Focused-ultrasound mediated anti-alpha-synuclein antibody delivery for the treatment of Parkinson’s disease

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Background: Parkinson’s disease (PD) is associated with the selective death of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc). While the specific cause of the neuronal loss remains elusive, the abnormal accumulation of alpha synuclein (α-syn), a major constituent of Lewy bodies, is considered to play a central role in the pathology of PD. Previous passive immunization studies remain ineffective due to the presence of the blood-brain barrier (BBB), which hinders most therapeutic agents to diffuse to the brain parenchyma. Focused ultrasound in conjunction with microbubbles (MB) is a technique to achieve noninvasive, transient, and localized BBB opening to enhance drug delivery to the brain. Therefore, the objective of this study is to explore the potential of FUS-mediated delivery of anti α-syn antibodies for the treatment of Parkinson’s disease.

Materials and Methods: The study group consisted of ten A53T mice expressing the human α-syn which were divided into three groups: control, FUS-only, and FUS combined with anti-α-syn monoclonal antibodies (mAb). Mice were sonicated with FUS at the left hippocampus, left caudate putamen, and left substantia nigra at an acoustic pressure of 450 kPa, and the microbubbles were injected intravenously (with or without antibodies) through the tail vein immediately prior to sonication. To investigate neuroprotective effects of FUS/mAb, mice received three weekly treatments at 6-7 months of age before significant alpha synuclein aggregation and were euthanized one month after the last treatment for perfusion and immunohistochemical analysis.

Results: FUS mediated successful delivery of mAb by a 3-fold in a transgenic mouse model of Parkinson’s disease (Figure 1), demonstrating its potential role in elevating to therapeutic dose.

![Antibody Delivery](image)
Figure 1. Fluorescence intensity quantification for antibody delivery. There is a ~3 fold increase in fluorescence in FUS-treated side with 100 µg mAb.

Quantification using a minimum error thresholding technique demonstrated reduced α-syn load in mice treated with both FUS and antibody compared to the control mice (p<0.01 by one-way ANOVA and multiple comparison test), without a significant change in neuronal cell counts (p>0.5 by one-way ANOVA, Figure 2).

Figure 2. Quantification of α-syn (left) and NeuN (right). Repeated ANOVA was performed to obtain statistical significance. ***: p<0.001.

Microglia were found not to be activated neither as a result of the pathology at the age of the mice studied nor as a result of the FUS. The FUS treatment was found to be safe as assessed by both MRI and TUNEL staining.

Conclusions: Repeated FUS in conjunction with MB can successfully deliver anti-α-syn mAb and achieve neuroprotective effects such as reducing α-syn in transgenic mouse models of PD. The results demonstrated the potential role of FUS-mediated drug delivery for the treatment of neurodegenerative diseases such as the Parkinson’s disease. Ongoing work aims at using more clinically-relevant MB dosages as well as a larger study group for the assessment of therapeutic role of FUS/mAb treatment.
Results from 72 transient blood-brain barrier (BBB) disruptions by an implantable low intensity pulsed ultrasound (LIPU) device: a safety and feasibility study in recurrent glioblastoma patients

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Background: The BBB limits the efficacy of drug therapies in the brain by blocking the passage of systemically administered drugs to diseased tissue. Two to four minutes of LIPU in combination with injection of micron-sized microbubbles can transiently disrupt the BBB to increase the passage of drugs such as carboplatin.

Materials and Methods: This first-in-man, single arm, monocentric trial was performed at Hôpital Universitaire Pitié-Salpêtrière, Paris, France from 2014-2018. GBM patients at any recurrence were implanted with (1) or (3) 1 MHz, 10-mm diameter cranial devices (SonoCloud®) in burr holes during debulking surgery or during a dedicated procedure under local anesthesia. Ultrasound dose was escalated over 7 patient cohorts in a 3+3 design. The implantable ultrasound device was activated monthly in a <15 minute procedure to transiently disrupt the BBB before intravenous administration of carboplatin (AUC4-6). BBB disruption was visualized using T1w contrast-enhanced magnetic resonance imaging (MRI) and patients were monitored clinically.

