administered in combination with chemotherapy (gemcitabine/nab-paclitaxel) and checkpoint therapy (nivolumab). This was a phase II trial at 7 centers. The immunotherapy combination (PD-1 inhibitor with CD40) did not produce anti-tumor activity. However, chemotherapy/immunotherapy combinations produced anti-tumor activity. Broad profiling with a 42-marker blood immune panel allowed the identification of specific cell populations.³⁸

The quality of the T cells matters and a blood assay panel for T cells and natural killer (NK) cells was developed. Bioinformatics were used to integrate clinical data with the biomarker information. The analysis demonstrated that the immunotherapy treatments hit their targets. Higher baseline levels of CXCR5+ effector-memory CD4+ T cells were associated with improved survival for PD-1 inhibition, but decreased survival for CD40. The two immunotherapies have very different mechanisms of action. In the CD40 group, there were lower baseline levels of effector-memory CD4+ T cells with improved survival. Immunosuppressive proteins in circulation correlate with shorter survival in response to CD40/chemotherapy treatment. The circulating tumor (ctKRAS) assay showed decreases in response to treatment, and specific KRAS mutations correlated to different responses with immunotherapy. Multi-omic assays allow the integration of large quantities of data to understand the rich interplay between the tumor and the immune system.

Moderated Open Discussion

Metrics to Predict Clinical Success & Roadmap

Frédéric Padilla, PhD | Moderator

- Participants discussed the clinical use of imaging as a surrogate biomarker. Imaging is not yet ready for use as a surrogate and more work on immunoassays are needed. Imaging is already used with FUS, and this imaging can likely be leveraged for deeper analysis. Going forward, both imaging and immunoassays should be collected in parallel for use with functional or quantitative imaging metrics in development. PET imaging in conjunction with FUS is also under consideration as an imaging modality.
- There was a question on radio-labeled checkpoint inhibitors. Panelists responded that there are publications in human patients with radio-labeled checkpoint inhibitors.
- A participant asked a question on repeated use of imaging. Imaging can be used to measure a lot of factors, such as drug targeting, etc. However, it is difficult to conduct a clinical trial with an experimental imaging agent and an experimental therapeutic agent.
- Patients may initially show immune system activation to immune therapy but become resistant over time. During combination radiation/immunotherapy, those patients that showed immune activation in non-irradiated sites had greater overall survival.
- A suggestion was made to consider administering intralipids prior to immunotherapy treatment to block the scavenging receptors on the macrophage cells of the liver. This might help to increase circulation time of an antibody.
- There was some discussion on the bioinformatics details for the PRINCE pancreatic trial. When tumor biopsies were not sampled from the same site, there was a great deal of variation in the pharmacodynamics. Parker Institute for Cancer Immunotherapy (PICI) has bioinformatics tools available, but the PRINCE data is not publicly available yet.
- A comment was made that the immune system is important, and potential benefits may not always be related to T cells. Participants were encouraged to look beyond CD8+ T cells during analysis.
- Multi-center trials that do not have a centralized analytical plan are difficult to carry out and harmonize the results. It is important to have a centralized repository for samples and coordination of assay analysis. It is also important to have a team of people to carry out the bioinformatics needs related to multi-center trials. Centralized trials will help to move the field forward faster compared with non-coordinated single-center trials.

Moderated Open Discussion

Knowledge Gaps, Funding Gaps, Other Gaps and Pharma Partnerships, Wish List

Jessica Foley, PhD | Moderator

- Chrit Moonen, PhD discussed challenges for early phase clinical trials. A phase I study with histotripsy and checkpoint inhibitors is in the planning phases. Pharmaceutical companies have rejected proposals to provide therapeutic agents for the trial and the out-of-pocket expense for the drugs is prohibitive.

