An international, randomized, controlled trial of Focused Ultrasound thalamotomy for essential tremor

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Objectives: Recent advances in transducer technology have allowed ultrasound to be transmitted with precision through the human skull. Pilot studies suggest that MR guided Focused Ultrasound can be used to successfully generate stereotactic thalamic lesions. We present the one year results of a double-blinded, randomized, controlled trial of FUS thalamotomy for essential tremor.

Methods: Seventy-six patients with medication-refractory essential tremor were randomized 3:1 to receive a unilateral Focused Ultrasound thalamotomy or a sham procedure. Tremor (CRST) and quality of life (QUEST) measures were obtained at baseline, 3, 6, and 12 months. Safety was determined adverse event monitoring throughout the study. All tremor assessments were videotaped and then rated by an independent core lab of neurologists who not involved in the procedures.

Results: Contralateral hand tremor, the primary endpoint, was improved by 49% at 3 months (p<0.001) with the treatment (18.09 +4.81 to 9.55 + 5.06) compared to sham procedures (16.00 + 4.42 to 15.75 + 4.90) and the effect was durable at one year (10.89+4.86).

Similarly, functional measures of disability and quality of life were statistically improved whereas there was no change in the sham cohort.

The most common side effects were paresthesia and gait disturbances which persisted at 12 months in 14% and 9%, respectively.

Conclusions: Focused ultrasound can be delivered effectively through the intact skull to make precise ablations deep in the brain. MR guided Focused Ultrasound thalamotomy improves hand tremor and quality of life in ET with an acceptable safety profile.
Treatment of essential tremor and Parkinson’s disease tremor by MRI guided Focused Ultrasound: A report of 38 consecutive cases in a single center

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Objectives: Thalamotomy of the ventral intermediate nucleus (VIM) is effective in alleviating medication resistant tremor in patients with essential tremor (ET) and Parkinson’s disease (PD). MRI guided Focused Ultrasound (MRgFUS) is an innovative technology that enables non-invasive thalamotomy via thermal ablation.

Methods: Thirty eight ET and PD patients with severe medication resistant tremor underwent MRgFUS underwent unilateral VIM thalamotomy using MRgFUS. Effect was evaluated using clinical Rating Scale of Tremor (CRST) in ET patients and Unified PD Rating Scale motor part (UPDRS) in PD patients. Quality of life was assessed by Quality of life in ET Questionnaire (QUEST) and PD Questionaire (PDQ-39).

Results: Tremor stopped in the treated hand in 37 patients immediately following the treatment. In one patients tremor was modified but not abolished. At one month post-treatment, the ET patients’ CRST score decreased from 38.6±12.0 to 9.3±7.7 (p<0.001) and QUEST scores decreased from 44.8±17.8 to 13.1±15.9 (p<0.001). In PD patients UPDRS-motor part decreased from 26.2±8.7 to 16.3±11.0 (p=0.0087) and PDQ39 decreased from 40.8±18.2 to 26.5±15.1 (p=0.027). During follow up of 1-24 months (mean 10.9±8.1 months) tremor reappeared in seven of the patients, but in all but three, to a lesser degree than before the procedure.

Adverse events that transiently occurred during sonication included: Headache (n=11), short lasting vertigo (n=17) and dizziness (n=4), nausea (n=4), burning scalp sensation (n=3), vomiting (n=3) and lip paresthesia (n=2). Adverse events that lasted after the procedure included gait ataxia (n=5), unsteady feeling when walking (n=4,) unilateral taste disturbances (n=3) and hand ataxia (n=3). All adverse events were transient and none lasted beyond 3 months.

Conclusions: MRgFUS VIM thalamotomy to relieve medication resistant tremor was safe and effective in ET, and PD. Current results emphasize its low adverse events profile and high efficacy in treating tremor. Large randomized studies are needed to assess prolonged efficacy and safety.
MRI guided High Intensity Focused Ultrasound surgery for Parkinson’s disease

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Objectives: The field of MRI guided High Intensity Focused Ultrasound surgery (MRgFUS) is evolving and offers the new hope for the treatment of many neurological disorders through both ablative mechanism and non-ablative mechanisms such as drug delivery, neuromodulation and etc.

Thus, I also want to evaluate the role of MRgFUS for the management of Parkinson’s disease especially for those who are struggling with drug related dyskinesia. And I had an approval of the feasibility study from the Korean FDA and the IRB of Yonsei University College of Medicine.

As well, all the patients had fully informed written consent for making unilateral pallidotomy to control the dyskinesia and other parkinsonian symptoms.

Methods: The treatment was performed in a 3.0 T MRI (Sgina, GE) using the Exablate 4000 device (Insightec), which features a 30 cm diameter hemispherical 1024 elements phased array transduced operating at 650 KHz. And the patient’s head was immobilized by fixation in an MRI compatible frame (Radionics).

Results: In this presentation, I will present the long-term follow up results of patient with Parkinson’s disease after MRgFUS with our imaging studies.

As well, I will discuss the several important unsolved issues which were related with the unilateral pallidotomy with MRgFUS.
Focused Ultrasound likely dominates deep brain stimulation and stereotactic radiosurgery for medically-refractory essential tremor: An initial decision and cost-effectiveness analysis

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Objectives: Essential Tremor (ET) is one of the most common neurologic conditions, and conservative measures are frequently suboptimal. Recent data from a multi-institution, randomized controlled clinical trial demonstrated that Magnetic Resonance-guided Focused Ultrasound (MRgFUS) thalamotomy improves upper limb tremor in medically refractory ET. This study assesses the cost-effectiveness of this novel therapy in comparison to existing procedural options.

Methods: PubMed and Cochrane Library searches were performed for studies of MRgFUS, Deep Brain Stimulation (DBS), and Stereotactic Radiosurgery (SRS) for ET. Pre- and post-operative tremor-related disability scores were collected from 32 studies involving 83 MRgFUS, 615 DBS, and 260 SRS cases. Utility (defined as percent change in functional disability) was calculated, and Medicare reimbursements were collected as a proxy for societal cost – costs of MRgFUS for ET were derived from a combination of available costs of approved indications and SRS costs where appropriate. A decision and cost-effectiveness analysis was then constructed, implementing meta-analytic techniques.

Results: MRgFUS thalamotomy resulted in significantly higher utility scores compared with DBS and SRS based on estimates of Medicare reimbursement (p< 0.001). MRgFUS was also the most inexpensive procedure out of the three (p<0.001).

Conclusions: Preliminary experience with MRgFUS for ET suggests that this novel therapeutic may be more effective than available alternatives and potentially less costly for society. It thus will likely “dominate” DBS and SRS as a more cost-effective option for medically refractory ET. Our findings support further investigation of MRgFUS for ET and broad adoption.
Tractography-based VIM identification for Focused Ultrasound thalamotomy: Initial Results

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Objectives: The ventral intermediate nucleus (VIM) is not visible on conventional Magnetic Resonance Imaging (MRI). A novel method for tractography-based VIM identification has recently been described. We report the short-term clinical results of prospective VIM targeting with tractography in a cohort of patients undergoing Focused Ultrasound thalamotomy.

Methods: All patients underwent structural and diffusion weighted imaging (60 diffusion directions, 2 mm isovoxel) with 3 Tesla MRI scanner (Philips Ingenia CX). The images were processed using streamline tractography (Stealth Viz, Medtronic Inc.). The lateral and posterior borders of VIM were defined by tracking the pyramidal tract and medial lemniscus respectively. A VIM region of interest (ROI) was placed 3 mm away from these borders. The structural connectivity of this VIM ROI was confirmed to the motor cortex (M1) and cerebellum. The coordinates of tractography-based VIM in relation to posterior commissure were noted for surgical targeting. The parameters analyzed include a clinical tremor scale (pre-, intraoperative, and post operative), operative time, and number of sonications.

Results: Tractography-based VIM targeting was successful in 7 out of 8 patients. The coordinates of tractography-based VIM were significantly different from the standard coordinates (3-D distance 3.9±2.4 mm). Therapeutic sonication (>55 °C temperature, 10 seconds) at the tractography target resulted in >50% tremor improvement with intraoperative objective tremor assessment without any motor or sensory side-effects. The mean operative time was 78±3.3 minutes with 12.8±3.9 average sonications. Overall the tremor scores significantly improved one month after surgery (preop CRST total 62.1±15.5 versus 30.3±14.1, two tailed t-test p=0.006). None of the patients experienced sensory deficits or motor weakness during follow-up.

Conclusions: We report that prospective tractography-based VIM targeting is safe and feasible. The short-term clinical results are satisfactory. Long-term tremor efficacy outcomes are desirable to further assess the usefulness of this technique.

Figure 1. Axial T1 projection showing the relation of VIM target 3 mm medial and anterior to pyramidal tract and medial lemniscus respectively

Figure 2. Postoperative sagittal T1 projection demonstrating the relationship between pyramidal tract and medial lemniscus in relation to thalamotomy lesion

Figure 3. Postoperative axial T1 projection demonstrating the relationship between pyramidal tract and medial lemniscus in relation to thalamotomy lesion
Targeted delivery of brain-penetrating non-viral GDNF gene vectors to the striatum with MRI-guided Focused Ultrasound reverses neurodegeneration in a Parkinson’s disease model

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Objectives: Parkinson’s disease (PD) is characterized by the degeneration of dopaminergic neurons in the motor control pathways of the brain. Gene therapy using glial cell derived neurotrophic factor (GDNF) has shown some limited promise for treating PD; however, we hypothesize that outcomes could be further improved by enhancing gene vector distribution. We previously developed a gene therapy approach that entails delivering systemically administered non-viral gene-bearing nanoparticles (BPN) across the Blood-Brain Barrier with MRI-guided Focused Ultrasound (FUS). BPN rapidly penetrate brain tissue due to a dense coat of polyethylene glycol, and this approach mediates efficient and localized transgene expression in the brain of healthy rats. Here, we tested whether the FUS-mediated delivery of GDNF plasmid-bearing BPN (GDNF-BPN) reverses neurodegeneration in the rat 6-OHDA PD model.

Methods: 6-OHDA rats were ultrasonically coupled to a 1.15 MHz MRI-compatible FUS transducer. T2 and T2* pre-treatment scans were obtained to allow FUS targeting of striatum. Microbubbles (2x10^5/g) and 100 μg of ~50nm non-viral GDNF plasmid-bearing BPN (polyethylene glycol/polyethylenimine) were co-injected i.v. and FUS was applied at 0.6 MPa, with a 0.5% duty cycle, for 2 min. Contrast T1 and T2* images allowed semi-real time confirmation of BBB disruption and safety, respectively. Efficacy was assessed using an ELISA for GDNF, tyrosine hydroxylase (TH) and VMAT2 immunolabeling for neural degeneration, HPLC for dopamine, and behavioral analysis (i.e. apomorphine-induced rotational asymmetry and forepaw use bias in 6-OHDA rats).

Results: Striatum-targeted delivery of GDNF plasmid-bearing BPN with FUS led to an ~80% reduction in apomorphine-induced rotational asymmetry, eliminated forepaw use bias (Fig 1a,b), and fully restored TH+ dopaminergic neuron density in both the substantia nigra pars compacta (SNpc) and striatum compared to untreated 6-OHDA rats (Fig 1c,d). T2* MRI confirmed safety of the BBB opening approach.

Conclusions: FUS-mediated delivery of systemically circulating non-viral GDNF-BPN to the striatum of 6-OHDA rats confers a significant behavioral benefit as well as a restoration of TH+ cell number in the nigrostriatal pathway, indicating cessation and/or reversal of neurodegeneration. Our studies indicate that delivery of GDNF-BPN with FUS may provide a powerful, non-invasive and highly tailorable gene therapy approach to slow or stop the neurodegenerative process in PD.

Graphs of rotational bias (A) or forepaw use bias (B) following 6-OHDA injection. (C) Representative images of TH-immunolabeled sections through the SNpc. Graphs represent TH+ cell number in SNpc (D) and staining intensity in the striatum (E). * p<0.05
Focused ultrasound facilitated gene delivery for neuro-restoration in Parkinson’s disease mice

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Objectives: Not released for publication
Methods: Not released for publication
Results: Not released for publication
Conclusions: Not released for publication

MRI-g-FUS for the treatment of Alzheimer’s Disease

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Objectives: Over the past ten years consistent effort has been put forward at the University of Toronto to develop Focused Ultrasound methods for the treatment of AD. This talk will review the progress made so far.

Methods: The first studies demonstrated safe antibody delivery in AD mouse model with significant reduction in the plaque load. A follow up studies with two-photon microscopy showed that blood vessels with plaque deposits showed a different type of opening than vessels in normal brain but large molecule delivery into the brain was still possible in these animals. Another study demonstrated that plaque reduction can be achieved by just opening the BBB with microbubbles.

Results: The histology revealed stimulation of neurogenesis. Multiple treatments of old mice resulted in memory rescue without any observable side-effects. A follow up study demonstrated that this neurogenesis was not induced with exposures that did not cause observable BBB opening even with the presence of the microbubbles. An ongoing study in large animals has shown that half-brain BBB opening can be safely and repeatable performed indicating the feasibility of clinical translation.
**Scanning Focused Ultrasound disruption of the Blood-Brain Barrier as an Alzheimer’s disease therapy**

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**Objectives:** Alzheimer’s disease is the most common form of dementia. Pathological abnormalities in the Alzheimer’s disease brain includes the presence of amyloid-beta plaques, hyperphosphorylation and intracellular aggregation of tau and synaptic degeneration. Focused ultrasound combined with intravenous injection of microbubbles has been shown to reversibly open the blood-brain-barrier (BBB). By moving the focus in scanning mode we are able to open the BBB throughout the brain of a mouse. Here we tested the effects of repeated scanning ultrasound (SUS) in APP23 amyloid plaque-bearing mice, pR5 tau mice and wild-type mice to determine the effects of SUS on amyloid, tau and dendritic spines.

**Methods:** The device used was the Therapy Imaging Probe System (TIPS, Philips Research), which has an eight-element annular array transducer with a focal length of 80 mm a radius of curvature of 80 mm, a 33 mm central opening, and a motorized 3D positioning system. The focus 6 dB size was 1.5 mm x 1.5 mm x 12 mm at 1 MHz. Settings that were applied were 1 MHz centre frequency, 0.7 MPa peak rarefractional pressure applied outside the skull, 10 Hz pulse repetition frequency, 10 ms pulse length and 10% duty cycle immediately after retroorbital injection of in-house made microbubbles. APP23 mice that accumulate amyloid beta, pR5 mice that overexpress FTD-mutant tau, and wild-type C57Bl/6 mice were treated weekly by scanning ultrasound (SUS) for periods of 4 to 7 weeks.

**Results:** In APP23 mice we used repeated scanning ultrasound (SUS) treatments of the mouse brain to remove amyloid-beta. Spinning disk confocal microscopy revealed extensive internalization of Abeta into the lysosomes of activated microglia in mouse brains subjected to SUS. Plaque burden was reduced in SUS-treated AD mice compared to sham-treated animals. Treated AD mice also displayed improved performance on three memory tasks.

In PR5 mice we investigated the efficacy of a novel tau isoform-specific single chain antibody fragment, RNX, delivered by passive immunization in the P301L human tau transgenic pR5 mouse model. When administration of RNX was combined with scanning ultrasound (SUS), RNX delivery into the brain and uptake by neurons were markedly increased, as were reductions in tau phosphorylation and anxiety-like behavior.

In wild-type mice we investigated the effects of SUS on neuronal excitability and morphology. We performed patch-clamp recordings from hippocampal CA1 pyramidal neurons in wild-type mice 2 and 24 hours after a single SUS treatment, and one-week and three months after six weekly SUS treatments. No change in CA1 neuronal excitability was observed compared to sham-treated neurons at any time-point. Multiple SUS treatments had the effect of preventing the loss of CA1 synapses that occurred in sham-treated neurons.

**Conclusions:** We show that scanning Focused Ultrasound disruption of the BBB has multiple biological effects in the brain which make it an attractive candidate for an Alzheimer disease therapy. SUS reduced plaque burden and amyloid-beta levels in APP23 amyloid mice, through activation of microglia, and improved performance on tests of memory function. In pR5 tau mice SUS alone reduced hyperphosphorylation of tau, and enhanced the delivery of anti-tau antibodies resulting in improved reductions in pathology and behavioral abnormalities. In wild-type mice SUS was shown to have no effect on the firing of hippocampal neurons or their morphology, but prevented spine loss at 3 months after six weekly SUS treatments. If these effects on Abeta and tau pathology, and dendritic morphology are recapitulated in human patients SUS may emerge as a promising AD therapy.
Scanning ultrasound as a treatment tool of proteinopathies including Alzheimer’s disease

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Objectives: Neurological disorders constitute a substantial social and economic burden, as they cause considerable ill health but few direct deaths. Treatment strategies for neurodegenerative diseases are hampered by the fact that the Blood-Brain Barrier (BBB) establishes an efficient barrier for therapeutic agents (Leinenga et al., Nature Reviews Neurology 2016). We have recently shown that scanning ultrasound (SUS) allows microglial-mediated clearance of extracellularly deposited amyloid-beta in APP mutant APP23 mice and restores memory functions in three cognitive tests to wild-type levels, in the absence of overt damage to the brain (Leinenga and Götz, Science Translational Medicine 2015). However, it had not been determined whether SUS treatment reduces the intracellular tau pathology that together with amyloid deposition characterizes Alzheimer’s disease.

Methods: We investigated the efficacy of a novel tau-specific single chain antibody fragment, delivered by passive immunization in the human tau transgenic pR5 mouse model, a model for the tau pathology of Alzheimer’s disease (Götz et al., Science 2001). To further assess the efficacy and drug-delivering ability of SUS, we established four experimental groups, using the novel anti-tau antibody that was injected weekly over four weeks, either on its own, or together with SUS. A third group used SUS only, and a fourth was the anaesthesia control group. The mice were analysed on the elevated plus maze, histologically and biochemically. Furthermore, uptake of the antibody by the brain was determine using fluorescently labelled single-chain antibody fragments.

Results: A histological and biochemical analysis of the pR5 tau transgenic mice revealed that SUS as well as the employed antibody ameliorated the tau pathology that characterizes the pR5 mice. In addition, the anxiety-like behaviour that characterizes pR5 mice was significantly reduced. We furthermore found enhanced delivery of the antibody using SUS yielding a synergistic therapeutic effect as determined by histology and using the elevated plus maze.

Conclusions: Our study suggests that SUS is a method that benefits diseases with protein aggregates more generally, whether they are intra- or extracellular. The therapeutic delivery combined with SUS could offer significant clinical benefits for the treatment of patients with Alzheimer’s disease and related tauopathies. Considering that the yearly costs of passive immunotherapy for AD is expected to exceed $25,000 per patient, combining SUS with antibody delivery could drastically reduce these costs.
MRI guided High Intensity Focused Ultrasound surgery for obsessive compulsive disorders

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Objectives: Surgery for intractable psychiatric illness has generated considerable controversy for a variety of scientific, social and philosophical reasons. However, the surgical treatment of obsessive compulsive disorders (OCD) by lesioning techniques such as cingulotomy, capsulotomy was well accepted in the clinical field throughout the world. However, the lacks of direct neuroanatomical and pathophysiological rationales for how lesions in specific limbic areas alleviate specific OCD symptoms have been consistent criticisms of lesioning procedures. Recently, because the anatomical and neurochemical substrates of brain function in health controls and disease patients are slowly being elucidated by various functional neuroimaging techniques, these criticisms are becoming less valid. Furthermore, by using new technique such as deep brain stimulation (DBS) and by making more precise targets, it enables to treat the patients without making serious complications.

However, as we recognize, DBS also has many disadvantages along with procedures and etc. Currently, MR-guided Focused Ultrasound (MRgFUS) has been developed as a non-invasive surgical tool of generating precisely placed focal thermal lesion in the brain. The authors underwent a feasibility study of MRgFUS for the treatment of medically refractory OCD. Patients with OCD were treated by making bilateral thermal lesions in the anterior limb of the internal capsule (capsulotomy) with MRgFUS. In this presentation, I would like to demonstrate the not only therapeutic effects but also technical & practical issues of the current MRgFUS for medically refractory OCD.

In this presentation, I will present the long-term follow up results of patient with obsessive compulsive disorders after MRgFUS with our imaging studies.
Enhancement of FUS mediated delivery of stem cells to the brain

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Objectives: FUS mediated Blood-Brain Barrier disruption (BBBD) can enable even large therapeutics such as stem cells to enter brain from the bloodstream and could be a major advance in cell delivery over current invasive methods of brain injection. The efficiency of cellular entry after FUS mediated BBBD alone however is low. We hypothesized that this process could be enhanced by combining it with a complementary strategy termed magnetic targeting. Stem cells can be safely loaded with super-paramagnetic iron oxide nanoparticles (SPION) in culture, allowing cells to be attracted by an external magnet. Our previous study showed SPION loaded stem cells to have enhanced brain retention near a magnet on the skull in a rat model of traumatic brain injury, where BBBD also occurs. The goal of our current project was to determine if magnetic attraction of SPION loaded stem cells would also enhance their delivery to brain after FUS mediated BBBD.