Results: Twenty-seven patients were implanted with LIPU devices and 25 per-protocol were sonicated from 2014-2018. A total of 19 patients were treated with (1) US emitter, SonoCloud-1 (SC1), and six patients were treated with (3) US emitters, SonoCloud-3 (SC3). In 85 ultrasound sessions, BBB disruption was visible on post-sonication T1w MRI for 72 sonications was ultrasound pressure dependent. Reported device or procedure-related adverse events were transient and manageable: two cases of transient edema (H1 and D15) and one transient facial palsy. No carboplatin-related neurotoxicity was observed nor infection.

In the SC1 group, patients with no or poor BBB disruption (n=8) had a shorter median PFS and OS than patients with clear BBB disruption (n=11). Two patients in the SC1 group had 10 monthly sonications and 1 patient in the SC3 group had 12 monthly sonications. Patients treated by repeated sonications showed no signs of attenuation of BBB disruption over time or evidence of additional toxicity.

Conclusions: LIPU was well-tolerated and may increase the effectiveness of drug therapies in the brain. The sonication of larger volumes of brain in recurrent GBM will be investigated in a future trial using a larger SonoCloud device and may further enhance the observed effectiveness of this treatment modality. Clinical trial information: NCT02253212.
Focused ultrasound therapy combined with pembrolizumab in metastatic breast cancer

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Background: Focused ultrasound (FUS) perturbs the microenvironment of a tumor by mechanical, thermal and vascular permeability effects. Carefully controlled FUS can induce apoptotic cell death and/or sub-lethal cellular damage as means of immune stimulation. FUS induces heat shock proteins, cytokine release and cellular mediated mechanisms resulting in T cell activation and recognition of tumor antigens. FUS has been demonstrated to be an effective method for inducing tumor antigen exposure and presentation to dendritic cells, thus acting as an auto-vaccine. Pembrolizumab (PBZ) is a PD-1 targeted antibody used in the treatment of multiple solid tumors to augment T cell activation. It is hypothesized that the combination of these two modalities will result in T cell infiltration into breast tumors as well as systemic immune responses.

Materials and methods: In this pilot study, PBZ therapy is combined with FUS to assess for immune stimulation and antitumor effects at local ablation sites, distant non-treated sites and in the blood. Biopsy before, after and 10 weeks post-FUS will examine the tissue in the peripheral zone of ablation, central ablation zone, and distant metastatic sites for CD8 and CD4 T cells, MDSC’s, T-regulatory cells and cytokine responses. Twelve patients will be randomized to receive either PBZ 14 days before or 7 days after a single time FUS partial tumor ablation on day 15. FUS will be delivered by Theraclion Echopulse device at 45W power, with a skin cooling device. Biopsies will be on days 1, 22 and 64 and tumor imaging will be every 12 weeks after baseline. Patients must have metastatic or unresectable breast cancer, adequate organ function, and prior therapy in the metastatic setting. They must also have a tumor in the breast or axilla amenable to FUS and biopsy.

Results: Five patients have been treated to date. The median age is 62. All patients had metastatic breast cancer and accessible primary tumors in breast or axilla. Ultrasound ablations of 30-42% of each lesion was accomplished at 45W power using checkerboard patterns. Increases in CD8+, FOXP3- tumor cells were observed in the periablation zones. Increased numbers of PD-L1+ cells were also observed in peri-ablation zones. Clinical responses will be measured by RECIST criteria. The treatments were reasonably well tolerated and no auto-immune toxicity is observed to date. The trial is ongoing.