- Timothy Bullock, PhD mentioned that pembrolizumab (Merck) was obtained by writing an investigator-initiated trial. Receipt of the drug was likely due to the fact that pembrolizumab was new to the market and the company wanted to explore different modalities. A formal proposal may be required. Another consideration is whether the drug is approved and/or standard of care for the indication.
- The FUSF is forming partnerships with pharma companies to educate them on the potential for FUS.
- Timing can be important and allow opportunities for combination treatments. Pharma companies are also concerned with having high-quality trials done to industry standards that will not need to be repeated if they have positive results. It is also important to note that many immunotherapy agents will be coming off patent in a few years.
- The FUSF typically provides funding for translational work and first-in-human clinical trials. They also fund trials to help gather data that would garner interest for further commercialization, such as safety and preliminary efficacy data that can be used to design larger-scale trials.
- There may also be a role for the FUSF to help with both patient recruitment and oncologist recruitment.
- Another issue is the harmonization of FUS equipment across institutions; each institution differs in the technology that they use making multi-center trials a challenge. The FUSF is working on guidelines regarding this issue.
 - The concept of dose will not work for FUS guidelines. However, the parameters of the device itself can be standardized.
 - A suggestion was made to put out a RFA for biophantoms, a biologically-activated pathway marker, that can serve as a readout for the effects under investigation. The endpoint needs to be well defined for this approach to be successful.
 - Another comment was made that thermal ablation is easier to standardize than other FUS modalities. The Foundation is holding a workshop series for standardizing cavitation dosimetry later in the year.
 - A list of comprehensive biological endpoints that could provide information on the changes caused by FUS could help design trials to investigate whether those changes lead to alteration in immune function.
- Standardization of imaging parameters will define the ability to find imaging biomarkers later. Scanner differences and contrast agent administration vary widely. Parameters could be controlled in preclinical guidelines, and this is an area of opportunity for FUSF to create guidelines on imaging parameters.
- There was also a proposal for a centralized database for clinical FUS platforms that are broadly used. Parameters to include were calibration data that includes pressure and other data points.
 - For preclinical platforms it would be possible to create a database of if/then situations that would allow the user to select intensity/depth/length of pulse in average tissue and estimate the resulting temperature elevation. Another example was acoustic radiation force and associated displacement.
- A suggestion was made to look at FUS modality combinations in sequence. Histotripsy could be used on the tumor mass, followed by FUS-guided CAR T administration.

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Roadmap and Action Items

As a result of the robust discussions the following recommendations for preclinical and clinical projects as well as more general projects were formed as next steps for the community (see Table 1 below).

Table 1

2021 Focused Ultrasound and Cancer Immunotherapy Workshop Projects and Action Items

Category	Project	Timeline	Action Items
General	Repository for data sets	January 2022	Schedule webinar to introduce OSF data repository.
General	Create forum for community communication	January 2022	Newsletter story introducing forum.
General	Precision medicine efforts in collaboration with Q2 and PICI	June 2022	Schedule discussion on this topic.
General	Identify central lab to perform immune analyses	2022	Solicit and review research proposals.
Preclinical	Lymphatic drainage in response to FUS	2022	Solicit and review research proposals.
Preclinical	Detailed analysis of partial vs. total ablation	2022	Solicit and review research proposals.
Preclinical	Replicate clinical conditions such as a fatigued immune system	2023	Solicit and review research proposals.
Clinical Trials	Clinical trial design roundtable	March 2022	Schedule clinical trial design roundtable.
Clinical Trials	Add on study: effects of FUS on vasculature and immune response	2023	Solicit and review clinical trial add-on.
Clinical Trials	WOO trial: immunologically responsive cancer with well-understood biology	2024	Solicit and review clinical trial proposal.
Clinical Trials	Longitudinal genomics study after FUS	2024	Solicit and review clinical trial add-on.
Clinical Trials	Phase 1 study: integrate FUS into immunotherapy standard of care regimen	2024	Solicit and review clinical trial proposal.

Pre-workshop Education Content

Introduction to Participating Organizations

Focused Ultrasound Foundation

Jessica Foley, PhD, provided an overview of the FUSF and why cancer immunotherapy is important in the field of FUS. Many questions remain about the role of FUS in activating the immune system for treatment of cancer. FUS can enhance the anti-tumor-immune response as well as enhance the delivery of immunotherapeutics.