Methods: With a small animal MRI guided FUS device (Image Guided Therapy, IGT and 7T Bruker MRI), we sonicated young adult rats (~ 120 g) with both radiologic (enhancement of the target region with gadolinium on post-sonication T1 MRI), and histologic (staining with Evans’ blue dye) evidence of BBBD, without tissue damage or hemorrhage. Confirmation of the cells within brain as those injected was performed by staining with Perl’s reagent for iron and by immuno-histochemistry with a human specific antigen. The procedure was then combined with the application of a powerful magnet to the head directly after IV injection of hNPCs.

Results: With BBBD alone human neuro-progenitor cells (hNPCs) loaded with SPION were observed in rat brain after intravenous (IV) injection directly after sonication only within the treated regions. To demonstrate the effect of magnetic attraction, we injected equal numbers of SPION and non-SPION labeled cells, where each cell type was labeled with a different fluorophore. In animals that had FUS mediated BBBD followed by a magnet applied to the head, significantly greater numbers of SPION labeled cells were observed compared to the non-labeled cells. This result was most pronounced in regions of the brain close to the skull (cerebral cortex) and magnet surface. More powerful magnets including magnetic arrays resulted in more effective retention of SPION labeled cells in even deeper brain regions such as the striatum. There, 90% of hNPCs observed contained SPIONs compared to 60-70% with a less powerful magnet.

Conclusions: These results demonstrate that the use of magnetic attraction can substantially enhance delivery of stem cells after BBBD. In prior published work, stem cells were delivered to brain after FUS mediated BBBD using cells injected directly into the carotid artery. In an effort to accomplish this goal in a safer and less invasive manner, our study utilized IV cell injection (tail vein), supporting the view that the combination of FUS mediated BBBD and magnetic attraction can allow stem cells to enter brain with a minimally invasive strategy.
Fluorescent lipid microbubbles for targeted brain drug delivery through the Focused Ultrasound-induced Blood-Brain Barrier opening \emph{in vivo}

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Objectives: Focused ultrasound (FUS) in the presence of lipid microbubbles can induce non-invasive, transient and reversible Blood-Brain Barrier (BBB) opening. This study entailed assessment of the feasibility of fluorescently loaded microbubbles, labeled with the fluorophore 5-dodecanoylaminfluorescein (C-12), as a vector for targeted brain drug delivery. Compared to prior studies by our group, where fluorescently-labeled dextrans were co-administered with microbubbles, this new methodology improves the safety and allows a more targeted drug delivery with potentially lower toxicity, avoiding systemic exposure. The main objective was thus to determine feasibility and safety of using the loaded microbubbles as carriers towards targeted brain drug delivery with simultaneous cavitation monitoring.

Methods: A spherical, single-element, FUS transducer (center frequency 1.5 MHz) was used. A pulse-echo transducer (center frequency 10 MHz), confocally mounted at the center of the FUS transducer, was utilized for passive cavitation detection (PCD). FUS (pulse length 10,000 cycles; pulse repetition frequency 5 Hz; duration 5 minutes; acoustic pressure 450-750 kPa) targeted mouse brains \emph{in vivo}, in combination with fluorescent microbubbles for C-12 delivery, which was evaluated by \emph{in vivo} transcranial PCD, through the quantification of inertial (ICD) and stable harmonic (SCDh) and ultraharmonic (SCDu) cavitation doses at 30, 60 and 300 s; together with \emph{ex vivo} fluorescence imaging. The BBB opening was verified using \emph{in vivo} T1-w Magnetic Resonance Imaging (MRI). The safety of this technique was assessed through \emph{ex vivo} hematoxylin & eosin staining for microhemorrhage detection and immunohistochemistry (Iba-1 for microglial activation) together with \emph{in vivo} T2-w MRI for edema assessment.

Results: Successful targeted C-12 delivery was achieved at 600 and 750 kPa in six out of 14 cases (Image 1). Comparison of ICD, SCDh and SCDu between successful and unsuccessful cases yielded a statistically significant linear relationship between the successful targeted drug delivery and CD and specific thresholds for efficient delivery were identified.

No edema was detected in mice sacrificed on Day 0 but edema appeared on Day 1 on mice sacrificed on Day 7. In all cases cases (except one) it was repaired within a week. Microhemorrhages were observed after sonication in some cases but were also cleared within the first week. However, a higher number of cell nuclei was observed in the sonicated region compared to the unsonicated side in some mice survived up to one week after opening. Iba-1 immunohistochemistry also showed microglial activation.

Conclusions: FUS was applied in conjunction with fluorescent microbubbles and, for the first time, the existence of CD thresholds for assessing successful drug delivery was defined. For CD above these thresholds, significant fluorescent enhancement was observed, demonstrating C-12 targeted delivery. One week after sonication, edema was cleared out but microglial activation was observed in certain cases. Therefore, this study indicates the feasibility and safety of a new methodology of FUS-induced BBB opening for targeted albeit potentially riskier brain drug delivery and provides a platform for predicting successful delivery via PCD.
BBB opening and fluorescence delivery: T1-w MRI showing BBB opening at pressures (a) 450, (b) 600 and (c) 750 kPa. Fluorescence delivery (green) and DAPI (blue) in two horizontal sections of mouse brains sonicated at (d) 600 and (e) 750 kPa.
Ultrasound-mediated delivery of gadolinium and fluorescent–labelled liposomes through the Blood-Brain Barrier

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Objectives: The main objectives of this study were: 1) to examine whether gadolinium and fluorescent labelled liposomes can extravasate into the brain parenchyma after ultrasound mediated Blood-Brain Barrier disruptions; and 2) to test whether extravasated liposomes were size dependent or not. The liposomes were labelled with gadolinium (Gd) and fluorophore, thus enabling detection of extravasated liposomes via MRI in vivo and fluorescence methods in tissue, respectively.

Methods: Liposomes labelled with gadolinium and fluorophore were prepared using lipid film hydration and extrusion to two different sizes; ~70-85 nm and ~130-150 nm. Animals were divided into two different groups based on the use of particle sizes; group A (~70-85 nm) and group B (~130-150 nm). Focused ultrasound mediated Blood-Brain Barrier disruption (BBBD) was produced in one hemisphere in 15 mice. Particles were injected before sonication. Sonications (0.69 MHz at 0.42 MPa) were performed in two locations combine with Definity (10 μl/kg). Acoustic emissions were recorded during FUS. T1-weighted contrast enhanced and T2*-weighted MRI were used to confirm Gd leakage and damage detection respectively. Mice were euthanized 5-24 hours after FUS and post-process for fluorescence measurement.

Results: In T1-weighted contrast enhanced MRI, gadolinium-leakage was able to detect on sonicated area at 5-24 after FUS but not on non-sonicated area (control). Detection of fluorescence signal from brain tissue homogenates confirm the liposomal particles extravasation on sonicated locations. On group A, gadolinium and fluorescence signal intensities on sonicated locations were increased by 26% and 62% respectively as compared with control and signal enhancement were statistically significant compared with control (p = 0.017 and p = 0.02 respectively, two-tailed, paired ttest). On group B, gadolinium and fluorescence signal intensities on sonicated locations were increased by 24% and 40% respectively as compared with control. Comparison of fluorescence signal intensities between two groups on sonicated location was statically significant whereas it was not significant on controls (p < 0.05 and p = 0.07 respectively, one-tailed unpaired ttest).

Conclusions: Overall, this work demonstrates that ultrasound can deliver up to ~150 nm liposomes that labelled with gadolinium and fluorophore through the Blood-Brain Barrier. The results indicate that the extravasation of liposomes were size dependent.
Sterile inflammatory response (SIR) in the brain following exposure to low intensity pulsed Focused Ultrasound and microbubble infusion

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**Objectives:** Magnetic Resonance Imaging (MRI)-guided pulsed Focused Ultrasound (pFUS) in combination with systemic injection of microbubbles (MB) is being advocated to increase drug or gene delivery by causing localized Blood-Brain Barrier (BBB) disruption (D). The objective of this study is to investigate the molecular and cellular responses following pFUS+MB associated with BBBD in the rat brain.

**Methods:** Female Sprague Dawley rats (\(<200\text{g}\)) were sonicated at 0.3MPa acoustic pressures with 10ms burst length and 1\% duty cycle (9 focal points, 120sec/9 focal points) using a single-element spherical FUS transducer (589.636kHz; FUS Instruments). 100\textmu l Optison\textsuperscript{TM} MB (GE Healthcare) was administered intravenously. Gadofosveset-enhanced T1w images were obtained with a 3.0T MRI (Phillips). Proteomic and mRNA expression in the brain following pFUS+MB were analyzed with ELISA, Western blot, quantitative real-time PCR or immunofluorescent staining (Figure 1a). Proteomics were normalized to sham and statistical analysis was performed by one-way ANOVA corrected for multiple comparisons. Rats were also injected with 8mg/kg Rhodamine encapsulated magnetic polymers (MicroTRACK\textsuperscript{TM}; BioPal) 3 days prior to sonication to label splenic macrophages (CD68) to monitor tropism to the brain.

**Results:** Post contrast T1w MRI and histology showed open BBB without evidence of microhemorrhage. Within 5 minutes following sonication, increased expression of pro-inflammatory and anti-inflammatory cytokines, chemokines and trophic factors (CCTF) was detected in the parenchyma lasting up to 24 hours (Figure 1b). Increases in heat shock protein 70 (HSP70), tumor necrosis alpha (TNFa), and interleukin (IL) 1a, 1b and 18 consistent with damage associated molecular patterns (DAMP)1 and activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NFKB) inflammatory pathways were observed with SIR to injury\textsuperscript{2} (Figure 1b). NFKB pathway-related gene activation along with anti-apoptotic genes, immune cell chemoattractants, selectins, and cell adhesion molecules showed significant (>2fold) increases in mRNA expression (Figure 1c). Histological analysis showed significant increases (p<0.05) in the following: number of TUNEL positive cells within 6hrs, GFAP and Iba1 staining for activated astrocytes and microglia (1-24hrs) and increase of ICAM up to 24hrs post sonication. We also detected a >4 fold greater (p<0.05) CD68 positive cells on day 6 post sonication containing intracellular fluorescent beads within the pFUS+MB treated hemisphere compared to contralateral hemisphere.

**Conclusions:** The temporal molecular response to pFUS+MB is indicative of SIR\textsuperscript{2} originating from the parenchyma. The pattern of cytokines immediately after pFUS+MB is initiated by cellular release of DAMPs and TNFa observed with mild cerebral trauma or ischemia\textsuperscript{1,2}. Increases in monocyte chemoattractant protein (MCP-1), vascular endothelial growth factors (VEGF), stromal derived factor 1 (SDF-1), erythropoietin (EPO) and brain derived neurotropic factor (BDNF) are associated with BBBD stimulating angiogenesis, neurogenesis and stem cell migration observed with ischemia and trauma. These results indicate that pFUS+MB rapidly affects the cerebral vasculature as evident by BBBD in addition to the shockwave from MB collapse that induces mild stress within various cellular elements in the parenchyma inducing a SIR.

**References**

Figure 1. pFUS+MB elicits a transient microenvironmental response in the brain that reflects a sterile inflammation.
Volumetric MR thermometry in a clinical transcranial MR guided Focused Ultrasound system

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Objectives: Transcranial MR-guided Focused Ultrasound (tcMRgFUS) applied within a small central brain volume has achieved excellent outcomes in treatment of movement disorders (Elias NEJM 2013). Although transducers used in tcMRgFUS have been designed with large apertures to spread the beam energy over as much skull as possible to reduce skull and cortex heating, currently used MR temperature imaging (MRTI) methods cannot monitor the temperature increase over the entire insonified brain volume. Instead, temperatures are typically measured in one 2D slice, leaving the majority of the insonified brain volume unmonitored. We have previously developed and published methods to achieve fully 3D MRTI covering the entire insonified brain with good spatial and temporal resolution (Todd MRM 2009/2010) but the techniques have not been evaluated on clinical tcMRgFUS systems. In this work-in-progress study we demonstrate the value of volumetric MRTI with two different pulse sequences applied during heating on a clinical tcMRgFUS system.

Methods: PRF MRTI was performed with a product 3D gradient recalled echo (GRE) pulse sequence and a custom-implemented 3D GRE segmented echo planar imaging (EPI) pulse sequence on a 3T MRI (Discovery 750T, GEMS). tcFUS heatings were performed in an ex vivo human skull filled with tissue-mimicking gel (ATS Laboratories) in a clinical tcMRgFUS system (ExAblate Neuro, Insightec).

Pulse sequences parameters are listed in Table 1. All data were zero filled interpolated (ZFI) to 1-mm isotropic spacing (GRE data additionally ZFI to 0.5-mm spacing). The skull was physically positioned in the FUS system, and the focus electronically steered, to target deep brain-structures located outside the normal treatment envelope. FUS sonications were applied at 940 W for 30/60 s while imaging with the GRE/EPI sequences, respectively. The EPI sequence used an echotrain length of 16 with bi-polar readout gradient, sampling 192 phase encodings in each direction, so that full “positive only” and “negative only” images could be reconstructed.

k-space data were retrospectively down sampled by a factor of R=4 and 8 (split into multiple time-frames without throwing any data away) for the GRE and EPI experiments, respectively, giving acquisitions times of 3.6 and 7.8 s, and reconstructed with a temporally constrained reconstruction algorithm (Todd MRM 2009).

Results: Figure 1 shows three orthogonal views of temperature maps overlaid on magnitude images in the GRE experiment. Attempting to focus this far outside the normal treatment envelope results in severe near- and far-field heating. Heating on both the cortex and in the far-field along the petrous bone is visible. In Figure 2 the temperature evolution at the focal spot is compared to that near the petrous bone. The gel near the bone shows a delayed and greater maximum temperature compared to the focus.

Three orthogonal views of the larger FOV in the segmented EPI experiment are shown in Figure 3. Heating along large parts of the cortex can be seen. In the underlying magnitude images it can be seen that the EPI sequence experiences more artifacts than the GRE sequence.

The focal spot position (evaluated as the temperature center-of-mass) was tracked as a function of time during the heating in the GRE data ZFI to 0.5 mm (data not shown). The focal spot did not experience any shift to within the finer ZFI spacing.

Conclusions: This study shows that volumetric thermometry over a FOV covering the focal spot and the skull base can be achieved with readily available pulse sequences. With custom implemented pulse sequences the fully insonified FOV (from skull cap to skull base) can be covered. Even though the reconstruction is done retrospectively, the described methods are valuable as research tools in e.g. treatment envelope evaluations. By utilizing 3D imaging
ZFI can be performed in all directions to minimize partial volume effects, and very accurate dynamic focal spot localization can be performed.

Future studies will compare the accuracy and precision of the described methods to standard 2D MR thermometry. Experiments comparing 3D MRTI with fiber optic probe measurements at potential target positions outside the currently available treatment envelope will also be performed (Monteith JNS 2016).

<table>
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<tr>
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<th>BW (kHz)</th>
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</tr>
</tbody>
</table>

*Table 1. MR scan parameters. TR – Repetition time, TE – Echo time, FA – Flip angle, BW – Bandwidth (readout), FOV – Field of view, Res – Resolution, Tacq – Acquisition time (before subsampling).*

Figure 1. Three orthogonal views of GRE temperature maps overlaid on magnitude image.

Figure 2. Temporal evolution of heating comparing focal spot to far-field next to petrous bone.

Figure 3. Three orthogonal views of EPI temperature maps overlaid on magnitude image.
**Multi-echo MR thermometry compared to single echo MR thermometry in the treatment of essential tremor**

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**Objectives:** The choice of receive bandwidth in MR thermometry acquisitions needs to balance two competing choices. Low bandwidths improve SNR, while high bandwidths reduce spatial shift of the hotspot due to the temperature off-resonance. The low bandwidth MR thermometry sequence in use in essential tremor MRgFUS treatments required repeated swapping of phase and frequency directions since the location of the hotspot could only be trusted in the phase encode direction.

A solution is to use multiple high bandwidth acquisitions, which when averaged, regain much of the SNR of the lower bandwidth image. Such a multiecho sequence has recently become available for clinical use. The purpose of this work was to compare the performance of the multi-echo (ME) MR thermometry to the single echo (SE) MR thermometry in clinical treatments of essential tremor.

**Methods:** Fifteen patients were treated for essential tremor, 12 with only single echo thermometry (TE and BW/pix=12.8 and 44), 2 with only multi-echo thermometry (TE and BW/pix=3, 8, 13, 18, 22 and 278), and 1 with both sequences. All other image parameters remained the same, including TR=100. All thermometry images were processed offline in Matlab with a single baseline subtraction (α=−0.00909), followed by referenceless processing for constant and linear terms. The multi-echo thermometry was further processed with a phase unwrapping algorithm in the TE dimension. The multiple echoes were then combined with a weighting by the square of the temperature SNR of each image.

**Results:** The decrease in sampling time for the ME thermometry dictates a theoretical decrease in temperature SNR of only 11% due to the decrease in sampling time alone. In the one patient that had both sequences in the same scan planes for two sonications, there was a 9% decrease in SNR in the ME thermometry as measured in the frame used for the referenceless processing, comparing well with theory.

A review of all sonications with ME thermometry when phase encoding was S/I revealed much reduced artifacts over SE thermometry with phase encoding in the S/I direction. Example images are provided in Figure 1. The region of interest measurement indicated a 30% improvement in temperature SNR for the ME over the single echo, due to this artifact reduction.

The spatial shifts should theoretically be reduced by a factor of 6.3 in the ME thermometry, as compared to the SE thermometry, due to the increase in receive BW. For example, a 23°C temperature rise would result in a 0.6 mm shift with the single echo, and only a 0.1 mm shift with the multi-echo.

**Conclusions:** When phase encoding is S/I, multi-echo thermometry is superior to single echo thermometry due to a reduction in ghosting artifacts and spatial shifts. Alignment of the focal spot should not require swapping phase and frequency directions. Future work will include a prospective study to verify this. In the other directions, ME thermometry is comparable to SE thermometry with the reduction of spatial shifts coming with a loss of temperature SNR of about 10%.

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Figure 1. Comparison of MR thermometry images when phase encoding is S/I. The single echo thermometry (a,b) demonstrates numerous ghosting artifacts (yellow arrows) that are not seen in ME thermometry (c,d). (a,b,d) are from the same patient.
High-resolution whole-brain MR thermometry with a 3D EPI stack-of-stars pulse sequence

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Objectives: Real-time whole-brain MR thermometry is needed for transcranial MR-guided Focused Ultrasound to accurately track rapid heating at the focus and to monitor for unsafe heating in the near- and far-field. Previous efforts to meet this need have been based on 3D segmented echo planar imaging (EPI) (Todd et al., 2012) and spiral MR readouts (Fielden et al., 2014). Here, we propose a 3D EPI stack-of-stars temperature mapping pulse sequence that enables greater volume coverage than has been reported with previous approaches. The sequence allows flexible adjustment of the scan acceleration factor and does not require a high density of receive coils for high frame rate thermometry.

Methods: Readout Trajectory: Figure 1 shows the proposed readout trajectory. Each TR comprises a 2D EPI plane that is frequency-encoded in the axial (x-y) and phase-encoded in the slice (z) dimensions. The plane is rotated between TRs by 111.25 degrees to sample a golden angle stack-of-stars k-space. The sequence was implemented on a Philips Achieva 3 Tesla scanner with parameters: field-of-view = 28.0 x 28.0 x 11.9 cm, 43 slices, in-plane resolution = 1.50 x 1.50 x 2.75 mm, 17 ms TE/45 ms TR, 14.5° flip angle, spectrally-selective fat suppression.

In vivo Experiment: To confirm the achievable brain volume coverage and image quality, with local IRB approval, brain images in a healthy volunteer were acquired without heating using the pulse sequence with one, two, and three interleaved shots and with an 8-channel receive coil array. Fully-sampled images were reconstructed using the density-compensated conjugate gradient method.

Phantom Heating Experiment: A tissue-mimicking gel phantom was sonicated using a clinical MR-HIFU system (Philips Sonalleve) operated at 1.2 MHz and 80 W for 12 s, while scanning with the proposed sequence with one shot and with 5 abdominal receive coils. Temperature maps were reconstructed in one second increments using the k-space hybrid method (Gaur et al., 2015) at three scan acceleration factors.