Conclusions: To date, focused ultrasound ablation in combination with pembrolizumab is safe and results in observable changes to the immune microenvironment in patients with metastatic breast cancer.
Histotripsy mediated immunomodulation in a mouse gl261 intracranial glioma model

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Introduction: Glioblastoma (GBM) is the most common and most malignant primary brain tumor in adults. Despite aggressive standard therapy including surgery, radiotherapy, and concomitant and adjuvant temozolomide the prognosis is poor with a median overall survival (OS) of less than 15 months. GBM is a highly invasive and diffuse tumor with no clear margin between tumor and healthy brain tissue, making full surgical resection impossible. Recent success of immunotherapies in treating cancers such as melanoma has sparked interest in applying such therapies to GBM. Although beneficial effects of immunotherapies in treating mouse models of GBM have been demonstrated, their limited efficacy has hampered immediate clinical translation. In an effort to enhance anti-tumor immunity in GBM, we hypothesized that histotripsy, due to its ability to induce tumor ablation in a safe and targeted manner, could be used to increase tumor permeability and activity of the anti-tumor immune response. In this initial study, we investigated the immune response of a mouse GL261 intracranial glioma model after histotripsy ablation of a fraction of an intracranial GBM.

Methods: A total of 27 male mice were inoculated with GBM according to a GL261-luc2 C57 BL/6 albino mouse model protocol. Tumor monitoring was performed using T2-weighted MRI and bioluminescence imaging. Histotripsy treatment was applied to a portion of the tumor in 15 of the 27 mice. The remaining 12 were left untreated as controls. A 1 MHz, 8 element transducer with aperture diameter of 58.6 mm and focal length of 32.5 mm was used for treatment. A phased array imaging probe was inserted coaxially within the transducer to allow tumor targeting and treatment monitoring. Tumors were targeted using features visible on both pretreatment MRI and B-mode ultrasound. 50 histotripsy pulses were applied through the skull to a single point within the tumor at a pulse repetition frequency (PRF) of 1 Hz and an estimated peak-negative pressure of 40 MPa. Preliminary experiments in healthy mice showed well-defined lesions using these treatment parameters (Fig. 1). Treatment was applied 2 weeks after inoculation and mice were survived for 1 week after histotripsy treatment after which point they were euthanized. Directly following euthanasia in both treatment and control mice, brain tumors, spleens and lymph nodes were harvested for flow cytometry. In addition, for both groups, immunohistochemistry staining was performed on the primary tumor. The overview of the study and specific time points is shown in Figure 2.

Results: Of the 15 mice treated with histotripsy, 14 survived treatments, all with lesions visible in post-treatment MRI. 12 of these mice were confirmed to have lesions in a portion of the bulk tumor volume as observed via MRI. Of the 12 control mice, 11 survived until the euthanasia date with 1 mouse dying 3 days prior to euthanasia. Figure 3 shows the pre- and post-treatment T2-weighted MR images from tumor monitoring of a treated mouse. Flow cytometry results yielded a two-fold decrease ($\alpha = 0.05$) in the number of myeloid derived suppressor cells (MDSCs) in the brain tissue of mice treated with histotripsy relative to that of the control mice (Fig. 4a). Additionally, a large increase in interferon gamma (IFN-γ) was observed in mice treated with histotripsy (Fig. 4b). As interpreted by a pathologist,
Giemsa staining of sections of brain tumors from treated mice sampled from regions away from the histotripsy lesion showed shrunken cells with shrunken and pyknotic nuclei separated by edema with insufficient cells to assess the mitotic index (Fig. 4c). This contrasted with the sections of brain tumors from control mice where intact tumor cells and a high mitotic index were observed (Fig 4d).