Most immunotherapies are only effective in 20-40% of patients. Known limitations include the fact that between 60-80% of patients are non-responders, some patients respond but relapse over time, and/or side effects and toxicities lead to stopping treatment. FUS disrupts tumor cells, releasing antigens and proteins. Opportunities for FUS in the immunotherapy landscape include stimulating an immune response to convert cold tumors into hot tumors, augmenting the effectiveness of immunotherapy, and enhancing the delivery of immunotherapeutics to tumors.

FUSF has become a catalyst to accelerate the development and adoption of FUS for a variety of disease states. In general, the FUSF works to identify critical unmet clinical needs, set research priorities, and change the culture towards patient-centric urgency and collaboration. The FUSF also fosters collaboration between academia, government, and industry. Other activities include organizing, conducting, and funding research. FUSF has previously held three workshops in partnership with the Cancer Research Institute (CRI) on the topic of FUS in combination with immunotherapy, established an advisory board and working group, and secured key partnerships with several collaborators.

FUSF is currently funding 6 clinical trials and 11 preclinical projects involving cancer immunotherapy. Key projects include FUS in combination with immunotherapy in a variety of cancer types, capturing immune assessment during ongoing FUS trials, and comparing immune effects induced by different FUS “modes” in preclinical models.

Cancer Research Institute

Jill O'Donnell-Tormey, PhD, explained that the mission of the CRI is to save more lives by fueling the discovery and development of powerful immunotherapies for all types of cancer. CRI has four mission pillars: to power basic and translational research, accelerate innovative clinical collaboration, convene global thought leadership, and educate as a trusted source of immunotherapy information. They fund a full spectrum of research from fellowships, clinic/laboratory integration program, a clinical accelerator program that serves as an incubator for multiple clinical trials testing a variety of immunotherapy combinations, a technology impact award that focuses on promising technologies related to immunology, and a mid-career funding program for high-risk/high-reward research.

Briefly, O'Donnell-Tormey described the types of immunotherapies. These include adoptive cell therapy, cancer vaccines, immunomodulators, oncolytic viruses, and targeted antibodies. The field is focused on several key areas of immunotherapy such as understanding the evasion or suppression of immune response by tumors, finding solutions to checkpoint resistance, predicting response to immunotherapy, and designing rational immune-based combination therapies. The future of immunotherapy lies in mechanistic discoveries, correlative science, and big leaps to personalized combinations as well as synthetic biology and gene editing.

Parker Institute for Cancer Immunotherapy

Theresa LaVallee, PhD explained that the PICI was founded as a collaboration organization to bring together a cross-functional team to break down barriers using resources and technology to ask questions on how to treat patients with immunotherapy. The mission of PICI is to translate scientific immunotherapy findings to cancer patients with urgency. PICI purposefully focuses on hard-to-treat and underserved tumor populations to look at novel scientific approaches without the constraints of time to market and pharmaceutical timelines.

PICI has developed a clinical trial platform to take promising hypothesis to clinical trials in a collaborative way without being bound by corporate portfolios. Samples (tumor, blood, and stool) are collected at baseline and during treatment for every patient enrolled in the study. The platform studies are easily expandable to new treatment options, expanding the patient cohort, or ending the trial when a treatment does not work. The trials take a multi-omic approach for every patient in the trial and look at biomarkers in addition to clinical data. The immune system is quite complex, and the crosstalk between the tumor and the immune system may differ for each tumor. While T cells are important to the immune system, the tumor microenvironment and other immune cell types are also important. LaVallee suggested that future work with FUS should look at the type of FUS and the interplay with the immune system and tumor microenvironment.

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Immune Effects by FUS Modality

Treatment Reporting Guidelines

Gail ter Haar, PhD discussed the importance of treatment reporting guidelines. Reporting guidelines improve comparison of treatments across institutions and different devices. Important parameters to look at include the transducer and system, treatment protocol, power and intensity (field distribution), cavitation monitoring methods, thermal dosimetry methods, bubble information, numerical simulation details, and quality assurance methods.^{39,40} Reporting categories have also been developed with the labels of minimum (minimum level of detail acceptable for reporting), medium (more inclusive list of details required with some measurement of the acoustic field), and optimum (details of everything, expected levels of detail for research labs) levels. A number of relevant standards have also been published, please see presentation recording for full list of resources. These reporting categories need the collaboration of clinicians, biological scientists, and physicists.