Results: In vivo Results: Distortions and signal dropouts caused by off resonance are visible in the sagittal and lower axial single-shot brain images in Figure 2 (white arrows). The distortions can be reduced by increasing the number of EPI shots per angle, which increases the pixel bandwidth in z. An axial slice positioned for monitoring Focused Ultrasound thalamotomy (red arrow) is also shown, which contains no visible distortions at any multishot factor.

Phantom Heating Results: Figure 3a shows reconstructed sagittal and axial temperature maps through the middle of the phantom at peak heat. Significant temperature aliasing does not appear until the scan is accelerated by a factor of 14.1 or a 1 second window width. The hotspot temperature curve plots in Figure 3b show that the reconstructed temperatures all coincide, though the 14.1x curve appears noisier. Importantly, at all acceleration factors, there is agreement with a hotspot measurement obtained by a 2D multislice Cartesian EPI temperature mapping pulse sequence (7 slices, same in-plane resolution) which was used as a reference standard.

A video of the entire sonication monitored using this pulse sequence can be viewed at https://www.youtube.com/watch?v=z3cVXjkgyLg.

Conclusions: A 3D EPI stack-of-stars temperature mapping pulse sequence was proposed and validated against a 2D multislice Cartesian EPI temperature mapping pulse sequence. The sequence enables fine spatiotemporal resolution with large volume coverage, without requiring temporally-regularized reconstruction or a large number of receive coils.
Illustration of the 3D k-space trajectory. A 2D EPI plane is scanned each TR and rotated by 111.25 degrees between TRs to sample a 3D volume. Scan acceleration is achieved by using a small number of consecutive rotated planes/TRs for reconstruction.

Brain images acquired with the proposed sequence. The sagittal and lower axial slice images contain distortions and dropout in regions with large frequency offsets (white arrows), which are diminished when multiple shots are used to shorten each readout.

Phantom temperature map reconstructions (a) and hot spot temperature curves (b) at three acceleration factors. Significant aliasing does not appear until the scan is accelerated by 14.1x. There is good reference standard agreement at all accelerations.
Ultrafast and sensitive volumetric passive acoustic mapping

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³Harvard Medical School, Boston, Massachusetts, United States
⁴Cleveland Clinic, Cleveland, Ohio, United States

Objectives: Not released for publication
Methods: Not released for publication
Results: Not released for publication
Conclusions: Not released for publication
Tissue stiffness imaging with interleaved multiple-point MR-ARFI

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Objectives: Although Focused Ultrasound (FUS) has the potential to treat a number of pathologies the methods used to guide treatment are still limited in the ability to identify effective treatment endpoints. Changes in MRI parameters such as proton density and T1 and T2 relaxation times may result from reversible edema instead of tissue death. While dynamic contrast enhanced and late Gd-enhanced MRI are more specific, Gd contrast cannot be administered multiple times during a procedure to monitor treatment progression. MRI acoustic radiation force impulse imaging (MR-ARFI) can monitor tissue stiffness, but the acquisition is relatively slow, and must be repeated to measure tissue displacement at more than a single point.1 The purpose of this work was to develop an efficient method to use MR-ARFI to measure tissue displacement volumetrically with an array of points as a first step towards monitoring tissue stiffness changes during MRgFUS procedures.

Methods: A 3D segmented gradient echo (GRE) echo-planar pulse sequence, designed to simultaneously measure tissue displacement with MR-ARFI and the corresponding tissue heating,2-5 was further modified to allow interleaved acquisition of multiple point MR-ARFI (mpMR-ARFI) volumes. Phase change due to displacement was separated from that due to temperature by complex subtraction of an interleaved volume acquired with no FUS applied. Temperature was obtained using the proton resonance frequency method with a referenceless background phase subtraction.6

Experiments were performed in a gelatin phantom and excised pig brain on 3T MRI scanners (Siemens Tim Trio and PrismaFit). Pulse sequence parameters were: TR/TE = 73/43ms, FA = 30°, readout bandwidth = 752 Hz/pixel, echotrain length = 7, FOV = 160x114x55mm, matrix= 128x91x22 giving voxel dimensions of 1.25x1.25x2.5 mm before zero filled-interpolation. Bipolar motion-encoding gradients (MEG) of 15 ms duration (each bipolar lobe) were applied prior to signal readout. Ultrasound pulses of 50 acoustic watts were applied during the second 15ms bipolar MEG lobe. Navigator echoes were used to measure and compensate for B0 field drift due to gradient heating caused by high MEG strength and duty cycle. The same navigators echoes can be used for respiratory correction in vivo.7

Results: Results from a13-point mpMR-ARFI acquisition in a gelatin phantom using the Siemens Tim Trio and MEG=20mT/m are given in Figure 1. An example cropped single slice from 10 of the 13 acquired displacement volumes is shown in Figure 1a with a cropped slice of the mpMR-ARFI composite of all 13 displacement volumes shown in Figure 1b. The corresponding temperature increase measured during the mpMR-ARFI acquisition is shown in Figure 1c and was relatively low (<3 °C). (For all mpMR-ARFI images, point separation is 5mm).

Figure 2 shows the improved image quality achieved by the phase-navigator correction in mpMR-ARFI measurements obtained using a PrismaFit 3T MRI scanner with MEG = 40 mT/m. The ghosting artifact in Figure 2a due to B0 field drift caused by high amplitude (40mT/m) and duty cycle (40%) MEG gradients during mpMR-ARFI acquisition. After correction (Figure 2b) mpMR-ARFI displacement images have negligible artifact.

Results of the displacement measured in excised pig brain using the Siemens PrismaFit 3T MRI scanner and MEG=40 are shown in Figure 3 before (Figure 3a) and after (Figure 3c) FUS ablation. An estimate of the cumulative thermal dose is shown in Figure 3b. Note the decreased displacement at central point.

Conclusions: Measuring tissue stiffness (displacement as a function of ultrasound intensity) and changes in displacement before, during, and after the procedure provides the unique potential to remotely palpate the ablated volume to monitor the formation of lesions created with MRgFUS. It will also provide a crucially needed assessment of tissue change in regions of fat where conventional MR thermometry fails.
Ghosting (a) is corrected by navigator echoes (b) allowing clean mpMR-ARFI displacement measurement (c,d)

mpMR-ARFI displacement measurements in an excised pig brain before (a) and after (c) MRgFUS ablation with estimated cumulative dose in CEM (b)

References

Binary localization of cavitation activity based on harmonic content for transcranial brain therapy

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²CENIR, ICM, CNRS U7225, INSERM U975, Paris, France

Objectives: Cavitation activity may occur during BBB disruption (due to UCA injections) and also during HIFU treatment due to nucleation under high negative pressure. The corresponding microbubble activity has to be monitored to assess the safety and efficiency of brain treatments by ultrasound. The purpose of this study is to binary discriminate the position of microbubbles, inside or outside the skull, in order to know whether cavitation occurs in the brain of the patient or not.

Binary localization is achieved here by taking advantage of the attenuation properties of the skull. The skull acts indeed as a low pass filter for acoustic signals. Thus we hypothesize that the harmonic content of signals from cavitation events could be used as a binary indicator of their localization: inside or outside the brain case.

Methods: A wideband Passive Cavitation Detector (PCD) recorded the acoustic signals from microbubbles activity.

An in vitro setup (Fig. 1) mimics a BBB opening configuration (contrast agent flow with 0.6MPa sonication during 10ms at focus) or a thermal ablation (calf brain sample at focus with 3-4MPa sonication during 0.3s at focus). Experiments were performed either with no skull in place, or with human (6 samples) or monkey (1 sample) skull in front of the PCD.

In vivo BBB opening were conducted on macaque (900kHz, 0.6MPa at focus) with the same PCD mounted on the monkey head.

The spectra are computed from data recorded by the PCD and reveal harmonics, subharmonic and ultraharmonics of the excitation frequency (900kHz in all cases).

The ratio of an high frequency harmonics over a lower frequency harmonic was then calculated. This ratio is expected to decrease when the acoustic signal from cavitation activity crosses through the skull. In order to achieve binary localization, two types of ratio have been computed: ultraharmonic ratios (e.g. 5/2 over 1/2) and harmonic ratios (e.g. 4 over 2).

Results: The ultraharmonics ratio obtained during in vitro thermal-like ablation is plotted in Fig. 2. This ratio significantly decreases when acoustic signal crosses through the skull. Thus a threshold can be introduced to binary localize cavitation inside/outside the skull.

Table 3 summarises the results for harmonic ratio 4 over 2 and ultraharmonic ratio 5/2 over 1/2 obtained in vitro and in vivo during BBB opening and thermal necrosis. For BBB opening, neither the sub- nor the ultraharmonics appears in vivo, probably due to the confinement of the largest microbubbles by the vasculature. Thankfully in vitro experiments point out that the harmonic ratio remains relevant to binary localize microbubbles in the human case. Indeed the harmonic ratio 4 over 2 exhibits a -30 ± 3 dB decrease when crossing through human skull.

In order to ensure the repeatability of this method, each configuration was statistically investigated by plotting receiver operating characteristics (ROC) curves. These plots (Fig. 4) illustrate the performance of this approach. Except for in vivo BBB opening on monkey, ROC curves demonstrate that a sensitivity and a specificity which are simultaneously nearly 100% can be obtained for the binary localization of cavitation activity.

Conclusions: This preliminary study, mainly done in vitro, shows that a low-cost and easy-to-use PCD can binary localize cavitation activity inside/outside the skull using the filtrating effect of the skull on the harmonic content of the spectra. The statistical study shows that the method is able to localize microbubble activity inside or outside the skull with high sensibility and high sensitivity. We look forward to testing extensively this technique in vivo for both BBB opening and thermal necrosis.
In vitro experimental setup for mimicking BBB opening

Ultraharmonics ratio (3/2 to 15/2 over 1/2) recorded during in vitro thermal ablation

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Table: ultraharmonic (5/2 over 1/2) and harmonic (4 over 2) ratio during thermal necrosis and BBB opening in dB scale.

Receiver operating characteristics for ultraharmonic (5/2 over 1/2) ratio and harmonic ratio (4 over 2) during thermal ablation and BBB opening.
Inflection of temperature vs. power curve in tcMRgFUS: Correlation with lesion location

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2University Children’s Hospital Zurich, Zurich, Switzerland

Objectives: During tcMRgFUS, power levels are progressively increased to align, verify treatment location, and create a durable ablation. It is predicted that the measured temperature at the focus for a consistent timepoint will increase monotonically with power. However, the temperature rise is often observed to actually decrease with increasing power, creating an inflection in the peak temperature vs. power graph (Figure 1). The purpose of this work was to show this behavior in the temperature rise is correlated with a lack of alignment between the thermal lesion and the monitored scan plane.

Methods: Fifteen ET datasets were included in this study. Post-treatment 3D T2-weighted FSE images (FOV 24cm, matrix 320x320, slice thickness 1mm) were thresholded at the signal intensity of zone 1 (Figure 1). Measurements from the targeted ACPC plane to the top and bottom edges of zone 2 were made in the sagittal plane.

MR thermometry (TE/TR=12.8/100 (n=13); TE/TR=3,8,13,18,22/100, (n=2)) was processed with a single baseline subtraction (\(\alpha=-0.00909\)), followed by referenceless processing for constant and linear terms. The maximum temperature of the third time point (k-space center 8.8s after sonication initiation) in the axial scan plane was plotted vs. power. This time point was chosen because all sonications were at least 10s in duration. The number of sonications after any inflection was noted.

In 12 cases, temperature-power curves from early sonications in the axial scan plane were extrapolated to higher power levels. The amount that the measured temperature was below the estimated temperature was measured.

Results: The results are shown in Figure 2. While the distance from the treatment plane to the inferior aspect of the lesion remains essentially constant, the distance from the treatment plane to the superior aspect of the lesion increases with number of sonications after inflection. While it is expected that multiple sonications at the same location may increase lesion size, this data demonstrates that lesion size increases preferentially in the direction of the transducer as we increase sonications after inflection.

The movement of the lesion superiorly from the AC-PC plane is correlated with the difference between the actual and estimated temperature (\(\star\)). Although this was quantitated only in the axial scan plane, the inflection was seen in all three scan planes. Prior work demonstrated that the lesion is double oblique (the superior aspect of the lesion is posterior and medial); therefore, movement of the focal spot towards the effective transducer aperture moves it also out of the monitored sagittal and coronal scan planes.

Conclusions: One interpretation of this data is that an increase in the acoustic absorption effectively shields the focal spot, with subsequent hotspots located closer to the transducer. A second explanation is that the acoustic properties of the skull may be changing during treatment.

An additional implication of this work is for comparison of simulation with thermometry. To simplify this comparison without the complication of the alignment of the thermometry plane, a sonication early in the treatment should be used for comparison.
Figure 1. a) Temperature in the third temperature image (∗) does not always rise monotonically with power. b) The distance from ACPC plane to the top and bottom edges of zone 2 were measured on images thresholded at the zone 1 signal intensity.

Figure 2. a) The lesion top edge is moving superior to the ACPC plane with increasing sonication number. b) As the lesion is moving out of the measured scan plane, the temperature is increasingly underestimated, giving rise to a temperature inflection.
Objectives: This study is to investigate the effects of Magnetic Resonance-guided Focused Ultrasound (MRgFUS) on in vivo pig brain tissue by comparison of the tissue damage in histology with the changes in MR images as a function of thermal dose (TD) up to 200 cumulative equivalent minutes (CEM) at 43 °C.

Methods: We have implemented a PI (proportional-Integral) control system in a laptop with an Arduino-based controller to modulate pulse duration of a FUS system (ExAblate 4000 Neuro 650 kHz system, InSightec) based on the temperature difference between target temperature and focal temperature as measured by an MRI system (Discovery MR75-3.0T, GE Medical systems) with proton resonant frequency (PRF) thermometry. Accumulated thermal dose in CEM was calculated every 3.7 seconds and used to stop the sonication when a prescribed thermal dose of interest was reached and delivered to the target brain tissue. After tuning of a closed loop control system in a phantom, one acute and seven chronic pig experiments with three-day survival were conducted to investigate the correlation of lesions between the MR images of pig brain tissue with the corresponding histology. Craniectomy was performed to create an acoustic window, and sonication was applied on 4 spots in the thalamus of each pig. Absolute temperature in pig brain tissue was computed based on the MR thermometry using the rectal temperature as a baseline. TD varied from 7 to 195 CEM with target temperature between 46 and 52 °C at appropriate acoustic powers. This pig study was approved by the University of

Results: From the acute pig experiment, we observed one large and 2 small lesions on MR images 1 hour after a sonication and subsequent histology showed 4 lesions of target. For the chronic pig experiments, 22 sonication spots in 6 pigs were analyzed through MR images and histology. One pig was excluded due to air bubbles introduced between the dura and scalp during the surgery procedure, and 2 sonication spots were failed to generate due to technical problems. Results show that large brain tissue damage was observed in MR images in all 7 spots with doses larger than 100 CEM and the corresponding histology results confirmed infarction with necrotic center for all except one with a dose of 101 CEM. The diameter of those lesions on T2-weighted axial MR images was measured to 2.9 ± 0.4 mm (mean ± SD) with a mean volume of 30.7 ± 12.9 mm3. All with TD lower than 17 CEM produced no visible lesions in either MR images or histology. There was a discrepancy in generating lesions with TD between 18 and 100 CEM, so that six smaller lesions (3 in volume) were shown except one large change in MR images at

Conclusions: In conclusion, large tissue damages were observed on MR images and histology for all TD above104 CEM, but no change was shown for all TD below 17 CEM. There is a variability in tissue changes between these TD levels. These results may contribute toward prescription of thermal dose rather than peak temperature or acoustic power for brain treatments, and expand the treatment envelope beyond the current limitations in selecting targets and patients.
Visualization tools for transcranial Focused Ultrasound procedure planning, simulation and analysis

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Objectives: The interactions between particular transcranial transducer and skull geometries are challenging to understand without interactive visual computing tools. Such a tool has been created to allow the visual exploration of the estimated treatment envelope of a transcranial transducer given a patient specific imaging dataset. The impetus for developing such a system is to aid in understanding treatment envelope constraints and to serve as a tool for specifying the input to various acoustic simulation systems.

Methods: A visualization application was created which makes heavy use of a modern GPU to interactively display a transcranial Focused Ultrasound transducer, patient specific brain and skull anatomy, and the interaction of each transducer element beam path with the skull. The incident angle of each transducer element beam axis with the outer table of the skull is calculated and displayed in an interactive fashion as the natural focus of the transducer is moved within the intracranial volume. Skull geometry is derived from a treatment compatible CT dataset. An MR dataset is registered with the CT for targeting. Due to the interactive nature of the tool, presumptive targets can be rapidly and intuitively explored and understood in terms of geometric constraints and estimated treatment efficiency.

Results: The visualization system functions will be demonstrated including targeting, transducer positioning and assessment of transducer efficiency in terms of effective transducer element count.

Conclusions: An interactive visualization system has proven valuable for facilitating understanding of the complex interaction of transducer and skull geometries. Future applications of this system may include patient screening, post-treatment analysis, indication feasibility screening and acoustic simulation initialization.
**HIFU for Pediatric Operations (HOPE) – A pediatric neurosurgical treatment system**

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**Objectives:** Pediatric patients have distinctive neuroanatomic features and specific disorders that make them unique candidates for transcranial MR-guided Focused Ultrasound treatment. Children have thinner skulls, and neonates in particular, possess a natural acoustic window through their fontanelle. This results in lower phase aberration and decreases the need for the larger hemispherical dome transducers used for current adult transcranial procedures. These systems also require fixation of the head in a stereotactic frame, which is dangerous for their fragile skull. In addition, neonates often require MR-compatible incubators and dedicated neuro-interventional coils for imaging. Focal brain ablation/disconnection for medical refractory epilepsy and lysis of intra-ventricular hemorrhage are some of the proposed noninvasive treatment options for this population. To provide such treatment, we have developed HOPE – HIFU for Pediatric Operations – an integrated neonatal HIFU treatment system.

**Methods:** HOPE is composed of multiple elements: a 5 degree of freedom MR-compatible robot positioning device, a 256 element phased-array transducer, an 8 channel neuro-interventional coil, and a real-time Python-based treatment planning and delivery software system. The specifications for HOPE were determined by a team of clinicians and researchers as follows: compatible with clinical neonatal incubators and MRI techniques, imaging coil integrated into the incubator, positioning system to couple and deliver the HIFU treatment and real-time control/monitoring of the treatment (Fig. 1). The hardware elements are designed and tested with a Philips Achieva 3.0TX MRI with an Imasonic 256 element transducer. For coupling, a custom designed water bag system was created to interface with the patients’ head. The software platform includes robot kinematics, robot visualization, registration, transducer control and MRI communication. A calibration procedure was developed using a series of Vitamin E markers visible in a 3D gradient echo sequence without fat suppression; the markers were attached to an extension of the robot that recreates the acoustic cone of the HIFU beam. A user-interface module was developed to calculate the center of mass of each marker and to co-register the coordinate systems of the MRI and the robot.

**Results:** The hardware system of HOPE has been designed and tested to show it can perform T1, T2 and DTI imaging that is comparable to clinical coils while delivering HIFU treatment in a phantom model. The neuro-interventional coils provide high resolution images for treatment planning (Fig. 2). The MR-conditional robotic system positions the transducer to an accuracy of 0.59 +/- 0.25mm and delivers thermal ablation treatment to targets in a Philips HIFU quality assurance phantom. The custom water coupler bag provided a transmission path for the HIFU with minimal energy loss. The HOPE software platform controls each of the robotic positioning axes with hardware safety switches in a real-time Python interface (Fig. 3). The software registration of the patient, MRI and robot frame allows the user to select specific brain targets. During treatment, real-time thermometry is displayed.

**Conclusions:** HOPE is an MR-guided Focused Ultrasound system aimed at delivering HIFU therapy (both thermal ablation and cavitation-based treatment) for neonatal and pediatric patients. The system has been designed to operate within an incubator and clinical MRI system which minimizes the impact to the patient. Future work involves characterizing the treatment accuracy and performing in vivo animal studies to test the overall system feasibility and usability for treatment of epilepsy and IVH clots. Other treatments that will be investigated are thermal ablation of brain tumors, hyperthermia and targeted drug delivery.
HOPE Concept (1 – incubator, 2 – coil, 3 – neonatal patient, 4 – transducer, 5 – robot and 6 – MR bore)

Coil comparison in a T1-TFE image of a porcine brain in vivo (Left: Prototype 8 channel neuro-interventional coil, Right: Philips clinical 32 channel head coil)

HOPE software interface with robot, treatment planning and real-time monitoring of HIFU exposure in phantom material.
Simultaneous stimulation of the human primary and secondary somatosensory cortices using transcranial Focused Ultrasound

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Objectives: Low-intensity transcranial Focused Ultrasound (FUS) is making progress as a new mode of non-invasive brain stimulation, having potential for superior spatial selectivity and depth penetration compared to transcranial direct current stimulation (tDCS) or transcranial magnetic stimulation (TMS). With accumulating evidences of the FUS-mediated neuromodulatory effects in small and large animal models, the sonication given to the primary somatosensory cortex (SI) has recently shown to be capable of eliciting tactile sensations in humans. Here, we further investigated the creation of tactile sensations induced by simultaneous FUS stimulation of the secondary somatosensory cortex (SII) and SI of the hand.