Conclusions: In this initial study, we investigated the immune response of a mouse GL261 intracranial glioma model after treating a portion of the brain tumor with histotripsy. Flow cytometric analysis showed a decrease in highly immunosuppressive MDSCs in histotripsy treated tumors compared to untreated brain tumors. In addition, the IFN-γ production was found to be extremely low in the untreated control tumors (consistent with literature), whereas histotripsy treated tumors showed relatively large amounts of IFN-γ, suggesting that the decrease in MDSC resulted in a decreased immunosuppressive intratumoral environment. Analysis of tumor immunohistochemistry revealed that histotripsy treated tumors showed shrunken tumor cells with shrunken and pyknotic nuclei separated by edema which contrasted with the untreated brain tumors, which appeared relatively healthy. These initial results provide a compelling rationale for the safety of GBM therapy using histotripsy, while changes in immunomodulatory cell populations provide evidence of potential mechanisms that may be at work in increasing the immunogenicity of the tumors following histotripsy treatment.

Figure 1. The brain of a healthy mouse after applying 50 1 MHz histotripsy pulses to a single point through the skull at a PRF of 1 Hz. The lesions as observed in both T2-weighted MRI (top) and H&E stained histology (bottom).
Figure 2. The overview of the study and specific time points.

Figure 3. The pre- and post-treatment T2-weighted MR images from tumor monitoring of a treated mouse. A well defined lesion is evident within the tumor in the first post-treatment image (day 15).

Figure 4. (a) Flow cytometry results for brain tissue (BT), spleen, sentinel lymph node (SLN) and draining lymph node (DLN). (b) IFN-gamma concentration in control vs. treated mice. Giemsa staining of tumor from (c) treated and (d) control mice.
Blood brain barrier opening in Alzheimer's Disease: Safety data from a phase I trial

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Blood brain barrier opening in Alzheimer’s disease using MR-guided focused ultrasound

Background: MR-guided focused ultrasound (MRgFUS) in combination with intravenously injected microbubbles (MB) has been shown to transiently open the blood-brain barrier (BBB), and reduce beta-amyloid and tau pathology in animal models of Alzheimer’s disease (AD). Here, we investigated the safety and feasibility of MRgFUS to open the BBB within the right prefrontal cortex, on two occasions, in six patients with mild-to-moderate AD.

Materials and Methods: We enrolled patients diagnosed with Alzheimer’s disease with Mini-Mental Status Exam score between 18 and 28 and [18F]-florbetaben PET positivity in the right frontal lobe, area of target. Patients underwent two BBB opening procedures using the ExAblate 220 KHz system (InSightec, Haifa, Israel), one month apart, and followed for a total of three months. Primary outcome measures were safety, in the number of adverse events, and feasibility by the leakage of gadolinium contrast on MRI due to increased BBB permeability. Secondary outcome measures were change in [18F]-florbetaben tracer uptake in the region of interest, and change in neuropsychological tests (e.g. ADAS-cog). Exploratory measures included changes in resting state connectivity following BBB opening.

Results: In all patients, the blood-brain barrier within the target volume was safely and repeatedly opened, with closure observed on follow-up MRI within 24 hours. Opening the blood-brain barrier did not result in serious clinical or radiographic adverse events. The average maximum sonication power was 4.6W with an average of 3.6 sonications administered for stage 1 and 4.5W for 7.5 sonications for stage 2. Changes in group ADAS-cog scores at three months and baseline, as well as tracer uptake after stage 1 and 2 were not statistically different (p > 0.05).

Conclusion: The results of this safety and feasibility study support the continued investigation of focused ultrasound as a potential novel treatment and delivery strategy for patients with Alzheimer’s disease.
Objectives: The blood-brain barrier (BBB) has long limited therapeutic access to brain tumor and peritumoral tissue. Pre-clinical evidence of low-intensity MR-guided focused ultrasound (MRgFUS) has shown precision in disrupting the BBB without surgery. The objective of this Phase I, single-arm open-label study is to investigate the safety and feasibility of BBB opening with chemotherapy delivery using MRgFUS for the first time in patients with glioma.

Methods: Five patients with previously confirmed or suspected high-grade glioma underwent a single MRgFUS BBB opening with concurrent chemotherapy (n=1 liposomal doxorubicin, n=4 temozolomide) administration. Surgical resection of the tumor was performed the following day, and tissue samples of ‘sonicated’ and ‘unsonicated’ tissue were obtained for analysis where accessible. Participants continued with standard neuro-oncology care and followed for three months.