Turning up the Heat: Using Non-thermal Histotripsy to Shift the Immunosuppressive Tumor Microenvironment from “Cold” to “Hot,” Augmenting Systemic Anti-Tumor-Immune System Activation

Irving Coy Allen, PhD, discussed both preclinical and clinical work using histotripsy with immune-oncology therapy. Histotripsy creates acoustic cavitation in a bubble cloud that leads to ablation. Histotripsy increases progression-free survival in a mouse model of pancreatic cancer (Pan02).⁴¹ Histotripsy results in reduced immunosuppressive cells in the ablation zone, and shifts the local tumor microenvironment from immunosuppressive to pro-inflammatory through decreased damage-associated molecular pattern (DAMP) signaling.⁴¹ Non-thermal ablation increases T cell proliferation and IFN γ -induced PD-L1 expression. Allen also mentioned that the lab has developed a working model of immune system modulation following focal tumor ablation therapy.¹³ Future directions are to develop a porcine model of pancreatic cancer to test histotripsy with the objective to eventually treat human patients.

Immunotherapy and FUS for Thermal Ablation

Kathy Ferrara, PhD, described her research combining ablation with chemotherapy or immunotherapy with the overall goal of developing personalized strategies. There are around 469 clinical trials ongoing or not yet recruiting for combining agonist therapies; this is a very diverse area of active research. The lab is focusing on pancreatic cancer because the 5-year survival rate is quite low at 10.0%. Preclinical work following tumor ablation in breast or pancreatic cancer models showed that tumor ablation was constrained by the tissue surrounding the tumor.⁴² They have developed PET imaging for gene therapy allowing the tracking of pharmacotherapeutics in the whole body. Preclinical work demonstrated that ablation enhances therapeutic accumulation in the tumor.⁴² Additionally, ablation combined with doxorubicin extended survival.

Based on the preclinical work, a clinical trial is now ongoing in patients with pancreatic cancer.⁴³ Tumor biopsy is carried out before and after ablation and the patient is then treated with

standard-of-care chemotherapy.⁴³ A preliminary study of 6 patients treated with FUS ablation reduced tumor volume without recurrence for 6 months.⁴⁴ Evidence in the literature suggests that reduction in tumor burden combined with checkpoint inhibitors can further improve patient outcomes.⁴⁵ In the B16-OVA mouse model, adding immunotherapy (anti-PD-1) before ablation enhanced lymph node, blood, and spleen antigen levels.⁴⁶ A combination of immunotherapy (anti-PD-1) and thermal ablation in an orthotopic syngeneic model of metastatic mammary carcinoma extended survival.⁴⁷ Ongoing work involves single-cell sequencing to identify differences in pancreatic and breast TME (tumor microenvironment).

Hyperthermia and Cancer Immunity: Some Brief Comments with Implications for Focused Ultrasound

Elizabeth Repasky, PhD, presented an overview on hyperthermia effects on the immune response. Very few clinical studies in hyperthermia have utilized radiofrequency heating methods similar to those in patient studies, with the exception of a few clinical trials in canine patients with cancer. Strong preclinical data links thermal signals to regulation of improved immune cell activation and function. While measurement of tissue temperatures achieved upon thermal therapy is critical and must be included for understanding the impact on immunity, there are many new and exciting emerging biomarkers. Recent preclinical data shows a significant positive effect of MRgFUS on the immune system. The most important concern for FUS in clinical trials is to choose at least one rational endpoint for the effects of hyperthermia on immunity and test for it in clinical trials. It is also important to keep in mind that brain heating may result in significant thermoregulatory responses to the immune system.