Methods: Ten healthy volunteers (two females, ages = 23–34, average of 27.8 ± 4.1 yrs) participated, and all procedures were conducted under the approval of the local Institutional Review Board of the Catholic University of Korea. For targeting of FUS focus to the individual functional neuroanatomy, each participant’s brain image was acquired using a 3-T MR scanner with anatomical (T1-weighted) and functional MRI (fMRI, T2*-weighted) protocols. The SI and SII areas were mapped while using four types of external tactile stimuli to the palm of the right hand—vibrotactile, pressure, warmth, and coolness. CT scan of the head was also acquired for the planning of transcranial sonication path. As guided by these individual-specific neuroimage data, FUS sonication (210 kHz, single-element FUS transducer with focal length of 38 mm) was administered to the SI and SII simultaneously (or separately), with an incident acoustic intensity of 35 W/cm² Isppa, tone-burst-duration of 1 ms, pulse-repetition frequency of 500 Hz (yielding a 50% duty cycle), and a sonication duration of 500 ms. The SII was targeted as divided into four sub-regions that are specifically activated by the external tactile stimuli.

Results: Across the differential selective stimulations (i.e., SI only, SII only, or SI and SII simultaneously), participants felt various types of elicited tactile sensations (while ‘tingling’ was dominant) from the hand areas contralateral to the sonication, such as the palmar/dorsal side of the hand or as single/multiple adjacent fingers. These results were similar to our previous study on FUS stimulation of the SI, while the elicitations of ‘vibrotactile’ and ‘warmth’ sensations were newly reported in the present study. The types of tactile sensations did not match to the sensations that are associated with the specific sub-regions in the SII. However, two individuals reported matching types of sensations (‘vibrotactile’, ‘pressure’, and ‘warmth’) during stimulation of the SI/SII simultaneously or the SII only. The stimulatory effects of the FUS were transient and reversible, and the sonication procedures did not induce any discomforts or adverse changes in the subjects’ physical/mental status.

Conclusions: Simultaneous stimulation of the SI/SII in the same hemisphere was achieved by using multiple FUS transducers, which elicited various types of tactile sensations. Stimulation of the SII only also induced the creation of tactile sensations. The ability to stimulate multiple region-specific brain areas may shed light on examining the causal relationships between regional brain activities and subsequent behavioral/cognitive outcomes.
Transcranial Focused Ultrasound stimulation of the primary visual cortex in humans

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Objectives: Transcranial Focused Ultrasound (FUS) has been suggested as a new non-invasive modality of regional brain stimulation, with potential to be more spatially-selective and to reach deep cortical/subcortical areas compared to the conventional methods of transcranial magnetic stimulations (TMS) or transcranial direct current stimulation (tDCS). In humans, low-intensity FUS sonication has been demonstrated to temporarily change the neural activities in the primary somatosensory cortex (SI), based on the observations of subjective sensory manifestations and electrophysiological responses. However, functional neuroimaging evidence of increased neural activity in the stimulated region, as well as the associated network-wide brain responses, has not yet been shown in humans. Here, we administered stimulatory FUS to the primary visual cortex (V1) as guided by the individual-specific neuroanatomy. Concurrent functional MRI was acquired to assess the brain regions that were activated due to the stimulation.

Methods: 19 healthy volunteers (five females, ages 20–45, average 26.1 ± 5.4 yrs) participated, and all procedures were conducted under the approval of the Institutional Review Boards of both the Catholic University of Korea and Korea University. Functional MRI (fMRI; for mapping of the visual areas) and cranial CT were obtained from each participant to provide the individual-specific V1 location for sonication planning/targeting. Then, in a separate session, an MR-compatible sonication setup (270 kHz, single-element FUS transducer with radius-of-curvature of 30 mm) was used to deliver FUS to the V1 under a clinical 3-T MR scanner for the image-guidance and the simultaneous acquisition of fMRI data. Separate from the FUS-fMRI session, electroencephalographic (EEG) potentials elicited by the FUS stimulation were also measured. We used a pulsing scheme having a sonication duration of 300 ms with a tone-burst-duration of 1 ms repeated at a pulse repetition frequency of 500 Hz (yielding a 50% duty cycle). The incident acoustic intensity at the FUS focus was 16.6 W/cm² Isppa. Retrospective numerical simulation of the transcranial acoustic wave propagation was performed proximal to the sonicated area to estimate the in situ acoustic intensity and spatial accuracy of sonication.

Results: Simultaneous acquisition of fMRI during FUS sonication to the V1 revealed the elicited activation not only from the sonicated brain area, but also from the network of regions involved in visual and higher-order cognitive processes. Accompanying phosphenes perception was also reported. The EEG responses showed distinct peaks associated with the sonication, having similarities with the classical visual evoked potentials (VEP) generated by photic stimulation. The procedures did not induce any discomforts or adverse effects from the participants, based on the subjective reporting and neuroradiological/neurological examinations. Retrospective numerical simulation of the transcranial FUS suggested the variability in individual responsiveness to the stimulation.

Conclusions: Simultaneous fMRI acquisition during FUS application to the V1 revealed the functional neuroimaging-based evidence in humans that the FUS stimulation activates the sonicated brain area and concurrently elicits the associated phosphenes perception. Successful stimulation of the V1 was also supported by the presence of the evoked EEG potentials associated with FUS. The individual variability in responsiveness to the stimulation suggested needs for an elaborate image-guidance.
Focused Ultrasound modulation of visual search performance and associated EEG in monkeys

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Objectives: Focused ultrasound (FUS) is a promising tool for neuromodulation because of its noninvasiveness and better spatial precision compared to other noninvasive methods, such as transcranial magnetic stimulation (TMS) or transcranial direct current stimulation (tDCS). FUS neuromodulation has been demonstrated in multiple animal models, including a prior study where ultrasound was applied transcranially over macaque frontal eye field (FEF) to influence saccade response time in an anti-saccade task. In this work, we applied FUS through a craniotomy over the macaque FEF while measuring saccade response times and EEG signals associated with selective attention.

Methods: A single element focused transducer was positioned through a craniotomy over FEF, a cortical area that plays a key role in the eye movement and attention systems. FUS stimulation was applied during a complex visual search task (Figure 1) where the monkey was required to shift its gaze to a target among distractors. We alternated blocks of trials with or without FUS stimulation (300 ms of pulsed FUS with a 50% duty cycle starting 150 ms before search display onset, center frequency 500 kHz, repetition frequency 2 kHz, pulse duration 0.25 ms, peak negative pressures of 250 kPa or 425 kPa, warming of brain tissue < 1.5°C). Saccade response time and intracranial EEG recordings were acquired in two monkeys performing 9 sessions each.

Results: In both monkeys, event-related potentials (ERPs) associated with selective attention (N2pc) were significantly reduced during stimulation with both intensities. FUS stimulation attenuated the N2pc over the entire session block, rather than on a trial-by-trial basis. In one monkey with a craniotomy positioned directly over FEF, the mean saccade response times were reduced by 5 ms by FUS stimulation at 425 kPa (p<0.001) when the target appeared in the upper hemifield contralateral to the FUS stimulation, while another animal with a craniotomy more ventrally did not show such a systematic behavioral modulation.

Conclusions: We are continuing to explore potential spatial relationships between stimulation location and behavioral modulations in ongoing work. Overall, our findings demonstrate prolonged FUS modulation of attention ERPs and suggest potential spatial selectivity based on the location of stimulation.
Ultrasound-mediated modulation of motor and ocular responses in anesthetized mice in vivo

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Objectives: Focused ultrasound has been identified as a non-invasive technique for modulating brain activity. Most studies involving sedate rodents utilize frequencies in the kilohertz-range, which allow for optimal transmission of acoustic power through the skull. The tradeoff with using lower frequencies involves producing larger acoustic foci and resultant poor target-specificity. Megahertz-range frequencies can therefore be used to improve target-specificity. This study demonstrates that Focused Ultrasound in the megahertz range can be used to evoke motor and ocular responses in mice under deep anesthesia by targeting cortical and subcortical structures, respectively. Contralateral-paired hind limb movements were observed when stimulating cortical regions, demonstrating the ability of megahertz-range FUS to stimulate activity in highly-targeted regions. Additionally, pupil dilation was observed when deep-seated anxiety-related structures were targeted, demonstrating the ability of FUS to modulate activity in a small subcortical structures.

Methods: For this study, wild-type adult male mice were anesthetized with intraperitoneal injections of sodium pentobarbital (65 mg/kg) and fixed in a stereotaxic frame. A single-element FUS transducer with fundamental frequency of 1.94 MHz was fixed to a 3D positioning system for accurate navigation through the brain. A 6x6 mm grid centered +2 mm anterior of the lambda skull suture was sonicated in a random order using a center frequency of 1.9 MHz, pulse repetition frequency of 1 kHz, 50% duty cycle, 1 second pulse duration, 1 second inter-pulse interval for a total of 10 pulse repetitions. The acoustic pressure applied was varied in order to evaluate thresholds for eliciting physiological responses like motor movement, eye movement, or pupil dilation. Motor movements were validated using video recordings and intramuscular electromyography recordings from the biceps femoris in both hind limbs. Pupil movement and dilation from subcortical modulation were evaluated using a high-resolution camera aimed at the right eye and frame-by-frame processing technique.

Results: The minimum peak rarefractional pressure required to elicit hind limb movements was 1.45 MPa when targeting cortical regions, calibrated using an excised mouse skull. Higher pressures increased the success rate from 20% (at the 1.45 MPa threshold) to 70% (1.79 MPa). Targeting eye-motor and anxiety-related regions of the brain elicited eye movements and pupil dilations up to 20%. Sonication of the superior colliculus resulted in both eye movement and pupil dilation at a lower threshold pressure (1.20 MPa) than the hippocampus and locus coeruleus, which required pressures greater than 1.80 MPa. A histological evaluation performed in five mice at 1.93 MPa and 3 MPa peak rarefractional pressure resulted in no red blood cell extravasation.

Conclusions: This study successfully demonstrated that megahertz-range Focused Ultrasound can be used to elicit motor and ocular responses with high specificity in mice in vivo. It was also shown that the success rate of stimulation increased with acoustic pressure for motor movements associated with cortical modulation but depends greatly on the region of the brain targeted. These findings emphasize the complex and yet to be determined mechanism of action involved in ultrasonic neuromodulation.

Figure 1. Evaluation of the pressure threshold when applying FUS to location within the somatosensory cortex. This location resulted in contralateral hind-limb movement relative to the sonication site.

Figure 2. Histological evaluation of brain at 1.93 MPa (left) and 3 MPa (right) revealed now red blood cell extravasation.
Non-invasive neuromodulation via targeted delivery of neurotransmitter chemicals

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Objectives: Focused ultrasound (FUS)-microbubble treatment has been used to open the Blood-Brain Barrier (BBB) for targeted delivery of a wide variety of therapeutics. Here we propose to deliver neurotransmitter chemicals such as GABA or glutamate for the purpose of non-invasive neuromodulation. These chemicals function to transmit or suppress signals across the chemical synapses that connect neurons in the brain. This novel approach affects signaling between neurons, as opposed to existing neuromodulation techniques that affect the transmission of electrical signals along neurons. Such an approach could be an important new complimentary tool for basic neuroscience or lead to new therapies for neurological disorders.

Previously, we used electrophysiology measurements to demonstrate functional blockade via BBB disruption and GABA administration. Here we present initial results demonstrating the proof of concept in a rodent model using delivered GABA to modulate neuronal activity and functional MRI to measure the effects.

Methods: Sprague-Dawley rats underwent bilateral hindpaw electrical stimulation (1-5 mA, 0.3 ms duration, 2 Hz) to elicit a functional response of the somatosensory network. Varying levels of GABA were systemically injected under conditions No BBB opening and BBB opening. Functional activity in the thalamus and S1 was measured using fMRI to quantify any effects of neuromodulation.

BBB opening: Microbubbles injected (Optison, 200 μl/kg), 274 kHz dual aperture transcranial FUS with 32 ms bursts applied at 4 Hz for 60 seconds.

GABA delivery: Systemic tail vein bolus injection in doses from 10 mg/kg to 50 mg/kg.

fMRI: Images acquired on a Bruker 7T scanner with a single shot EPI sequence (TR = 1.5 s, TE = 18 ms, 18 slices, 300 images). Stimulation performed in a 40 s OFF, 20 s ON block design over 7.5 total minutes. T-scores obtained using general linear model analysis in SPM 12.

Results: BBB Closed: Figure 1 shows activation results in S1 for the case of No BBB opening. Compared to the baseline case of No GABA injected, a GABA injection of 10 mg/kg showed significant decrease in activity (p<0.05) but GABA injections of 25 mg/kg and 50 mg/kg did not.

BBB Open: Figure 2 shows activation results in the thalamus for the case of BBB opening. BBB opening was targeted, and confirmed through gadolinium imaging, in the right hemisphere. Bilateral activation was seen in the thalamus for the baseline case of No GABA injected. For GABA injection of 25 mg/kg, a significant decrease in activation was seen in the right (opened) ROI (p<0.001), but not the left (unopened) ROI. For GABA injection of 50 mg/kg, a significant decrease in activation was seen in both ROIs (p<0.001).

Conclusions: More experiments on a number of rats are necessary to confirm and expand these findings. However, these very preliminary results are a promising indicator that a neurotransmitter such as GABA can be delivered through the opened BBB for targeted manipulation of neuronal activity.
fMRI results without BBB opening. Top: T-score values overlaid on a T1w image. Bottom: Paxinos/Watson rat brain atlas with hindleg S1 area colored red and bar plots for t-score metrics comparing the activity for the various GABA doses. * = p<0.05.

fMRI results with BBB opening in right thalamus. Top: T-score values overlaid on a T1w image. Left: Extent of BBB opening. Bar plots show t-score metrics from right ROI (yellow/opened) and left ROI (green/closed). ** = p<0.001.
Initial experience in a pilot study of Blood-Brain Barrier opening for chemo-drug delivery to brain tumors by MR-guided Focused Ultrasound

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Objectives: Magnetic Resonance-guided Focused Ultrasound (MRgFUS) has been shown to reversibly open the Blood-Brain Barrier (BBB) for targeted drug delivery.¹ Research on animal models, including non-human primates,² has been conducted to investigate the effectiveness and characteristics of BBB openings. Here we describe our initial experience in a pilot clinical study to establish the feasibility, safety and preliminary efficacy of Focused Ultrasound to temporarily open the BBB to deliver chemotherapy to brain tumors.

Methods: This phase-one clinical trial of BBB opening by Focused Ultrasound was approved by Health Canada. A modified clinical MRgFUS brain system (ExAblate 4000, 230 kHz, Insightec, Tirat Carmel, Israel) was used with a 3T MR scanner (Signa MR750, GE Healthcare, Milwaukee, WI, USA). Two hours before the procedure, liposomal doxorubicin Caelyx (Janssen, Toronto, Ontario, Canada) was intravenously infused over 1 hour at a dose of 30 mg/m2. The patient’s head was then shaved and positioned in the FUS array with a stereotactic frame. Two targets close to the posterior margin of the glial tumor were chosen based on T2 images (Fig.1). Each target consisted of a 3x3 grid of 9 spots at 3 mm spacing. For each spot, 2.6 ms on, 30.4 ms off FUS pulses were repeated for 300ms before steering to the next spot. The pattern was repeated periodically resulting in an overall pulse repetition frequency (PRF) for each spot of 0.9%. A bolus injection of 4 ul/kg of Definity microbubbles (Lantheus Medical Imaging, N. Billerica, MA, USA) was applied simultaneously with each sonication (1/5th of the clinical dose for ultrasound imaging). With the first injection of microbubbles, 10s short sonications at 5W, 7W and 9W acoustic power were applied to find the appropriate power level based on feedback of cavitation signals. Cavitation signals were detected by two receivers and sampled at a rate of 2 MHz. Spectrum integration from 75 kHz to 155 kHz was calculated and two threshold levels of the spectrum integration were defined as a safety mechanism based on pre-clinical studies on a trans-human skull pig model.³ 9W was found to be adequate for these targets. 50 s sonications at 9W were then applied at each target, with a separate bolus injection of microbubbles for each. Post sonication, Gd (Gadovist, Bayer)-enhanced 3D FSPGR images were acquired to verify the BBB openings, and T2*-weighted GRE images (TE=15ms) were collected to detect potential hemorrhage. After the treatment, the patient was released from the head frame and MR scans were repeated with an 8-channel head coil for better quality images. The patient underwent routine tumor resection the next day and tissue samples at the two BBB opening targets were collected for quantification of chemotherapy drug concentration.

Results: The opening of the BBB was successful at both locations with clear Gd enhancement in the 3x3 grid pattern. (Fig.2). Despite using the same power level, the actual acoustic pressure at the 2nd target was lower than the first due to steering of the FUS beam. Low-level extravasation of red blood cells were seen as small dark signals within individual sonicated spots in the T2* image (Fig.3). The quantification of drug concentration is pending further analysis.

Conclusions: The 3mm spacing of the 9 spots was intentionally designed to form a grid pattern of Gd enhancement for easier confirmation in heterogeneous tumors for the initial cases. We do not expect an impact on other parameters if the spacing needs to be reduced for a more uniform drug distribution within the BBB opening volume. There was a small level of RBC extravasation but this was not a concern in the tumor environment. Our animal experiments have shown that the cavitation signal can be used during the sonications to control the power level for eliminating the RBC extravasations.⁴ The current system did not use this method during sonications.
The tumor in this patient was in the right temporal lobe adjacent to the skull. The two targets were ~4 cm lateral from the midline of the brain, and the 2nd target was also ~2.5 cm posterior. Thermal ablations by FUS at these off-centre locations are technically challenging due to excessive skull heating. However, successful BBB openings at these locations were demonstrated at low powers at 230 kHz. If these results can be repeated in other patients without complications, then the method may provide a new way to deliver therapeutic agents into brain for the treatment of tumors and other brain diseases.

References
Enhanced bevacizumab delivery to the CNS by Focused Ultrasound induced Blood-Brain Barrier opening for malignant glioma treatment

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\textbf{Objectives:} Malignant glioma is the most severe form of primary brain tumors with an extremely high recurrence rate and the poorest prognosis. Current anti-angiogenic monoclonal antibody (mAb) treatment failed to show therapeutic efficacy due to transient “vascular normalization” stage that restores BBB integrity in tumor regions and restricts anti-angiogenic mAb penetration, preventing angiogenic suppression of tumor cells in the CNS and diminishing the improvement in overall survival in clinical treatment observation. The purpose of this study is to demonstrate that transcranial Focused Ultrasound (FUS) enhances Blood-Brain Barrier (BBB) permeability of the antiangiogenic monoclonal antibody, bevacizumab, for glioblastoma multiforme (GBM) treatment.

\textbf{Methods:} Transcranial FUS in the presence of microbubbles was used to transiently open BBB, and enhance CNS penetration of bevacizumab in normal and glioma-bearing mice. Bevacizumab was quantitated by high-performance liquid chromatography (HPLC), and Western blotting confirmed bevacizumab in the CNS. Bevacizumab permeability was estimated \textit{in vivo} via contrast-enhanced Magnetic Resonance Imaging (CE-MRI), and glioma progression was longitudinally followed via T2-MRI. Morphological changes and vascular inhibition were confirmed histologically with H&E and CD-31 immunohistochemistry.

\textbf{Results:} HPLC confirmed that FUS significantly enhanced CNS delivery of bevacizumab from 5.7- to 56.7-fold. The high correlations between CE-MRI imaging indices and bevacizumab concentration ($r^2=0.56-0.7378$) suggested feasible non-invasive \textit{in vivo} imaging of large-molecule BBB penetration. FUS-enhanced bevacizumab delivery significantly inhibited glioma progression, and improved median survival (ISTmedian = 135\%, compared to 48\% in bevacizumab-administration alone).

\textbf{Conclusions:} In conclusion, anti-angiogenic glioma therapy is enhanced via our proof-of-concept study that FUS enhances large molecule bevacizumab BBB permeability, and combining Focused Ultrasound to open the Blood-Brain Barrier with bevacizumab delivery can overcome bevacizumab vascular normalization and potentiate bevacizumab’s anti-angiogenic tumor therapy effect.