Results: The BBB within the target volume showed radiographic evidence of increased permeability to gadolinium immediately following focused ultrasound procedure, resolving on the MRI approximately 20 hours. No adverse clinical or radiologic events occurred with BBB opening. Biochemical analysis of tissue from the tumor margin suggests evidence of chemotherapy delivery.

Conclusion: MRgFUS BBB opening of tumor and peri-tumor tissue with concurrent administration of chemotherapy was well tolerated by patients with glioma. The characterization of therapeutic delivery and clinical response to this treatment paradigm requires further investigation.

Funding sources: This study was supported by Focused Ultrasound Foundation.
Leveraging MR image-guided focused ultrasound to potentiate immunotherapy for glioblastoma


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Background: Glioblastoma (GB) is the most common and malignant brain tumor, its diffuse nature and proclivity for recurrence rendering it largely intractable. Immunotherapy (ITx) approaches (e.g. anti-PD1) may hold promise for treating GB; however, the blood-brain (BBB) and blood-tumor (BTB) barriers hinder delivery of systemically administered ITx drugs. A potential approach to enhancing ITx delivery is MRI-guided focused ultrasound (FUS), a non-invasive technique that, when combined with concomitant systemic injection of microbubbles (MB), can transiently disrupt the BBB/BTB and mechanically perturb the tumor microenvironment. Here, we investigate whether localized BBB/BTB disruption with FUS+MB enhances anti-tumor immune responses and inhibits tumor growth in an orthotopic murine glioma model.

Materials and Methods: At 14 days following intracranial implantation of GL261 cells stably transfected with luciferase (GL261-luc2), mice were ultrasonically coupled to a 1.1 MHz small animal MR image-guided FUS system. In animals bearing MRI-visible tumors, albumin-shelled MB were injected intravenously and a 4-spot grid of sonifications was applied to the tumor-bearing region immediately following (0.4-0.6 MPa peak negative pressure (PNP), 0.5% duty cycle, 120s period). BBB/BTB disruption was confirmed by post-treatment MR imaging. Tumor outgrowth was monitored serially by IVIS imaging. One week following treatment, whole brains and peripheral lymphoid organs were harvested for flow cytometry.

Figure 1. (A) Representative axial contrast-enhanced T1-weighted MRI images demonstrating BBB/BTB disruption in GL261-luc2 tumors. (B-C) Stable and inertial cavitation doses calculated from harmonic and broadband acoustic emissions, respectively. (D-E) MFI of CD86 on CD11c+ DC in DCLN and tumor one week following FUS+MB (groups: untreated GL261-luc2 tumors, GL261-luc2 tumors exposed to 0.4 MPa or 0.6 MPa FUS). (F) Absolute frequency of CD8+CD44+PD1+ T cells in GL261-luc2 tumors one week following FUS+MB. (G) Serial bioluminescence imaging quantification demonstrating early tumor growth inhibition. *p<0.05, **p<0.01.
Results: MR imaging of GL261-luc2 tumors treated at the stated parameters evidenced successful BBB/BTB disruption (Fig.1a). Analysis of acoustic emissions detected by passive cavitation monitoring discretized stable and inertial cavitation activity as a function of PNP (Fig.1b,c). Seven days following BBB/BTB disruption at 0.6 MPa, CD86 (a marker of maturity) mean fluorescence intensity (MFI) on dendritic cells (DC) increased ~3-fold in deep cervical lymph nodes (DCLN) (Fig.1d). Accordingly, intratumoral CD86+ DC trended towards increased absolute frequency while CD86 MFI on intratumoral DC was significantly different across the two PNPs evaluated (Fig.1e). One week following BBB/BTB disruption, intratumoral CD4+ T cells doubled and intratumoral CD8+ T cells increased by ~17%. Within the intratumoral CD8+ T cell population, a significant differential increase in antigen-experienced (CD44+) PD1+ T cells was observed as a function of FUS+MB treatment at both 0.4 and 0.6 MPa (Fig.1f), thereby inciting a rationale for future implementation of anti-PD1 therapy. Bioluminescence imaging revealed a significant reduction in total photon flux as early as 6 days following FUS+MB at 0.6 MPa (Fig.1g), indicating early growth inhibition of GL261-luc2 tumors.