Repasky pointed out that skin heating results in a rapid and strong thermoregulatory response.⁴⁸ Mild hyperthermia has a variety of effects on the TME and immune contexture.⁴⁸ Fever supports T cell responses by promoting mitochondrial translation.⁴⁹

Immunological Effects of Boiling Histotripsy Tumor Ablation

Tatiana Khokhlova, PhD, provided an overview of boiling histotripsy and immunomodulation in preclinical models. Boiling histotripsy is a pulsed HIFU regime for non-thermal, mechanical soft-tissue ablation, mediated by vapor and cavitation bubbles. Boiling histotripsy releases tumor cell antigens that allow the immune system to identify and respond to the tumor.¹⁷ Boiling histotripsy ablation has been explored in several preclinical models of cancer including melanoma, renal cell carcinoma, neuroblastoma, colon adenocarcinoma, and pancreatic cancer.^{16, 50-53} These studies suggest that boiling histotripsy mechanical ablation of tumors, even if partial, promotes lymphocyte infiltration and pro-inflammatory milieu within a short time frame (1–3 days). A sustained pro-inflammatory microenvironment (e.g., with checkpoint inhibition) promotes the proliferation of tumor-specific effector-memory T cells over a longer time span, which may potentiate tumor regression as well as an abscopal effect. In tumors that are considered “cold” (e.g., pancreatic cancer), additional immunotherapies may be needed.

Low-Intensity Focused Ultrasound's Effect on the Immune Response

Petros Mouratidis, PhD, discussed low-intensity FUS and cancer and the immune cycle. The cancer immunity cycle may be influenced by FUS by promoting the release of antigens from damaged cells, the release of danger signals in the TME, and induction of local inflammation.⁵⁴ The immunological changes following low-intensity FUS in several preclinical cancer models were described.^{55, 56} Low-intensity FUS can induce an acute inflammatory response after BBB disruption.^{56, 57} Low-intensity FUS combined with immunomodulatory agents produced an effective anti-cancer immune response.⁵⁸ Low-frequency FUS enhanced T cell recruitment at local and distant tumor sites and may also overcome tumor-induced tolerance in CD4+ T cells.^{58, 59} In summary, immune responses may be temporal and tumor dependent and low-intensity FUS parameters should be carefully selected to avoid unwanted immune responses.

Immune Response to BBB/BBB Opening with Focused Ultrasound

Richard Price, PhD, reviewed immune response to FUS in BBB/BBB opening in preclinical models of melanoma (B16F10/B16F10) and glioma (GL261). In a preclinical model of melanoma brain metastases, FUS increased pro-inflammatory transcripts and immunity gene sets suggesting sterile inflammation.²¹ There was also increased dendritic cell maturation in the meninges, but there was no activated T cell homing nor increase in adhesion cell molecules.²¹ Price also discussed using FUS as a way to deliver immunotherapy to the brain.²⁶ BBB/BBB opening with FUS followed by delivery of anti-CD47 restricted the growth of GL261 and improved survival.²⁶

Overview of Radiotherapy's Effect on the Immune Response

Chandan Guha, PhD, presented on radiotherapy in combination with immunotherapy as way to create an in-situ tumor vaccine. Radiotherapy was given in weekly small doses in combination with chemotherapy followed by immunotherapy (durvalumab) in patients with lung cancer and significantly increased progression-free survival compared with placebo. However, another trial that administered immunotherapy (avelumab) concurrently with radiotherapy and chemotherapy, had no effect on PFS compared with placebo. Non-ablative radiotherapy followed by immunotherapy (durvalumab) in patients with NSCLC improved pathological response in an open-label phase II trial.⁷ Guha also described a clinical trial using a FLT3 ligand (CDX-301) in combination with SBRT to a single pulmonary lesion in patients with advanced NSCLC.⁶⁰ The hypothesis was that SBRT will cause immunogenic cell death and promote dendritic cell activation and maturation and that CDX-301 will expand dendritic cells in tissues and tumor. Results from the phase 1 study showed that 9 out of 29 patients had an abscopal effect and PET responses at 2 months were prognostic of survival.⁶⁰ Surviving patients had a 13-month median follow-up duration.⁶⁰

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Immune Effects by Tumor Type

Overview of the Conclusions from the GBM Consortium

Pavlos Anastasiadis, PhD, presented an overview of the FUS-GBM consortium. Extensive research has demonstrated local and systemic immune suppression in GBM, and there are a number of immunosuppressive factors that are specific to GBM. A protocol was developed in a mouse model of GBM (GL261) that examined a variety of factors after several different FUS modalities including BBB opening with and without PD-L1, thermal ablation, pFUS, hyperthermia, and histotripsy. Details for FUS parameters were described in the presentation. This research suggests that FUS and immunotherapy combinations may be synergistic. FUS may also be able to locally activate the tumor-immune microenvironment to drive an anti-tumor response.