Figure 1. (a) Representative imaging indexes obtained from DCE -MRI analysis (T1-WI, R1-AUC, K\textsubscript{trans}, and Ve). (b) CD-31 IHC fluorescent microcopies. Bar = 100\mu m.
Closed-loop control of targeted drug delivery across the Blood-Brain Barrier in rat glioma models

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Objectives: Microbubble-mediated Focused Ultrasound (FUS) can induce targeted drug delivery through Blood-Brain Barrier disruption (BBBD). Real-time feedback control of cavitation is critical to realize desired treatment outcome while avoiding tissue damage. Here, we propose an acoustic emissions-based controlling paradigm that can sustain stable cavitation (harmonic emission, HE) while suppressing inertial cavitation (broadband emission, BE). Our objective is to deliver desired drug dose by controlling HE strength during BBBD, while keeping the brain damage-free.

Methods: A dual-aperture FUS setup (f = 274.3 kHz) produced a sub-centimeter focal depth in rats’ brain (n = 50) in vivo, and a passive detector (fcentral = 650 kHz) monitored cavitation activity. HE and BE were analyzed during 32-ms bursts in real time and fed back for control of the next pulse. The impact of multiple FUS parameters and microbubble (Optison) injection protocol on the controller performance was studied. To avoid inertial cavitation, the pressure was reduced if BE was detected and terminated if it crossed a set threshold. Both wild type and F98 glioma (ATCC # CRL-2397) models have been used in this study. Delivery of a model drug (Trypan Blue; 960 Da) and chemotherapeutic drug (Doxorubicin) was assessed using fluorescent imaging of formalin-fixed tissue blocks 1-h post sonication.

Results: Pilot study demonstrated the HE-pressure linearity (R2 = 0.93) and found that the BE threshold decreased as bubble dose (up to 400 µl/kg) was augmented. To optimize controller performance in sustaining HE while suppressing BE, a Phase-1 study demonstrated that: 1) 4-Hz PRF (compared to 1-Hz, P < 0.001) significantly suppressed the HE signal variance; 2) Infusing microbubbles after an initial bolus prevented the decline of HE and a corresponding increase in pressure, and further improved HE stability (P < 0.05 for all comparisons, Fig 1A) while reducing the likelihood of BE (33.7% vs. 16.7%).

Using optimal settings, a Phase-2 study investigated HE control and Trypan Blue delivery (Fig 1B). Integrated HE was exponentially correlated with epi-fluorescence intensity (R2 = 0.82; red symbols in Fig 1C). Based on this calibration, a Phase-3 study tested if we could deliver a desired amount of drug by sonicating until the HE reached a preset goal. The resulting fluorescent intensity matched well with the reference curve for three different goals (n > 5 per group, green symbols in Fig 1C).

Conclusions: Our proposed controlling system and method has been demonstrated to effectively sustain the stable cavitation behavior while suppressing the inertial cavitation at a minimum level. Moreover, this real-time closed-loop controller can enable the reliable delivery of a pre-determined amount of drug to the brain.

Figure 1. A) Signal stability assessment (*: P<0.05, ***: P<0.001, ****: P<0.0001); B) Cavitation control profile (HE in black and BE in red) and Trypan blue delivery; C) Calibrated BBBD correlation (in red) and controlled delivery results (in green).
**Objectives:** HER2-targeting antibodies (i.e. trastuzumab and pertuzumab) prolong survival in HER2-positive breast cancer patients with extracranial metastases. However, the response of brain metastases to these drugs is poor and it is hypothesized that the Blood-Brain Barrier (BBB) limits drug delivery to the brain\(^1\). We aim to improve delivery by temporary disruption of the BBB using MR-guided Focused Ultrasound (MRgFUS) in combination with microbubbles. MR imaging is used to evaluate the treatment benefit of combining HER2-targeting antibodies with Focused Ultrasound-mediated BBB disruption in a breast cancer brain metastasis model.

**Methods:** Three groups of 10 nude rats were included: a control-group that received no treatment; an antibody-only group that was treated with trastuzumab and pertuzumab; and a FUS+antibody-group that received trastuzumab and pertuzumab in combination with BBB disruption using MRgFUS. At week 0, HER2-positive human cancer cells (MDA-MB-361) were injected in the right brain hemisphere. Six weekly treatments started at week 5. The ultrasound treatments took place in a 7T MR-system (Biospec, Bruker) using a single-element, spherically-focused 690 kHz-transducer. Before and after the sonications, T2-weighted (T2w), T1w and T2*w images were obtained for tumor localization and targeting, confirmation of BBB disruption and evaluation of hemorrhages. At the start of each sonication (duration 60s, 10-ms bursts, burst repetition frequency 1 Hz), the ultrasound contrast agent Optison (100 µl/kg) was injected. The complete tumor was treated in 4 to 14 sonications using peak negative pressures between 0.46 and 0.62 MPa.

After the sonications gadolinium (Magnevist) was injected to confirm BBB disruption. The difference in signal intensity change in pre- and post-contrast T1w images was determined between the tumor and contralateral brain region (=ΔSI%). In two animals tumor leakiness was studied before the tumors were sonicated and quantified in the same manner. Pre- and post-sonication T2*w images were qualitatively inspected for hypo-intense regions, which can indicate extravasated erythrocytes.

Every other week, high-resolution T2w imaging was performed to determine tumor volume. The volumes were fitted with: \(\text{volume}(t)=a\times\exp(rt)\), in which \(r\) is the growth rate and \(t\) is the time in days. \(r\) was determined for the treatment period (week 5 to 11) and the follow-up period (week 11 till sacrifice). An animal was classified as ‘responder’ if \(r\) was lower than the mean \(r\) of the control animals minus two standard deviations.

The animal was euthanized if its condition was poor or the tumor diameter exceeded 13 mm. Brains were stained for hematoxylin and eosin (H&E) and HER2.

**Results:** BBB disruption was successful in all sessions with an average ΔSI% of 21.2% (range 4.5–77.6%). The mean ΔSI% of two tumors before BBB disruption during the six treatment weeks were 0.4% and 0.6%, indicating that the tumors were not leaky before disruption. In 20/60 FUS-sessions, regions were present that were clearly more hypo-intense on post- than on pre-sonication T2*w images, suggesting hemorrhages. In the remaining 67% of the sessions, no or a small difference in hypo-intensity was observed.

In the FUS+antibody-group, 4/10 animals were classified as responders during the treatment period with an average growth rate of 0.010±0.007, compared to 0.043±0.013 for the non-responders. There was no difference in the average ΔSI% of the responding rats (21.8%±16.7) and the non-responding rats (20.7%±9.7). None of the control or antibody-only animals were classified as responder. For the follow-up period, none of the animals was classified as responder.

High-resolution T2w imaging showed that the tumor was homogenous in most animals till week 13-15, when cystic and necrotic areas started to develop. The tumors showed also a heterogeneous appearance on H&E-stained sections and the complete tumor was HER2-expressing in the examined brains.
**Conclusions:** In this study, we demonstrate that BBB disruption using MRgFUS in combination with antibody therapy can slow down the growth of breast cancer brain metastasis. As the tumors were not leaky before BBB disruption and there were no responders in the antibody-only group, the disruption of the BBB is necessary for drug delivery to these brain metastasis. Interestingly, only part of the rats responded to the treatment, the other animals had the same growth rate as the control-group. We did not observe a difference in tumor volume at the start of the treatment, in HER2 expression, or in contrast-enhancement on T1w images between the responders and non-responders to explain this. Better understanding of why certain animals respond is needed and will help in translating this technique to the clinic.

**References**


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**Figure 1.**

A) T1-weighted images before contrast administration. The red arrow indicates the tumor. B) No difference in enhancement of the tumor is observed after contrast administration (ΔSI=0.4%). C) After focused ultrasound-mediated Blood-Brain Barrier disruption, the tumor enhances after contrast administration (ΔSI=30.1%)

**Figure 2.**

A) The tumor of a control animal shows a heterogeneous appearance with cysts on T2w imaging. The animal was euthanized after imaging. B) The H&E-stained section corresponds well with MR-imaging. C) HER2-stained section shows that the complete tumor is HER2-expressing.
Phase I/IIa clinical trial of Blood-Brain Barrier disruption by pulsed ultrasound

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Objectives: The Blood-Brain Barrier (BBB) limits the delivery and efficacy of systemically administered drugs to the brain. Pre-clinical studies have demonstrated that ultrasound-induced disruption of the BBB can significantly increase the intracerebral concentration of chemotherapy. To harness this approach, our group has developed an implantable ultrasound device, SonoCloud®, that allows for simple and repeated disruption of the BBB. The goal of this work was to determine the safety of repeated disruption of the BBB using the SonoCloud® device in patients with recurrent glioblastoma prior to receiving carboplatin chemotherapy.

Methods: A Phase 1/2a clinical trial was initiated at the Hospital Pitie Salpetriere in Paris, France in July 2014. Patients with recurrent glioblastoma were implanted with an 11.5-mm diameter biocompatible 1 MHz ultrasound transducer. The device was fixed to the skull bone in a standard burr hole, after which the skin was closed. Once a month, the device was connected to an external generator system using a transdermal needle connection and patients received a 150 second ultrasound sonication (25,000 cycles/burst, 1 Hz) in combination with systemic administration of an ultrasound contrast agent. BBB disruption was monitored immediately after sonication using gadolinium-enhanced Magnetic Resonance Imaging (MRI). Systemic intravenous injection of carboplatin chemotherapy (AUC 4-6) was administered immediately following acquisition of each patient’s post-sonication MRI (<1 hr after BBB disruption). Patients followed a progression of ultrasound dose in which the acoustic pressure was increased from 0.5 to 1.1 MPa throughout the course of the study.

Results: Fifteen patients were included in this on-going study from July 2014-January 2016. A total of 41 BBB disruption sessions were performed. Contrast-enhanced MRI indicated that the BBB was disrupted at acoustic pressure levels up to 1.1 MPa without detectable adverse effects on MRI or clinical examination.

Conclusions: Our preliminary results from this Phase I/IIa study indicate that repeated disruption of the BBB using the SonoCloud® device is safe and well tolerated in patients with recurrent GBM and may improve chemotherapy delivery in the brain.
In vivo porcine histotripsy brain treatments

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Objectives: Focused ultrasound thermal therapies have been explored for brain applications including treatment of essential tremors, Parkinson’s disease, and stroke. Histotripsy, a cavitation-based ultrasound therapy, is being investigated for treatment of brain tumor and intracerebral hemorrhage, but there are concerns that excessive hemorrhage may be induced in the brain during histotripsy treatment by disrupting blood vessels. This study investigates the in vivo feasibility and safety of generating targeted lesions in the porcine brain using histotripsy. The goal is to demonstrate that lesion generation may be accomplished safely and without excess hemorrhage, edema, or other major complications associated with treatment. We also seek to investigate the histotripsy dose required to fully ablate target brain regions.

Methods: Histotripsy treatments were delivered to brains of 11 pigs using a 1.5MHz focused transducer following a craniotomy. Lesions generated were targeted in the cortex at depths of 5 to 20mm from the exposed surface of the brain. Using ultrasound imaging guidance, treatment regions were targeted to be contained within individual gyri to avoid perforations into the sulci. During dosage studies, single lesions were generated using between 1 and 200 histotripsy pulses delivered at ≤10Hz PRF with an estimated peak negative pressure of 45MPa. Large lesions were generated by incrementally repositioning the histotripsy target site during treatment to ablate entire target volumes. 7 acute pigs were sacrificed within 6 hours of treatment and brains were removed for MRI and histology. 4 sub-acute pigs were survived for 3 days after treatment. To assess lesion damage, hemorrhage and edema surrounding the lesions, MR images of the brains were acquired at 1 hour and at 3 days after treatment for sub-acute pigs. After euthanization brains were dissected for histology.

Results: In 4 acute pigs, with ultrasound guidance, histotripsy was used to generate precise single lesions of 1.5x2.5mm within the gyri. Dosage studies revealed that a single histotripsy pulse was sufficient to generate identifiable damage in the brain in MRI and complete cell fractionation in lesions was achieved by 50 pulses. In 3 acute pigs, 5 larger lesions measuring up to 7mm diameter were generated in the brain. MRI and histology revealed that lesions confined to within the gyri show no hemorrhage or other major complication associated with treatment. In 4 sub-acute pigs, 6 single lesions were generated by 10 and 50 pulses, and two larger square lesions of 3.5mm were created. MRI following treatment showed no hemorrhage or damage outside the target regions and at 3 days showed no additional hemorrhage and only minor edema near the lesion boundary. No midline shift or brain herniation was observed. Histology showed damage confined to within target volumes, with well demarcated boundaries between treated and untreated tissues and no evidence of hemorrhage, ischemic changes, encephalitis, or acute, sub-acute or chronic inflammatory infiltrate beyond the areas of the lesion.

Conclusions: The results of this study demonstrate that histotripsy may be used to generate targeted lesions in the brain without causing excess hemorrhage, edema, or other major complications associated with treatment. Lesions were observed to have well defined boundaries between treated and untreated tissues, with little damage beyond the confines of the lesion area. Tissues surrounding the lesions appear very viable with no acute, sub-acute or chronic inflammatory infiltrate, and tissues in adjacent gyri appear uninvolved and unremarkable. These results demonstrate that histotripsy may be applied in the brain without causing major complications and suggest the potential of histotripsy for use in brain therapy applications.
Neuronavigation-guided Focused Ultrasound and real-time acoustic mapping: Evaluation in non-human primates with Blood-Brain Barrier opening

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Objectives: Focused ultrasound (FUS) has shown great promise for noninvasive brain treatment, including surgical ablation, Blood-Brain Barrier (BBB) opening and drug delivery, and neuromodulation. Accurate and precise targeting with personalized planning is the key for treatment success. Furthermore, repetitive procedure is often required for BBB opening and drug delivery as well as neuromodulation, which entails the demand for a portable system apart from the current MR-guided FUS system requiring a dedicated and custom-built suite. In this study, a neuronavigation-guided FUS system was developed, combined with in silico preplanning based on CT and MRI as well as real-time acoustic mapping in visualizing the location and intensity of the cavitation events associated with BBB opening. The system was evaluated in non-human primates with BBB opening, with the treatment preplanning and targeting accuracy validated both in silico and in vivo.

Methods: Three rhesus macaques were sonicated (N=15) and the subcortical structure of the basal ganglia was targeted, which is associated with neurodegenerative diseases including Parkinson’s and Huntington’s disease. Both CT and MRI were acquired for personalized pre-planning and neuronavigation guidance (Brainsight). The 3D numerical simulation of the acoustic pressure field was performed for pre-planning and post comparison with the BBB opening. A single-element, 0.5-MHz FUS transducer (diameter: 64 mm) with in-house microbubbles were used for sonication, and a programmable data acquisition system (Verasonics) with an array of acoustic detectors for real-time passive cavitation mapping. Both the FUS and cavitation mapping were guided with the neuronavigation system in real time during the FUS procedure. After sonication, the contrast enhanced T1-weighted MRI was used to confirm the location and size of BBB opening. The accuracy of both the FUS targeting and cavitation mapping were assessed.

Results: The target shift was on average 2.0 mm laterally and 3.5 mm axially in the in vivo experiment, and the shift due to the skull was predicted to be 0-1 mm laterally and 1.0-5.5 mm axially in silico. Real-time cavitation mapping confirmed the sonicated area with and without BBB opening, and distance between the centroid of the cavitation map and that of the resulting BBB opening was under 2 mm. In order to achieve the desired BBB opening volume, the pressure and focal spot size in situ were estimated in silico and tailored for targeting in each subject. Simulation results showed a smaller focal spot size through the skull (2.6 mm laterally and 16.7 mm axially, compared with the original size of 4.0 mm laterally and 35.3 mm axially), which corresponded to the BBB opening volume under specific peak negative pressures (NHP 1 at 200 kPa, NHP 2 at 600 kPa). This pressure difference between individuals was due to the difference in skull attenuation, since the in silico pressure reduction was estimated to be 30.9%±12.4% and 53.9%±14.3% in NHP 1 and NHP 2, respectively.

Conclusions: In conclusion, the new portable neuronavigation-guided FUS with in silico preplanning and real-time cavitation mapping was shown feasible and could facilitate the BBB opening and neuromodulation applications while maintaining translational capability to a clinical setting.
Definition of basic properties of physical immunotherapy in pancreatic cancer using HIFU and immune checkpoint inhibition

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Objectives: The latest advances in cancer immunotherapy have improved our understanding of how to stimulate the patient’s immune system to fight their own disease. Notwithstanding the unparalleled success of treating melanoma tumours and non-small cell lung cancer with immune checkpoint inhibitors, patients are not all getting the same benefit. In this context, pancreatic cancer presents unique challenges. Its dense stroma characteristics result in limited immune cell infiltration in the tumour microenvironment. In addition the low number of mutations in pancreatic cancer cells result in few tumour antigens, thereby reducing their immunogenicity. HIFU may address some of these limitations by ablating segments of the tumour to induce a stronger inflammatory response and degrade the stroma to facilitate enhanced diffusion of tumour antigens out of the tumour, and increased penetration of lymphocytes to the tumour. We propose to co-treat pancreatic tumours with HIFU and immune checkpoint inhibitors to elicit an effective anti-tumour immune response.

Methods: In vitro characterization of the effects of heat on the presentation of cell surface receptors associated with the immune response has been undertaken in human colon cancer HCT116 cells and mouse pancreatic cancer Panc02 cells using FACS analysis. A KPC-derived pancreatic cancer cell line will be transplanted in C57BL/6 subjects to form syngeneic subcutaneous and orthotopic pancreatic tumours. These tumours will be treated with HIFU, and with in vivo monoclonal anti-CTLA-4 and anti-PD-1 antibodies. The phenotype of markers of the adaptive immune response will be assessed to identify whether HIFU exposures can initiate intrinsic anti-tumoural cellular immunity at the site of treatment. Analysis by flow cytometry and immunohistochemistry of both effector cells (cytotoxic CD8+ T cells, dendritic cells), and immunosuppressive cells (Tregs, myeloid derived suppressor cells), as well as cytokines and chemokines at various time points post HIFU will be performed. Tumour growth will be determined using high resolution ultrasound imaging, and the results will be correlated with overall survival.

Results: Thermal exposure of cancer cells results in a time-dependent regulation of the transmembrane receptor CD47. CD47 decreases immediately after, and 1 day after treatment, and increases 3 and 4 days after treatment relative to the sham-exposed cancer cells. Further data on the effects of thermal exposures on the regulation of immune-associated pancreatic cancer cell surface receptors will be presented.

Conclusions: Detailed pre-clinical studies in various mouse models of pancreatic cancer can provide a mechanistic understanding of the combinatorial effects of HIFU and immune checkpoint inhibitors. Strong evidence that HIFU stimulates endogenous immunity against pancreatic tumor cells, and augments the immunotherapy treatment of poorly immunogenic pancreatic tumours could be used as proof-of-concept for the initiation of Phase 1 clinical trials of HIFU-enhanced immunotherapy in patients with pancreatic cancer.
Melanoma growth control via ultrasound depends on the adaptive immune system and surpasses αPD-1

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Objectives: Melanoma incidence continues to rise, while Stage IV 5 year survival remains below 20%. Targeted immunotherapy approaches, designed to enhance natural anti-tumor mechanisms or inhibit the immunosuppressive tumor microenvironment, have shown promise in early clinical use. The programmed death receptor and its ligand (PD-1 and PD-L1) have drawn considerable attention. PD-L1 is known to inhibit the activity of anti-tumorigenic cytotoxic T cells, promoting a pro-tumor immune profile, and ultrasound (US) can promote the infiltration of immune cells into the tumor. Here, we tested the hypothesis that a combination of pulsed US, microbubbles (MBs) and anti-PD-1 will improve tumor growth control in a mouse model of melanoma in a manner dependent on the adaptive immune system. Melanoma incidence continues to rise, while Stage IV 5 year survival remains below 20%. Targeted immunotherapy approaches, designed to enhance natural anti-tumor mechanisms or inhibit the immunosuppressive tumor microenvironment, have shown promise in early clinical use.

Methods: Eight week old immunocompetent C57BL/6 male mice were inoculated unilaterally with B16-F10 melanoma cells and divided into four groups: Control (untreated), US+MBs, αPD-1, or US+MBs+αPD-1. Immunocompromised Rag1-/- mice were similarly inoculated and divided into two groups: Control (untreated) or US+MBs. On day 11 post-inoculation, animals receiving US were anesthetized and sonicated with a 0.75” unfocused 1MHz transducer as previously described for 60 minutes. MBs (105/g b.w.) were injected i.v. throughout the sonication. The αPD-1 groups were given an IP injection of 250 µg of αPD-1 on Days 10, 13 and 16. Tumors were monitored until Day 18, when tumors were resected for flow cytometry. Helper T cells (CD4+), cytotoxic T cells (CD8+), T regulatory cells (Treg), macrophages and natural killer cells (NK) were counted in immunocompetent mice, and dendritic cells (DC), natural killer cells, M1 and non-M1 macrophages were counted in immunocompromised mice.