Conclusions: These findings demonstrate that BBB/BTB disruption with FUS+MB can potentiate anti-tumor immunity against glioma, independent of drug delivery. Ongoing studies entail combining FUS+MB with checkpoint inhibitor (i.e. anti-PD1, anti-TIGIT) delivery to evaluate whether an allied treatment approach can promote an even more robust anti-glioma response.
A pre-clinical investigation of the immunological effects of pulsed focused ultrasound and immune checkpoint inhibitors in pancreatic cancer

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Objectives: The clinical benefit of immunotherapy has not yet been realised in pancreatic cancer, which is characterised by a low antigenicity and dense stroma profile. Focused ultrasound (FUS) can be used in the treatment of solid tumours, either by inducing necrosis (using ablative temperatures), or by creating cavitation which results in mechanical disruption of the stroma. Both of these processes may regulate the immune response and make the tumours more susceptible to immunotherapeutic treatments. In this study, pancreatic tumours have been exposed to FUS and co-treated with immune checkpoint inhibitors (ICI) to explore whether control of the tumour growth can be achieved.

Methods: Syngeneic orthotopic KPC pancreatic tumours (KrasLSL.G12D/+; p53R172H/+; PdxCre tg/+ ) were grown in immune-competent murine C57BL/6 subjects (> 15 weeks old). Tumours were exposed to pulsed FUS using the small animal Alpinion VIFU 2000 Therapeutic Ultrasound platform. Pulsed FUS exposure parameters were designed to result in cavitation (power = 250 W, duty cycle = 1 %, pulse repetition frequency = 1 Hz, 60 repeats) in the target tissue. A passive, weakly focused, broadband (0.1 to 20 MHz) sensor was used to detect cavitation. A combination of anti-CTLA4 and anti-PD-1 antibodies (200 mg per antibody per subject), or their respective isotypes, were administered intraperitoneally 3 days before treatment, and every 3 days thereafter. Tumour growth was estimated using high frequency ultrasound imaging, and with callipers at the time of culling. The biological effects of the treatments on the tumours and the extracellular matrix were investigated using H&E and trichrome staining of formalin-fixed, paraffin-embedded histological sections, and using immunohistochemistry to detect CD4+ and CD8+ tumour infiltrating lymphocytes. Results were quantified using ImageJ. The immunological effects of the treatments were investigated in the blood, spleen and the pancreaticoduodenal and gastric lymph nodes of the treated and sham-exposed subjects with multi-colour flow cytometry of white blood cells and splenocytes. Staining patterns typical of T helper, T cytotoxic, T regulatory, T effector and Myeloid-derived suppressor cells were used. Their relative abundances were quantified using Flow Jo.

Results: Pulsed FUS treatment of pancreatic tumours resulted in cell and collagen depleted regions in the tumours, associated with an extensive rearrangement of the extracellular matrix. Broadband signal (suggestive of cavitation) was detected. No skin damage was observed. Combination of a single pulsed focused ultrasound treatment with administration of ICIs resulted in improved control of tumour growth relative to the monotherapies and sham exposures. Survival of subjects treated with the combination treatment was extended relative to the survival of subjects treated with the monotherapies. Additional results for the systemic and localised relative abundance of immune cells will be presented.
Conclusions: FUS can be used in combination with antibody immunotherapy to control the growth of pancreatic tumours. These results suggest that focused ultrasound can turn an immunologically “cold” tumour into an immunologically “hot” tumour and should be trialled with additional immunotherapies.