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Abbreviations

BBB	Blood-brain barrier
CAR	Chimeric antigen receptor
CRI	Cancer Research Institute
FDA	US Food and Drug Administration
FUS	Focused ultrasound
FUSF	FUS Foundation
HIFU	High-intensity focused ultrasound
LIFU	Low-intensity focused ultrasound
NMH	Nanoparticle-mediated histotripsy
NNSCLC	Non-small cell lung cancer
PICI	Parker Institute for Cancer Immunotherapy
TIL	Tumor-infiltrating lymphocytes

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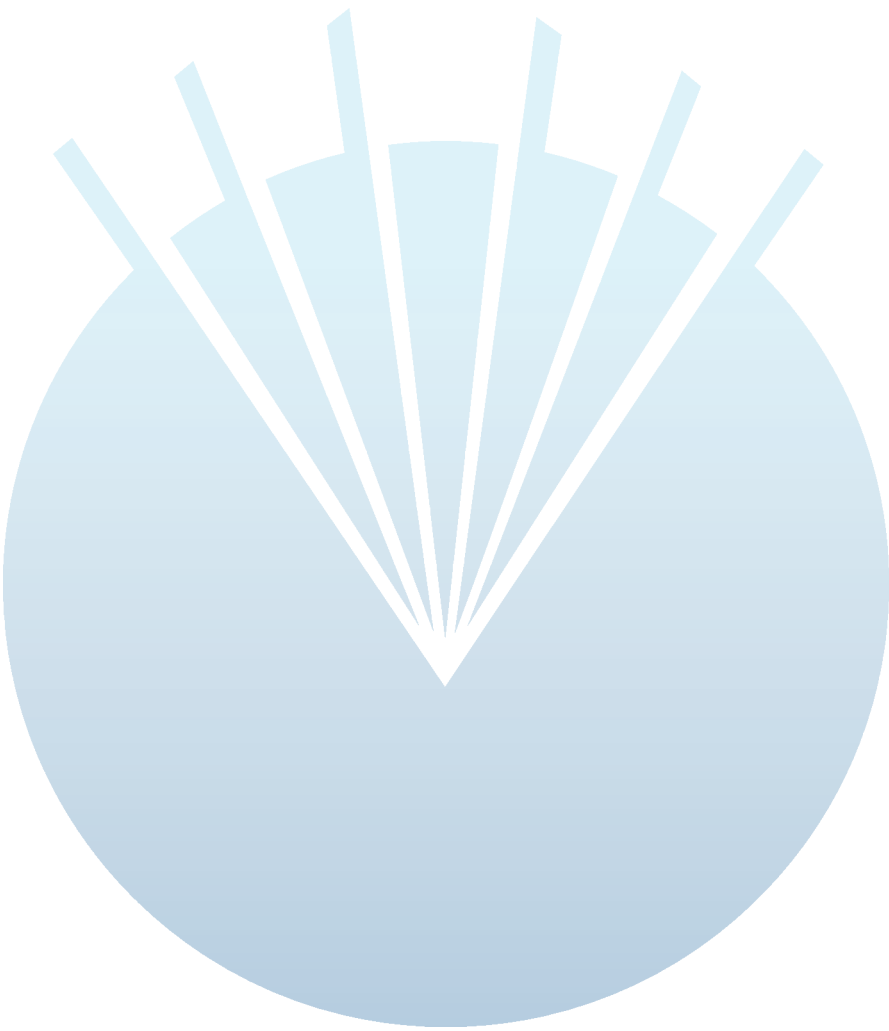
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