Results: In immunocompetent mice, treatment with US+MBs provided improved tumor growth control compared to all other groups (Figure 1A) as well as a significant survival benefit (not shown). Additionally, US+MBs generated a significant increase in macrophages (not shown) and Tregs (Figure 1B) compared to control. Interestingly, the αPD-1 and US+MBs groups performed similarly in CD4+ and CD8+ cell counts (Figure 1B), but the combined US+MBs+αPD-1 treatment generated fewer T cells than either treatment alone. In immunocompromised animals, treatment with US+MBs did not provide tumor growth control (Figure 1C) or a survival benefit (not shown), but did generate a significant decrease in M1 macrophages within the tumor (Figure 1D), although total myeloid cell counts remained the same (not shown).

Conclusions: US indiscriminately increases the numbers of immune cells within subcutaneous melanoma; therefore, we expected that US-enhanced delivery of αPD-1 would provide an additional benefit; namely, a shift towards an anti-tumorigenic immune profile. However, αPD-1 did not synergize with US+MB treatment. While the mechanism is not yet understood, low power noninvasive US+MBs provided excellent tumor growth control and may serve as a stand-alone clinical treatment. Furthermore, this anti-tumor effect is clearly dependent on the adaptive immune system, since US+MBs treatment in immunocompromised mice produced no benefit. A better understanding of this mechanism may identify adjunct treatments that would synergize with US and further enhance the anti-tumor effect demonstrated here.

Reference:
Figure 1. (A) Tumor growth in immunocompetant C57BL/6 hosts. *P<0.05 vs. control. (B) Immune cell representation in tumors in C57BL/6 hosts. (C) Tumor growth in immunocompromised Rag1-/- hosts. (D) Immune cell representation in tumors in Rag1-/- hosts.
Stress response of cancer cells after low intensity Focused Ultrasound

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Objectives: Low intensity Focused Ultrasound (LOFU) results in sublethal stress to tumor cells. We investigated the cell surface expression of immunomodulatory molecules in three murine cell lines (Lewis lung, breast and prostate adenocarcinoma). The immunomodulation was examined by flow cytometry of cell surface stress proteins: HSP70 & calreticulin, of immunomodulatory co-activating signals: CD40, CD80, CD86 and other receptors: MHC I, Fas). Surface expression of these molecules trigger activation of or phagocytosis by dendritic cells. Additionally, the activation of the unfolded protein response (indicated by phosphorylation of PERK or IRE1α or cleavage of ATF6α) can trigger apoptosis – a programmed cellular death pathway which can also activate the immune system. We hypothesize that by altering the total acoustic power of LOFU, we can modulate the stress signals both on the cell surface and within the cells.

Methods: LOFU treatment was performed on the Philips Therapy and Imaging Probe System (TIPS, Philips Research Briarcliff, USA) using 3W or 5W, 100% duty cycle, 1.5 seconds, 1 mm spacing. LOFU treatment was performed on a cell pellet which were then replated and incubated at 37°C, 5% CO2 for the specified time. At the end of the incubation time, cells were lightly scraped for flow cytometry staining or lysis buffer was added to the adherent cells and then scraped for Western blot analysis.

Results: The analysis of murine lung and breast cancer indicates that there is a significant surface localization of both HSP70 and calreticulin as early as 4 hours after LOFU treatment and this localization diminishes by 24 hours post treatment. For murine prostate cancer cells, however, the surface localization of HSP70 increases at 4 hours after treatment and increases further at 24 hours. Additionally, there is a significant increase in surface localization of both HSP70 and calreticulin with a higher output power of 5W compared to 3W. For immunomodulatory co-activating signals, there is increased surface expression in Lewis lung carcinoma and prostate adenocarcinoma, but not breast carcinoma. Results of the activation of the unfolded protein response are still pending at the time of submission, but preliminary results indicate the both phosphorylation of PERK and IRE1α are increased following LOFU treatment.

Conclusions: Tumor stress response to LOFU treatment can be altered by the total acoustic power of LOFU treatment. The increased surface localization of HSP70, calreticulin, CD40, CD80 and CD86 have the capacity to augment the immune response to the tumor. Therefore, LOFU can be used in combination with ablative therapies in order to induce anti-tumor immunity and destroy the primary tumor.
The “ABSCOPAL” effect after USgHIFU treatment of advanced pancreatic cancer

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Objectives: Pancreatic cancer is considered one of the main big “killers” in Oncology, with still a very poor prognosis, both in patients amenable to resection and of course in more advanced stage disease. More than 50% of patients are diagnosed with an advanced stage disease, where chemotherapy is usually the main therapy option. External radiotherapy is the most common palliative loco-regional treatment in pancreatic cancer, but in the last few years HIFU has been proposed as an alternative option for palliation in advanced stage.

From our specific experience of USgHIFU for pancreatic cancer, we report few cases of ABSCOPAL effect: where after a local treatment not only the target tumor but also the distant deposits will shrink

Methods: From 2008 until April 2016, 72 consecutive patients affected by pancreatic tumors were selected by a dedicated Tumor Board for receiving treatment with USgHIFU, for local tumor control and palliation. 64% of patients were affected by pancreatic adenocarcinoma and 84% of all the treated tumors were located at the level of pancreatic head. Majority of the patients had at least two lines of chemotherapy and 45% of them received concurrent and/or consequent external RT. USgHIFU treatment was performed always during general anesthesia with GI catheter and abdominal compression with a dedicated water balloon in order to reduce the distance between the HIFU probe and the target and for push the bowel loop away from the acoustic treatment window. Patients were than evaluated by clinical and instrumental follow up.

Results: All the procedures were technically successful, with only one main complication due to a complete portal thrombosis developed 24hrs after treatment. Objective response (OR) at MDCT, MRI and PET was 64% with a disease control (DC) up to 90% of all the patients. Pain was palliated in 23/27 of symptomatic patients. In four patients (4/46 with adenocarcinoma) the imaging follow up after the treatment revealed the shrinkage of metastases located in other sites: 2 retroperitoneal lymphnode (Fig1a,b), 1 liver and 1 lung.

Conclusions: In advanced pancreatic carcinoma, HIFU can have a role in locally advanced unresectable disease. Based on our limited experience, HIFU could be also suggested in a metastatic setting (Stage IV), together with systemic therapy, with the potential benefit of being effective also outside the treated target (abscopal effect). There are few papers reporting the effect of HIFU as a booster of immune response in cancer patients, but there are still no data supporting the use of this non-invasive approach in advanced cancer patient just for this purpose. Specific dedicated clinical trial should be conducted for better understand the biological mechanism behind it.

![Figure 1a: multiple retroperitoneal lymphnodes in patient with pancreatic cancer](image1.png)

![Figure 1b: 18months after HIFU, CT shows the complete response at the level of metastatic lymphnodes](image2.png)
Characterizing the immune response to Boiling histotripsy ablation of renal carcinoma in the Eker rat

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Objectives: Boiling histotripsy (BH) is an experimental non-invasive Focused Ultrasound (US) technology. BH uses milliseconds-long US pulses at low duty cycle to mechanically homogenize targeted tissue. We aim to evaluate the adaptive immune response to BH ablation of spontaneous renal tumors in the Eker rat hereditary renal carcinoma (RCC) model.

Methods: Genotyped Eker rats (Tsc2 heterozygotes) were monitored for de novo RCCs with serial US until tumors were ≥8 mm. Syngeneic Wild-type (WT) (planned total n = 36) and Eker rats (planned total n = 48) were then randomly assigned to BH (n=10 total to date) or an US SHAM procedure (n=22 to date). BH was performed extracorporeally using US-guided small animal FUS system (VIFU-2000, Alpinion). The 1.5 MHz transducer was operated at duty cycle of 1%, 10 ms pulses, 525-550 W electric power. Treatments targeted a ~0.5 cc area of the lower pole in WT rats and the largest RCC with a margin of normal kidney in the Eker rats. Blood samples were collected immediately before treatment and serially post-treatment. Rats were euthanized at 48 hrs, 7 days, 14 days, or 56 days following treatment. At euthanasia, ipsilateral and contralateral kidneys, hilar lymph nodes, and the spleen were collected. Intrarenal and circulating plasma cytokines were assessed using a 10 cytokine multiplex assay. Renal/tumor infiltrating leukocyte populations were assessed using immunohistochemistry. Leukocyte populations in tumor draining hilar lymph nodes and spleens were assessed with flow cytometry.

Results: BH treatment has been successful in all treated (n=4 Eker, n=6 WT to date) subjects, producing hypoechoic regions on US consistent with BH treatment effect. BH treatment was associated with significantly increased mean relative-plasma TNF-α vs. sham treatment at 0.25 (p=0.02), 1 (p=0.04), and 24 (p=0.05) hours. At 48 hours, significant differences in intra-renal cytokines were observed in BH (n=4) vs SHAM (n=4) treated rats: IFN-gamma, IL-10, and IL-8 with trend towards differences in TNF-alpha and IL-6 (Figure 1). On immunohistochemistry, BH treated Eker rats had increased CD8+ T-cells in both the treated and contralateral kidney compared to SHAM treated rats at 48 hours (Figure 2). Additionally, increased infiltration of f4/80+ M1 macrophages into the treated kidney, was observed in 2/4 BH treated rats vs. SHAM at 48 hours. Longer-term data is pending at this time.

Conclusions: These data represent preliminary findings indicating differences in cytokines and tumor infiltrating leukocytes between BH treated and SHAM treated Eker rats up to 48 hours post-treatment. Assessment of longer-term immune effects of BH treatment and their significance is on-going and will be reported.
Use of shock-wave exposures for accelerating thermal ablation of localized tissue volumes

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Objectives: In High Intensity Focused Ultrasound (HIFU) applications, nonlinear acoustic effects can result in the formation of high-amplitude shock fronts in focal waveforms, with amplitudes exceeding 100 MPa. The presence of such shocks leads to increased tissue heating which can be beneficial for HIFU thermal therapy. The goal of this work was to evaluate the efficacy of different shock-wave exposures to accelerate thermal ablation of tissue volumes while enabling safer conditions for intervening tissues.

Methods: Simulation studies were performed for a multi-element 1.2 MHz HIFU phased array of a clinical system (Fig. 1a, Sonalleve V1, Philips, Vantaa, Finland) for three different peak intensity levels at the array elements of 1.2, 8, and 15 W/cm². A pulsing scheme was combined with discrete electronic steering of the array focus over a series of foci separated about 1 mm and arranged in 4 concentric circles (Fig. 1b). The circles were positioned in a plane at 25 mm depth in liver tissue of 50 mm thickness (Fig. 1a). The period between consecutive pulses was 20 ms. Pulse duration was varied to keep a constant time-average intensity at the array elements: 20 ms at 1.2 W/cm², 3 ms at 8 W/cm², and 1.6 ms at 15 W/cm². The Westervelt equation combined with the bioheat equation was used for modeling temperature and thermal dose in tissue. Initial temperature in tissue was 20°C, sonication was performed starting from the center and spiraling outward until a thermal dose of 1.76 s at 56°C (equivalent to 240 CEM at 43°C) was reached at each circle of the trajectory.

Results: Shown in Fig. 2 are the focal waveforms simulated in tissue for different peak intensities representing quasilinear sonication (1.2 W/cm²), sonication with a fully developed shock of 95 MPa at the focus (8 W/cm²), and sonication with higher focal shock amplitude of 118 MPa (15 W/cm²). Corresponding temperature distributions obtained in the focal and axial planes at the end of the HIFU exposure are shown in Fig. 3. Red contours indicate boundaries of the ablated tissue volumes. Tissue ablation was achieved much faster at higher peak intensities with more clearly defined boundary between treated and untreated tissue. While temperature distributions and thermal dose contours in the focal plane were similar in shape for each trajectory and intensity level, corresponding distributions in the axial plane varied greatly. Slower heating at 1.2 W/cm² resulted in strong heat diffusion in the axial direction and much larger ablation volumes. On the contrary, with rapid shock-wave heating, the ablated tissue volume followed the geometry of the targeted heat deposition. Although overall ablated volumes were smaller at higher peak intensities, the ablation rate was the highest at 15 W/cm².

Conclusions: In comparison with conventional HIFU treatments, shock-wave exposures provide higher ablation rates at target sites with less heat diffusion to the surrounding tissues. Shock-wave heating regimes may therefore be clinically advantageous for accelerating thermal HIFU treatments, reducing the heating of surrounding tissues, and providing sharper margins of lesion volumes.
Acoustic pressure waveforms simulated at the focus in tissue for different intensities at the array elements. While the waveform is quasilinear at the lowest intensity of 1.2 W/cm², high-amplitude shocks are formed at intensities of 8 W/cm² and 15 W/cm².

Temperature maps in tissue in the focal (a) and axial (b) planes of the transducer at the end of HIFU exposure for different intensities at the array elements. Red contours depict final regions of thermally ablated tissue.
**Effects of dosing and focal spacing in rapid ablation of large tissue volume using histotripsy with electronic focal steering**

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**Objectives:** Current tumor ablation techniques, typically thermal-based, are either limited to treating tumors <3 cm in diameter (RFA and microwave) or are very slow (HIFU). Because histotripsy uses microsecond-length pulses separated by up to seconds of off-time for a given focus, it is possible to electronically steer the therapy focus of a phased array transducer to excite cavitation events throughout a large volume consisting of many foci during the off-time. We hypothesize that histotripsy combined with electronic focal steering can achieve rapid ablation of a large volume. This hypothesis was investigated in this paper, and the effects of number of pulses per focal location and focal spacing on tissue damage generated using histotripsy with electrical focal steering were studied.

**Methods:** Histotripsy was applied using a 250 kHz, 256-element hemispherical phased array transducer with a 15 cm focal distance, generating 1.5-cycle, 6-microsecond pulses. Each element produced ~0.5 MPa P-individually, but focal pressure produced by all elements could not be measured due to cavitation. To establish treatment parameters including pulse repetition frequency (PRF) and number of pulses (dose) to deliver, a single-focus lesion was generated in tissue-mimicking phantoms containing a thin layer of red blood cells (RBCs) and monitored by optical imaging. Based on these results, 35 *ex vivo* bovine hepatic tissue samples were treated by electronically scanning the therapy focus at 200 Hz over 1000 sites (or .2 Hz per focal site). To evaluate the dose required for complete tissue fractionation, dose was varied between 20-120 pulses per focus. Focal spacing was varied from 2.5, 3.15, and 3.5 mm in the lateral direction and held constant at 4.1 mm in the axial direction to assess the extent of overlap between adjacent foci required to achieve complete homogenization. Lesion size was assessed by gross morphology and Magnetic Resonance Imaging (MRI). The degree of tissue fractionation was examined by histology. Surrounding tissue temperature was measured by thermocouples 1 cm from the lesion.

**Results:** RBC phantom results suggested that fractionation efficiency was optimal near 0.2 Hz and that 120 pulses were sufficient to achieve complete homogenization. Using lateral spacing ≥ 3.15 mm or < 100 pulses, unfractionated tissue structures were observed between foci. Using 2.5 mm lateral focal spacing and 120 pulses per focal location, complete, homogeneous tissue ablation of 43 +/- 6 mL region was achieved within 10 minutes, resulting in an ablation rate of 4.3 mL/min. A temperature increase of ~4° C was measured at the surrounding tissue. This work demonstrates that histotripsy combined with electronic focal steering can achieve rapid ablation of large volume at a rate more than double the rate of current available ablation techniques such as RFA.

**Conclusions:** Treatment of large and multiple tumor nodules remains a challenge for current tumor interventions, which are mostly thermal-based. This work demonstrates that histotripsy combined with electronic focal steering achieved homogenous and complete ablation of a large target volume at a rate greater than two-fold faster than microwave and RF ablation. Since histotripsy is non-thermal, the treatment should not be affected by the heat sink effect and is expected to remain effective and efficient even in highly vascular organs. With the capability of achieving rapid, homogenous cell disruption, histotripsy has the potential to substantially improve upon current tumor ablation methods.
The TRANS-FUSIMO system — towards a prototype for HIFU treatment of the liver under breathing motion

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Objectives: The movement of the liver under breathing and its partial occlusion by the rib cage challenge the application of High Intensity Focused Ultrasound (HiFU/MRgFUS) for the thermal treatment of tumors. To explore the full potential of extracorporeal FUS to safely and precisely destroy tissue in the depth of the moving liver requires sophisticated technology. The European FUSIMO consortium (www.fusimo.eu) has shown in a proof of concept experiment that patient specific image processing, mathematical modeling of the organ's motion, ultrasound propagation in the moving organ, and tissue heating is such enabling technology that empowers a physician to perform safe, effective and efficient ablation of tumors and to facilitate prediction of the outcome. In TRANS-FUSIMO (www.trans-fusimo.eu) the consortium is currently developing a prototype of a fully integrated system for the FUS treatment of the liver, which will be evaluated in an animal study and a patient study.

Methods: The TRANS-FUSIMO system comprises three sub-systems:
1. a planning software that incorporates image processing, motion modeling and prediction, as well as FUS simulations. This part of the system allows to plan and to assess the feasibility of the treatment;
2. a treatment system that performs the actual treatment control using previously evaluated treatment plans, as well as model based MR/US motion tracking and prediction and MR thermometry;
3. a training system which acts as a treatment simulator that is based on real cases and the simulation of FUS propagation, tissue heating, and organ motion.

Thereby, the treatment system fully integrates with the GE MR scanner and the InSightec ExAblate Conformal Bone System (CBS). The control system and the model components have been validated in phantom and ex vivo experiments. Currently, the safety, efficacy and efficiency of the system are being evaluated in an in vivo animal study. Following, a two-arm study (neoadjuvant TRANS-FUSIMO MRgFUS + resection, TRANS-FUSIMO MRgFUS only) for human patients with metastases or HCC will show the feasibility of the TRANS-FUSIMO prototype for the clinical setting.

Results: The core of the TRANS-FUSIMO system comprises patient specific models for the motion of the liver under breathing, ultrasound propagation, and thermal damage. On the one hand the models are used for the planning of the treatment and in the training system. On the other hand the models are used to augment US/MR data during the treatment in order to achieve motion compensation and thermal monitoring.

Conclusions: The ex vivo validations show the safety and efficacy of the system under regulated motion of phantoms. Furthermore the validations show that the system fulfills all specification requirements. The currently running animal study will be the basis for a following two arm patient study.
The screenshot shows the TRANS-FUSIMO treatment system during the execution of a sonication in a phantom.
Multifunctional robotic platform for USgFUS

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Objectives: Despite its promising results, FUS suffers from limited flexibility in therapy delivery, which narrows the applicability to non-moving and non-essential organs, mainly under Magnetic Resonance Imaging (MRI). We strongly believe that a robotic-assisted approach may offer the chance to overcome the current limitations of FUS by guaranteeing high robustness, flexibility/adaptability and precision of therapy. In this framework, the FUTURA (Focused Ultrasound Therapy Using Robotic Approaches) project (www.futuraproject.eu) is developing an autonomous, multi-functional and multi-robotic assisted platform able to perform non-invasive FUS on abdominal organs under US monitoring (USgFUS).

The FUTURA platform (Fig. 1) consist of: i) two six degrees of freedom anthropomorphic manipulators, ii) a dedicated broadband 16 channels wave generator and a 16 channels annular array HIFU transducer; iii) two different US probes both connected to an acquisition system (SonixTablet, Analogic Ultrasound). The control architecture is implemented by using Robot Operating System software (ROS) with a purposely developed HMI.

Methods: The FUTURA architecture allows to maximize the flexibility of the procedure both for organs targeting and during the sonication procedure and lesion assessment. In addition, the FUTURA robotic integrated approach allows for safe interaction and cooperation between robots, patient and medical staff by using environmental reconstruction sensors.

In order to demonstrate the unique features of the FUTURA platform, several tests have been performed in \textit{in vitro} and \textit{ex vivo} static conditions by using a home-made tissue-mimicking phantom made up by a bulk of agar gel with internal cylinders (representing the targets of the therapy), based on polyacrylamide gel (PAA) mixed with egg white and \textit{ex vivo} material (i.e. porcine liver or breast chicken instead of PAA cylinders into an Agar structure).

The complete workflow of the FUTURA procedure is reported in Fig 2; more specifically, the effective use of two US imaging probes during different phases of the lesion treatment has been carried out. The sonication is composed by 10 repetitions of 1 second with 90% duty cycle. The value of frequency and acoustic power of the sonication are 1200 kHz and 120 Watts, respectively.

Results: During the target identification phase, both the US imaging probes are used. Although the US confocal probe is rigidly attached to the HIFU transducer, the FUTURA platform maintains US imaging flexibility thanks to the 3D US probe mounted on the second manipulator. The 3D US phantom reconstruction and the related 2D US confocal images acquired before sonication show the target without any lesion. In addition, the estimated HIFU transducer focus, located in the center of the target, assesses the correctness in positioning of the therapeutic manipulator (intersection of blue lines in pictures B.II-III). During the sonication process an on-line monitoring of the lesion is performed by exploiting a PWM signal for the HIFU transducer generator. Finally, the 3D US phantom reconstruction and the related 2D US confocal images acquired after sonication allow the lesion assessment (i.e., accuracy better than 1 mm). An optical assessment on the sectioned targets in proximity of the performed lesion was also carried out (Fig 3). Finally, a specific analysis of the HIFU properties has been performed by observing the typical dimensions of the lesions induced by the transducer into the breast chicken tissue at different output power and duration of exposure (Fig 4).

Conclusions: The most important features of FUTURA platform have been demonstrated. This work assesses the possibility to use a robotic platform for USgFUS treatment, using two US imaging probe for the different phases of the procedure. Synchronizing the HIFU transducer shot with the US imaging acquisition enables to monitor the lesion progress during the sonication phase, which is very important for patient safety. In addition, the use of both US probes during the sonication phase enables the tracking of moving 3D organs.

Future work will be carried out to assess the accuracy of USgFUS treatment under dynamic condition and a qualitative temperature estimation technique (e.g., by means of elastography) for the assessment of thermal effects will be integrated in the analysis.
Figure 1. FUTURA platform.

Figure 2. Workflow of the robotic-assisted FUS procedure that includes: A) Pre-Treatment, B) Treatment and C) Post-Treatment phases.

Each single sonication can be divided in three further stages: B.1) Target identification phase: both US probes are used to identify the point to be sonicated; B.2) Sonication phase: the HIFU is switched on and the lesion progress is assessed through US confocal probe. During the treatment phase a preliminary lesion assessment of the single sonication is carried on, even if the therapy evolution is performed in the next phase; B.3) Lesion assessment phase: the created lesion is assessed with both US imaging probes.

Figure 3. Optical images of the lesion induced by the HIFU transducer during the sonication phase into a tissue-mimicking phantom (A) and into ex-vivo tissues - porcine liver (B) and breast chicken (C).

Figure 4. Optical images and typical dimensions of the lesions induced by the HIFU transducer into breast chicken at different output power and duration of exposure. In the first image, sonication power of 64W for 5 sec, the lesion is imperceptible.
Real time MR and US motion tracking for abdominal US/MRgFUS

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Objectives: The ablation with HIFU/FUS would be a fantastic tool for liver tumor treatment and would lead to an invaluable benefit for patients suffering from malignant liver metastasis or hepatocellular carcinoma. However, FUS of the liver has still not yet reached a significant clinical spread, particularly in the western world. There are two major challenges for FUS ablation of the liver: the rib cage and the permanent motion of the liver, e.g. due to respiration. Both, MRI as well as diagnostic US imaging are suitable to provide real-time information about the liver position. For several years, we have been developing and improving US and MR based motion tracking for HIFU/FUS, e.g. currently in the European TRANS-FUSIMO project (www.trans-fusiomo.eu). A further goal of the presented work is the automated detection of suited tracking features and the assessment of feature quality on MR and US image data.

Methods: US or MR image streams are analyzed in real-time to track liver motion. In a first step, pronounced structures, like the diaphragm, or landmarks/features like liver vessels are automatically detected or manually defined on 2D US or MR-EPI images. For motion tracking, a particle filter-based algorithm evaluates state hypotheses of local affine transformations to follow these features through the image stream. Data fidelity is assured by not only tracking each landmark separately, but also incorporating information about global motion. For each landmark, an estimate of the current position and the current uncertainty of that estimate is provided. Features with high uncertainty may then be ignored in following processing steps or replaced by more stable landmarks by the algorithm. For use in an application, the output of the algorithm is sent to the HIFU/FUS treatment unit, which can incorporate the data into further motion compensating components. This may either be established through direct beam steering of the FUS focus or by gating the therapy.

Results: US as well as MR based liver tracking was evaluated in different phantom experiments and on data from volunteers. Figures 1 and 2 show US and MR liver images with sample data provided by the algorithm. The arrows show the displacement of the feature related to a reference image due to liver motion. The discs indicate a relative measure of positional uncertainty. Automated feature detection, e.g. of blood vessels in EPI liver images, enabled fast and reliable definition of tracking features. Automated tracking quality assessment allowed a reliable detection of poor quality features which were replaced to allow a stable and reliable tracking over longer time periods. The mean tracking error on US liver images was 1.5 mm in 2D and 2.79 mm in 3D with a processing time of about 2ms/frame. 1.7 mm was the mean error of MR based liver tracking with a computing time of 2ms/frame and feature.

Conclusions: Quasi real-time liver motion tracking in 2D and 3D based on diagnostic ultrasound image streams as well as on ultra-fast MR image data is feasible, reliable and offers a sufficient precision for motion compensated HIFU/FUS therapy. Besides regular and irregular respiratory motion also liver drifts due to peristalsis and gravity on longer time scales can be compensated, since this technique is based on live image data. Integration of the tracking into existing clinical USgFUS and MRgFUS therapy units should be possible without great efforts. The presented tracking method is currently being integrated into an MRgFUS therapy setup for the treatment of liver tumors within the TRANS-FUSIMO project. A first clinical trial is planned at the beginning of 2017.
Pancreatic tumor monitoring and treatment using Harmonic Motion Imaging for Focused Ultrasound (HMIFU) in a transgenic mouse model

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Objectives: Pancreatic ductal adenocarcinoma (PDA) is one of the deadliest cancers with the lowest prognosis due to late diagnosis. PDA is characterized by an unusually dense stroma limiting chemotherapy perfusion. Harmonic Motion Imaging (HMI) assesses tissue mechanical properties by inducing localized oscillation resulting from a periodic acoustic radiation force. The amplitude of the induced displacement is directly related to the underlying tissue stiffness. The sonication is kept short for imaging (HMI) without tissue damage or the duration is prolonged for simultaneous HMI and HIFU treatment (Harmonic Motion Imaging for Focused Ultrasound or HMIFU).

This study first aimed at using HMI for characterizing the development of pancreatic tumors as a function of size and fibrosis extent in the KPC genetically-engineered mouse model. Secondly, the tumors were treated using HMIFU. HMI measurements were then resumed to monitor the mechanical changes resulting from the treatment.

Methods: A 4.5-MHz Focused Ultrasound transducer (FUS) generated an amplitude-modulated beam resulting in harmonic tissue oscillations at its focus. Axial tissue displacement was estimated using 1D cross-correlation of RF signals acquired with a confocally aligned, 7.8-MHz diagnostic transducer (P12-5, ATL) using a plane-wave beam sequence at a framerate of 1 kHz. Imaging was performed with 0.2 s sonication for each scan position, when treatment required 60-s long sonications which were shown to generate lesion in this model according to previous work by our group. KPC mice were genetically-engineered to develop pancreatic tumors with pathophysiological and molecular features similar to those of human PDA. Pancreatic tumor growth was monitored for 15 days with HMI scans performed every two days. For the second part of the study, HMIFU was performed on 5-mm tumors. The success of the treatment was assessed by measuring both the tumor area and its elasticity up to 14 days.

Results: HMI demonstrated its capability to provide reproducible elasticity measurements in murine pancreatic tumors. Figure 1 shows that stiffening occurs progressively during pancreatic tumor growth from the very early stages. When plotting the HMI displacement against the tumor size, an exponential trend was fitted to the data with $R^2 > 0.87$ in accordance to general models. When ablated with HMIFU for 60s, the tumor stiffened displaying a decrease in HMI displacement (Figure 2). The lesion was confirmed by histology. The follow-up of the HMIFU treatment is performed with HMI to assess long-term tissue mechanical changes after treatment.

Conclusions: These results demonstrate the capability of HMI to provide elasticity measurements in the KPC murine pancreatic tumor model. The technique monitored the tumor growth as well as its stiffening when treated with HMIFU. Post-treatment mechanical changes could also be assessed with HMI. This study underlines the potential of HMI for monitoring tumor growth, treatment and follow-up changes in elasticity.
Preliminary investigation of integrating deployable reflectors and fluid lenses with endoluminal ultrasound to enhance and dynamically adjust focal gain and depth.

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Objectives: Endoluminal and endovascular catheter-based high-intensity ultrasound offer spatially-controlled thermal ablation in tissue targets adjacent to body lumens, such as the pancreas, liver, prostate, heart, etc. Due to the size constraints necessary for applicators to traverse through luminal passages, the integrated therapeutic transducers have minimal aperture size, limiting the depth of energy and thermal penetration typically to within a few centimeters beyond lumen borders. This study introduces concepts for a deployable applicator that features inflatable balloon reflectors and adjustable fluid-lenses to permit compact delivery and expansion at target sites (e.g. stomach, bladder, colon) to augment the effective therapeutic aperture for deeper and more selective heating generation. A preliminary theoretical analysis of potential designs, incorporating acoustic and thermal parametric studies, was performed along with benchtop proof-of-concept experiments as an initial investigation of the feasibility and capabilities of this design strategy.

Methods: Two applicator designs, representing end-firing and side-firing configurations, were conceptualized and modeled, as shown in Figure 1. The end-firing configuration consists of an array of tubular transducers surrounded by an expandable conical balloon that reflects acoustic energy into a fluid lens at the applicator’s distal tip. The side-firing configuration consists of an array of planar and tubular transducer segments surrounded by a parabolic reflector balloon that diverts energy through a fluid lens adjacent to the assembly. Acoustic simulations of the two assemblies were performed using a MATLAB implementation of the rectangular radiator method and incorporation of reflection/refraction of wave-fronts at material interfaces through the method of secondary sources. Thermal modeling, implemented in COMSOL, was used to generate resulting temperature distributions in homogenous and heterogenous tissue models. Parametric studies were performed to investigate performance of different potential lens fluids, and to evaluate acoustic focal gain and heating as functions of applicator/reflector/lens dimensions and geometries. Hydrophone beam plot measurements were made for a proof-of-concept end-firing applicator, consisting of a mounted tubular transducer centered in a conical brass reflector and covered by a perfluorocarbon fluid lens with adjustable radius-of-curvature (ROC).

Results: Of the three potential lens fluids investigated (perfluorocarbon, silicone oil, and chloroform), perfluorocarbon offers the best acoustic transmission at lens focal lengths ranging from 0-100 mm, due to its greater speed-of-sound mismatch as compared to water or tissue. As demonstrated in Figure 2, simulated achievable focal gain for either device configuration increases as a function of lens focal length to a maximum between ~20-50 mm, depending on the overall deployable reflector/lens size and tissue attenuation. Thermal simulations in homogenous muscle tissue with the end-firing configuration indicate capabilities of creating localized (~1 cm long X 5 mm wide) or volumetric (~3 cm long X 2 cm wide) ellipsoid thermal lesions at variable depths up to and beyond 5-6 cm in the tissue, as demonstrated in Figure 3. Hydrophone beam plots of the preliminary proof-of-concept assembly illustrated capability of dynamically adjusting the focal plane depth between ~1-5 cm by adjusting the lens ROC, as shown in Figure 4.

Conclusions: Preliminary theoretical and experimental investigations illustrate capabilities of using reflector and fluid lens assemblies coupled with endoluminal ultrasound to produce deeper and dynamically-variable acoustic focuses and thermal lesions.

Objective: To investigate the feasibility of integrating deployable reflectors and fluid lenses with endoluminal ultrasound for enhanced and dynamically adjustable focal gain and depth.

Methods: Two applicator designs for end-firing and side-firing configurations were conceptualized and modeled. Acoustic simulations were performed using MATLAB, and thermal modeling in COMSOL was used to investigate performance with different lens fluids. Hydrophone beam plots were taken for a proof-of-concept end-firing applicator.

Results: Perfluorocarbon fluid was found to offer the best acoustic transmission. Focal gain increases with focal length, reaching a maximum between 20-50 mm, depending on the overall deployable reflector/lens size. Thermal simulations demonstrated the capability to create localized or volumetric thermal lesions at variable depths.

Conclusions: The preliminary investigations illustrate the feasibility of using deployable reflectors and fluid lenses with endoluminal ultrasound for deeper and more selectively heated therapeutic effects.
Endoluminal ultrasound applicators with deployable balloon reflectors and adjustable fluid lenses, in either A) end-firing or B) side-firing configurations.

Intensity gain along the central longitudinal axis for the end-firing assembly as a function of lens focal length, as simulated in water. The 1.5 MHz tubular transducer was 8 mm OD x 20 mm height, leading to a 45 mm reflector/lens aperture diameter.

Simulated temperature distributions and 52 °C contour (black) in muscle tissue using the end-firing assembly, with specifications as in Figure 2. Sonication duration was for a) 10 s and b) 120 s at 5 W/cm² constant surface intensity input.

Hydrophone pressure-squared contours for the proof-of-concept end-firing assembly, showing tight focusing capabilities at two distinct lens focal lengths, a) ~20 mm and b) ~35 mm.
**Concurrent low dose Focused Ultrasound with chemotherapy in abdominal tumors**

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**Objectives:** Systemic therapies for advanced stage IV abdominal tumors as standard chemotherapy regimens usually lack efficacy, and often this is substantially reduced by means of toxicity. Hyperthermic therapies have demonstrated efficacy alone by themselves and as well as concurrent treatments along with chemotherapy and/or radiotherapy. Focused ultrasound has been increasingly used as a method to enhance drug delivery. Several previous experiences have used this approach to improve tumor control in different types of tumors and with different chemotherapy drugs.

**Methods:** This is a feasibility study to increase delivery of the chemotherapy drug to the tumor. We have employed Ultrasound guided High Intensity Focused Ultrasound (USgFUS) along with concurrent standard chemotherapy treatments in a clinical setting with abdominal tumors. We have included advanced stage IV liver metastases from colorectal tumors, advanced stage IV pancreatic tumors and advanced stage IV retroperitoneal sarcomas. All patients were previously treated with and failed at least to 2 chemotherapy lines. Patients should not be candidates for tumor ablation. Patients will receive 3 FUS treatments concurrent with their chemotherapy infusions. FUS therapies are delivered the day of the chemotherapy infusion, the day before and the day after the chemotherapy. FUS treatments are on the range of 100 Watts for 45 minutes average. The objective is to cover maximum volumetric treatment.

**Results:** We measure performance status, complications rate, quality of life, symptoms control, pain control, and time to progression. Blood samples for functional studies and tumor markers as well as immunological studies: lymphocyte profile, immunoglobulines, complement, CRP were taken at daily basis during FUS treatments. MRI images were obtained previously and immediate after FUS treatments.

**Conclusions:** Study is in progress. We will report updated results at the meeting.
Clinical experience of intra-operative High Intensity Focused Ultrasound in patients with colorectal liver metastases. Results of a Phase II study.

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Objectives: Managing colorectal liver metastases (CLM) is a major clinical challenge, and surgery remains the only potentially curative treatment. High intensity Focused Ultrasound (HIFU) has been proven effective in a wide range of clinical applications. However, extracorporeal treatment of the liver is difficult because presence of the ribcage may stop propagation of ultrasound waves and respiratory motion may cause targeting problems. HIFU treatment of CLM needs to be improved, and reducing the duration of surgical intervention by increasing the size of ablated fields is particularly important. A HIFU device enabling destruction of larger liver volumes has been developed based on toroidal transducers. Preliminary in vitro and preclinical work demonstrated the potential, feasibility and safety of such HIFU ablations. Such preclinical work is now translated into clinical practice. The aim of this study was to assess the feasibility, safety and accuracy of HIFU ablation in patients undergoing hepatectomy for CLM.

Methods: This study was a prospective, single-centre phase I/II study. The transducer has a toroidal shape. The diameter of the transducer was 70 mm and was divided into 32 ultrasound emitters of 0.13 cm2. The operating frequency was 3 MHz. The radius of curvature was 70 mm. A 7.5 MHz ultrasound imaging probe was placed in the centre of the device. The imaging plane was aligned with the HIFU focal zone. Nineteen patients were included in Phase I-IIa. In each patient two HIFU ablations were placed in a target previously identified in ultrasound images (step 1) and then at distance (step 2) from a target. Eight patients were included in Phase IIb until now and ten metastases were treated. HIFU ablations were created to ablate metastases (20 mm maximal diameter) with safety margins in all directions. The exposure time varied from 40 seconds to 370 seconds according to the diameter of the metastases to be treated. In order to demonstrate the safety and efficacy of rapidly ablating liver metastases, ablations were performed within the areas scheduled for resection. This study is registered with Clinical-Trials.gov (NCT01489787).

Results: The dimensions of ablations measured on ultrasound imaging were correlated (r=0.88, p<0.001) with dimensions measured during histological analysis. The average dimensions of HIFU ablations obtained in 40 seconds (Phase I) were a diameter of 21.0 ± 3.9 mm and a long axis of 27.5 ± 6.0 mm. The phase IIa study showed both that the area of ablation could be precisely targeted on a previously implanted metallic mark and that ablations could be created deliberately to avoid such a mark. Ablations were achieved with a precision of 1-2 mm. In Phase IIb, one metastasis of 10 mm in diameter was ablated in 40 seconds with safety margins. Using electronic focusing nine metastases of 2 cm in diameter were ablated with safety margins (> 3 mm in all directions) in 370 seconds. The dimensions of these HIFU ablations were a diameter of 48 ± 4.9 mm and a long axis of 51 ± 3.4 mm. No damage occurred to neighbouring tissues. Two patients had a post-operative complication (pneumonia with pleural effusion and a urinary tract infection) not related to the HIFU treatment. There were no substantive changes in hemodynamic and respiratory parameters.

Conclusions: This HIFU device safely achieved large volume liver ablations in short time, with a precision of one to two millimetres. HIFU ablations of small metastases (< 20 mm) were successfully created with planned safety margins of at least 3 mm in all directions. This study is the first clinical use of intra-operative HIFU in patients with CLM. Reducing the risk of potential important complications associated with an extracorporeal approach was among the reasons we developed an intra-operative device. An open procedure seems also more appropriate since 15-20% of additional metastasis are discovered intraoperatively. The size of the device does not limit ease of use. It was possible to visualize about 90% of the liver and all major anatomical structures with the integrated imaging probe. From these preliminary results, intra-operative use of a HIFU toroidal transducer appears feasible, safe and effective in ablating liver metastases but this needs to be confirmed.
[18F]-FDG PET and contrast MRI for enhanced guidance of Focused Ultrasound ablation in a syngeneic orthotopic model of murine pancreatic adenocarcinoma

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Objectives: Pancreatic ductal adenocarcinoma (PDA) is the most lethal cancer and there is a desperate need for new therapeutic strategies. Due to its characteristic hypovascularity, dense stromal architecture, rapid progression, and local invasiveness, conventional therapies fail to cure PAC. Magnetic Resonance guided Focused Ultrasound ablation (MRgFUS) offers a platform to build combination therapies due to its ability to debulk tumors, enhance delivery of chemotherapeutics, and stimulate an immune response. Here, the feasibility of MRgFUS is evaluated in a syngeneic orthotopic mouse model of PAC developed from the KrasLSL-G12D/+; Trp53LSL-R172H/+; Pdx-Cre (KPC) model 1. Further, [18F]-FDG positron emission tomography (PET) is used to enhance guidance.

Methods: Murine mT4-2D cells (gift from the Tuveson laboratory, Cold Spring Harbor, NY) were injected orthotopically by a sterile laparotomy (n=5, C57BL/6 female mice). Mice were treated with MRgFUS on day 16 post-implantation with an MR-compatible annular array (Imasonic SAS, 3 MHz center frequency, 4.7 Watt acoustic power, 5.6 MPa peak negative pressure, 0.5 x 0.5 x 1.5 mm3 focal volume) and positioning system (Image Guided Therapy) in a Bruker BioSpec 7T. Tumors were ablated with a diameter of 2 mm and scanning speed of 1 revolution per second for ~40s. In a separate group (n=6), on days 10 (n=3 + 1 wild-type) and 14 (n=3 + 2 wild-type), mice were injected with 200 uCi of [18F]-FDG, rested for 30 minutes, and scanned with a Siemens Inveon DPET for 30 minutes. Following PET acquisition, T1w images were acquired post gadolinium contrast (TE/TR/FA = 11.7ms/750ms/180°, 4.3 x 4.3 cm2 FOV, 256 x 256 matrix, 17 slices). Mice were euthanized, organs of interest were harvested, and radioactivity was measured by Wizard 1470 Automatic Gamma Counter (PerkinElmer). Maximum intensity projections were registered with MR images and regions of interest (ROIs) were drawn on the tumors and normal pancreases using Siemens Inveon Research Workplace.

Results: [18F]-FDG-PET images enhanced the visualization of the tumor rim and demonstrated the heterogeneity of uptake within the tumor (Figure 1A). In spite of the heterogeneity, ROI analysis indicated that primary pancreatic lesions (60.6 %ID/cc) exhibit a higher maximum uptake of [18F]-FDG than the normal pancreas (17.8 %ID/cc) at day 14 of tumor progression (p<0.05). Biodistribution confirmed these measurements, with 15.5 %ID/g and 16.9 %ID/g in the tumor-bearing pancreas at day 10 and day 14, respectively, as compared with 5.2 %ID/g in the normal pancreas (p<0.05). Contrast enhanced T1w MRI facilitated visualization of lesions within both the pancreas and spleen (Figure 1B-C). MRgFUS ablation was successfully completed under MR guidance (Figure 1B), mice recovered well from treatment and the effect was confirmed on histopathology at 24 hours (Figure 1D).

Conclusions: [18F]-FDG facilitated visualization of the tumor rim. Feasibility of MRgFUS ablation of a syngeneic orthotopic murine model of PAC was established. Future studies will combine [18F]-FDG-PET with MRgFUS to guide therapies and monitor disease progression.
Early experience with HIFU in the United States at Vituro Health, Sarasota

Stephen Scionti
Scionti Prostate Center, Sarasota, Florida, United States

Objectives: Not released for publication
Methods: Not released for publication
Results: Not released for publication
Conclusions: Not released for publication
MRI-guided transurethral ultrasound ablation (TULSA) of localized prostate cancer: 24-month follow-up of a prospective Phase I study

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1 UT Southwestern Medical Center, Dallas, Texas, United States
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6 Profound Medical Inc., Toronto, Ontario, Canada

Objectives: MRI-guided transurethral ultrasound ablation (TULSA) is a novel minimally-invasive technology for the ablation of benign and malignant prostate tissue, which aims to provide good control of local disease with low morbidity. A prospective, multi-national Phase I study was performed with objective to determine safety and feasibility of MRI-guided TULSA.

Methods: Thirty patients (pts) with low-intermediate risk prostate cancer were enrolled: cT1c-T2a; PSA≤10ng/ml; GS≤3+3 (USA/Europe) and ≤3+4 (Canada). Under general anesthesia, the ultrasound device (TULSA-PRO, Profound Medical Inc.) was inserted and positioned in the prostatic urethra with MRI guidance. Treatment planning was performed under MRI visualization with therapeutic intent of conservative whole-gland ablation, including 3mm margins around the periphery, and 10% residual viable prostate expected around the capsule. Ultrasound treatment was delivered under real-time active MRI thermometry feedback control.

Primary endpoints are safety and feasibility, with follow-up to 12 months (mo). Complete clinical monitoring is 5 years, including serial PSA, TRUS biopsies, IPSS (urinary symptoms) and IIEF (erectile function).

Results: Median (IQR) age was 69 (67-71) years and PSA 5.8 (3.8-8.0) ng/ml, with 24 (80%) low-risk and 6 (20%) intermediate-risk cancers (D’Amico). Treatment time was 36 (26–44) min and prostate volume 44 (38–48) cc. Spatial control of ablation was ±1.3mm on MRI thermometry, and correlated well with the non-perfused volume confirmed on CE-MRI. Complications (CTCAE:v4) included hematuria (13pts Grade1; 2pts G2), urinary tract infections (10pts G2), acute retention (3pts G1; 5pts G2), and epididymitis (1pt G3). There were no rectal injuries or intraoperative complications. Baseline IPSS of 8 (5-13) recovered to 6 (4-10) at 3 mo (n=29), stable to 8 (5-10) at 18 mo (n=22). The proportion of patients with erections sufficient for penetration remained relatively unchanged from 21/30 (70%) at baseline to 20/29 (69%) at 12 mo and 15/22 (68%) at 18 mo. PSA decreased 87% to 0.8 (0.5–1.1) at 1mo (n=30), stable to 0.7 (0.4–1.5) at 24mo (n=18). MRI at 12 months shows diminutive prosstates with median volume reduction of 88% (83-95%), consistent with the near whole-gland treatment plan. Positive biopsies at 12 months show 61% reduction in total cancer length, clinically significant disease in 9/29 patients (31%), and any disease in 16/29 patients (55%).

Conclusions: MRI-guided TULSA provides accurate treatment planning, real-time thermal dosimetry and precise control of prostate ablation, with well-tolerated side-effects. Study results support the primary objectives of conservative whole-gland prostate ablation. A larger trial with reduced treatment margins is scheduled to begin in 2016.
Investigation of the Therapeutic Effects of Pulsed Focused Ultrasound Combined with Encapsulated Chemotherapeutic Agents for Treatment of Prostate Cancer in vivo

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Fox Chase Cancer Center, Philadelphia, Pennsylvania, United States

Objectives: To investigate the improvement of prostate cancer inhibition by combining pulsed Focused Ultrasound (pFUS) exposures and chemotherapeutic agents encapsulated nanodroplets under MR guidance for prostate cancer therapy.

Methods: Prostate cancer (LNCaP) cells were implanted orthotopically (Figure1). First we developed nanodroplets encapsulated with chemotherapeutic agents for both paclitaxel (PTX) and docetaxel (DTX). Secondly, tumor–bearing mice were randomly divided into 5 groups (n=5). Group 1 animals were treated with an i.v injection of DTX-encapsulated nanodroplets (DTX-ND) + pFUS. Group 2 were treated with pFUS alone. Group 3 were injected (i.v) with DTX-ND alone, Group 4 received free DTX and Group 5 was used as control. Ultrasound treatment parameters were 1MHz, 25W acoustic power, 10% duty cycle and 60 seconds for each sonication. After treatment, animals were allowed to survive for 4 weeks. Tumor volumes were measured on MRI. Third, we repeated the experiment with PTX-ND. Finally we performed study on biodistribution of PTX-ND for prostate cancer and the treatment effects on tumor growth delay are being evaluated.

Results: With DTX-ND, significant tumor growth delay was observed in Group 1 with p=0.039. There was no significant tumor growth delay observed for Group 2 (p=0.477), Group 3 (p=0.209) and Group 4 (p=0.476) (Figure2). The results are consistent with earlier PTX-ND studies in which significant tumor growth delay was observed in Group 1 with p=0.004. There was no significant tumor growth delay observed for Group 2 (p=0.285), Group 3 (p=0.452) and Group 4 (p=0.158) (Figure 3). The peak of drug update in tumor appeared at 4h after injection in the biodistribution study.

Conclusions: Our results showed a great potential of targeted nanodroplets for prostate cancer therapy, which could be activated by pFUS. Our study also suggested that the optimal timing for applying pFUS is 4h after i.v injection of PTX-ND and the treatment effect is being evaluated.
Monitoring histotripsy ablation with passive cavitation imaging

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²University of Washington, Seattle, Washington, United States

Objectives: Histotripsy is a form of therapeutic Focused Ultrasound that mechanically ablates tissue. Pre-clinical studies have explored the potential for histotripsy to treat fetal septal defects, deep vein thrombosis, liver cancer, and benign prostatic hyperplasia (BPH). Histotripsy technology is also being tested in a clinical trial for the treatment of BPH. The bubble clouds generated by histotripsy are hyperechoic, enabling standard B-mode ultrasound imaging to be used for image guidance of tissue ablation. The mechanical oscillations of the bubbles within the cloud generate acoustic emissions. Information about the amplitude and location of these acoustic emissions would provide an additional means to monitor the treatment progress from histotripsy. Passive cavitation imaging (PCI) is an ultrasound imaging modality currently under development that spatially maps the power of acoustic emissions generated by cavitation. In this study, the ability of PCI and plane wave B-mode imaging to predict the location and spatial extent of histotripsy

Methods: Histotripsy pulses were generated in a prostate tissue phantom with a 1-MHz, 8 annular element array with a 10-cm aperture and 9-cm focal length. All elements of the transducer were simultaneously driven in parallel by a custom class D amplifier and matching network. Histotripsy pulses over a range of pulse durations (5-20 microseconds) and peak negative pressures (12-23 MPa) were explored in this study. During histotripsy insonation of the phantom, a 128 element linear array controlled with an ultrasound research scanner was used to monitor cavitation activity with both PCI and plane wave B-mode imaging. After application of the histotripsy pulses, the phantom was sectioned and stained with a periodic acid-Schiff stain to delineate the ablation zone. The passive cavitation images and plane wave B-mode images were co-registered with the histological images. A receiver operating characteristic curve was used to quantify the comparison between each imaging modality and the spatial extent of the ablation zone.

Results: The area under the receiver operating characteristic (AUROC) was significantly greater than 0.5 for both plane wave B-mode and PCI, indicating both approaches can be used to predict the spatial extent of the ablation zone. The AUROC and accuracy were significantly greater for PCI compared to plane wave B-mode imaging. Furthermore, the sensitivity for predicting lesion formation was a factor of 3 larger for PCI compared to plane wave B-mode imaging.

Conclusions: Overall, these results indicate PCI provides an improved prediction of lesion formation from histotripsy.
Real time Tissue Changes Monitoring during the treatment of prostate cancer with HIFU

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\textsuperscript{2}Ulthera, Inc., Mesa, Arizona, United States

Objectives: Backscattered RF ultrasound signals have been used clinically in differentiating normal and infarcted heart condition. Similarly, backscattered signals from pre and post hifu site are significantly different in magnitudes that provide correlation on degree of tissue changes. This is a validation study of Tissue Change Monitoring (TCM) algorithm performed with real-time thermometry. TCM performs spectral analysis of the RF backscattered (pulse-echo) ultrasound signals acquired before and immediately following the HIFU exposure. The RF signal analysis generates energy spectra of these signals and the difference of the energy magnitude is used as an estimator for tissue changes caused by the HIFU tissue ablation. TCM results are color coded and displayed on the each HIFU ablative site in Orange, Yellow and Green indicating tissue temperatures in the range from 75-100, 60-75 and 37-60 degrees C respectively.

Methods: Five (5) patients with histologically confirmed, organ confined prostate cancer were enrolled with approved protocol for this study. Four patients with focal cancer at stereotactic saturation biopsy had hemiablation only and one had a whole gland ablation. The Sonablate 500 HIFU device with Tissue Change Monitoring (TCM) software was used for the ablative treatment. Specifically designed 18 gauge needles containing three thermocouples per needle separated by a 1cm distance were placed transperineally under TRUS guidance in the prostate. 4 or 5 needles were placed to cover the ablative sites. Temperatures from all thermocouples were recorded simultaneously at 0.5 seconds interval by a multi-channel thermometry system. Temperature data were acquired from a total of 56 sites,(26 focal zone sites, 22 posterior sites to the focal zone and 8 sites in the lateral gland of the prostate where there was no HIFU applied). Sixteen RF signals lines, pre and immediately post HIFU, for each ablative site were acquired at 50 MHz sampling rate. All RF data lines were processed by the SB-500 computer in the frequency domain to derive average energy spectra and change in magnitude for pre and post HIFU. The magnitude change and temperature from the site were used to derive cross correlation function and color code to overlay on the ablative site.

Results: The measured temperatures (Average, Max, and Min) in the HIFU treatment zones were 84, 114 and 70 degree C respectively. TCM energy readings were 1.05, 2.6 and 0.4 resulting in 83 % orange (75-100 degree C) and 17% yellow (60-75 degree C) indicating an estimated average temperature of 91 degree C. Outside the focal zone, average recorded temperature was 50 degree C. The temperature recorded in the lateral lobe where no HIFU was applied was 40.7 degrees C.

Conclusions: The backscattered RF data analysis is capable of estimating tissue changes reliably during the HIFU procedures in real-time and can be used as an aid in the operating procedure. TCM was found highly sensitive to tissue motion. When the tissue reached boiling temperature, TCM output resulted in higher energy and was beyond the linear range of temperature monitoring from the RF backscattered signals.
MRI-US fusion guided High-Intensity Focused Ultrasound with Focal-One® system: Impact on PSA, complications and genito-urinary functions during initial experience

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²Institut Mutualiste Montsouris, Paris, France

Objectives: We report our initial experience in the treatment of prostate cancer (PCa) with high-intensity Focused Ultrasound (HIFU) using MR-US fusion guided Focal-One® system.

Methods: Between June 2014 to October 2015, 85 patients underwent HIFU (focal / whole-gland) treatment for localized PCa with low and intermediate disease.

Preoperative cancer localization was done with multiparametric Magnetic Resonance Imaging (mpMRI) and transrectal ultrasound–guided biopsy.

Treatment was carried out using the Focal-One® HIFU system under general anesthesia.

Oncological follow-up includes PSA measurement (1,3, 6 and 12 months) and control biopsy with mpMRI at 1yr after treatment. Questionnaire-based functional outcome assessment (Pre-op and 1,3,6 and 12 months) was done.

Complications were reported as Clavien-Dindo grade.

Results: The study cohort had a median age -70 yr (IQR 66-77 yr), and median PSA - 7.79 ng/ml (IQR 6.32-9.16 ng/ml). Median total cancer length was 7.50 mm (IQR: 4.25-14.50 mm). Biopsy characteristics are summarized in table 1. Mean prostate volume was 40 cc (range 15-70). Suspected lesions were observed in 71 pre-treatment mpMRI. Pre-treatment mpMRI. Pre-operative TURP was performed in 22 patients. Focal and whole-gland therapy was performed in 64 and 21 patients respectively. Ten patients received salvage HIFU. Complications were encountered in 15% of cases, all Clavien II graded. Mean hospital stay was 1.8 d (range 0-7) and bladder catheter was removed on day 2 (range 1-6). Mean percentage reduction of PSA was 53% for first 40 cases and 56% for last cases. Nine patients had higher PSA post treatment. Median follow-up was 3 mo (IQR: 2-8 mo). At last follow-up, 8 patients had protocol control biopsy, 4 having residual disease in the treated area. Functional outcomes: all patients were continents at 3 mo and potency was maintained in 83% of preoperatively potent patients.

Conclusions: Focal-One® HIFU treatment appears to be a safe procedure with few low grade complications. Functional outcomes proved no urinary incontinence and sexual function were maintained in 83%.

Table 1-Biopsy characteristics, no. (%)

<table>
<thead>
<tr>
<th>Variable</th>
<th>n=85</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gleason score</td>
<td></td>
</tr>
<tr>
<td>3+3</td>
<td>52 (61)</td>
</tr>
<tr>
<td>3+4</td>
<td>30 (35)</td>
</tr>
<tr>
<td>4+3</td>
<td>2 (3)</td>
</tr>
<tr>
<td>4+4</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Tumor Side</td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>29 (34)</td>
</tr>
<tr>
<td>Left</td>
<td>43 (51)</td>
</tr>
<tr>
<td>Bilateral</td>
<td>13 (15)</td>
</tr>
<tr>
<td>Localization</td>
<td></td>
</tr>
<tr>
<td>Base</td>
<td>30 (35)</td>
</tr>
<tr>
<td>Medial</td>
<td>20 (24)</td>
</tr>
<tr>
<td>Apex</td>
<td>13 (15)</td>
</tr>
<tr>
<td>Anterior</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Multiple one-side</td>
<td>8 (10)</td>
</tr>
<tr>
<td>Bilateral</td>
<td>13 (15)</td>
</tr>
</tbody>
</table>
High Intensity Focused Ultrasound hemiablation versus MRI guided 'lesion only' ablation of prostate cancer — genito-urinary functional outcomes and complications

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²Institut Mutualiste Montsouris, Paris, France

Objectives: Focal therapy (FT) for Prostate Cancer (PCa) aims to gradually shift from subtotal/hemiablation (HA) towards image guided 'lesion-only' ablation (LOA). We compared complications and early functional outcomes of High Intensity Focused Ultrasound (HIFU)-HA versus HIFU-LOA

Methods: We queried our prospectively maintained FT for PCa database (2005 – 2015) of 291 patients and identified 88 patients of HIFU-HA and 47 patients of HIFU-LOA. Patients eligible for FT were offered HIFU-HA (Ablatherm) between 2009 – 2013 which involved ablation of the cancer containing lobe. MRI fusion HIFU (FocalOne) was introduced since July 2014 after which all the eligible FT patients underwent HIFU-LOA defined as MRI-US fusion targeted ablation of the cancer 'lesion only' with a safety margin of 8-9mm. Due to shorter follow-up of LOA group, we compared complications and early genitourinary functional outcomes (pre-op and post-op – 1 month) between the two groups - using validated questionnaires (IPSS, ICS and IIEF).

Results: The clinical and cancer characteristics of the study population are shown in Table 1. We treated significantly larger cancer volumes in LOA group. There was no significant rise in IPSS score after the treatment in both groups and the mean IPSS rise (4.5 vs 3.7, p - 0.8) was also similar between them. HIFU-HA had significantly higher incontinence rate (15.9% vs 5.1%, p - 0.04) and decrease in the IIEF score (6.2 vs 4.2, p – 0.07) as compared to LOA. Complications and functional outcomes data are shown in table 2. The complication rates were similar between the groups, but LOA was not associated with any clavien grade 3 complications.

Conclusions: Our initial experience of HIFU-LOA had demonstrated significantly lower incontinence rates and better erectile function preservation compared to HIFU-HA. However, the oncological outcomes of LOA group are awaited.

Table1: Clinical and cancer characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>HIFU-HA</th>
<th>HIFU-LOA</th>
<th>p - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>88</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>Mean age (SD) years</td>
<td>68 (6.9)</td>
<td>67.6 (7.3)</td>
<td>0.8</td>
</tr>
<tr>
<td>Mean BMI (SD)</td>
<td>23.9 (5.9)</td>
<td>25.1 (5.8)</td>
<td>0.8</td>
</tr>
<tr>
<td>Mean PSA (SD) ng/ml</td>
<td>7.1 (2.9)</td>
<td>7.7 (2.6)</td>
<td>0.2</td>
</tr>
<tr>
<td>Mean Prostate volume (SD) cc</td>
<td>37.2 (12.2)</td>
<td>40.3 (11.6)</td>
<td>0.8</td>
</tr>
<tr>
<td>Median percent of positive cores</td>
<td>12.4</td>
<td>19.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Median percent of positive core length</td>
<td>4.9</td>
<td>8.6</td>
<td>0.01</td>
</tr>
<tr>
<td>Gleason Score (%)</td>
<td></td>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td>6 (3+3)</td>
<td>73 (83)</td>
<td>32 (68)</td>
<td></td>
</tr>
<tr>
<td>7 (3+4)</td>
<td>15 (17)</td>
<td>15 (32)</td>
<td></td>
</tr>
<tr>
<td>Median pre-op IPSS score (SD)</td>
<td>5.8</td>
<td>3.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Median pre-op IIEF score (SD)</td>
<td>17.8</td>
<td>22.6</td>
<td>0.1</td>
</tr>
<tr>
<td>Number of incontinent patient (%)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
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</table>
Table 2: Functional outcomes and complications

<table>
<thead>
<tr>
<th>Variable</th>
<th>HIFU-HA</th>
<th>HIFU-LOA</th>
<th>p - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median hospitalization time days</td>
<td>3.1</td>
<td>1.7</td>
<td>0.1</td>
</tr>
<tr>
<td>Median catheterization time days</td>
<td>2.9</td>
<td>2.4</td>
<td>0.5</td>
</tr>
<tr>
<td>Mean follow-up (SD) months</td>
<td>21.1 (6.3)</td>
<td>6.3 (3.1)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Follow-up at 1 month</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median drop in PSA (ng/ml)</td>
<td>2.5</td>
<td>2.5</td>
<td>0.7</td>
</tr>
<tr>
<td>Median increase in IPSS</td>
<td>2.4</td>
<td>2.2</td>
<td>0.8</td>
</tr>
<tr>
<td>Number of incontinent patients (%)</td>
<td>14 (15.9)</td>
<td>2 (5.1)</td>
<td>0.04</td>
</tr>
<tr>
<td>Median drop in IIEF</td>
<td>4.2</td>
<td>2</td>
<td>0.07</td>
</tr>
<tr>
<td>Complications (%)</td>
<td>12 (13.6)</td>
<td>3 (6.8)</td>
<td>0.2</td>
</tr>
<tr>
<td>Complications requiring hospitalization (%)</td>
<td>7 (8)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Complications requiring intervention (%)</td>
<td>2 (2.3)</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>
Focal therapy with High Intensity Focused Ultrasound in low risk prostate cancer

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²Klinikum Muenchen Harlaching, Munich, Germany
³Clinica Columbus, Milano, Italy

Objectives: Prostate cancer (PCa) therapy undeniably exposes patients to a risk of impotence and incontinence. Therefore, low-risk localized prostate cancer is increasingly managed with watchful waiting or active surveillance, although this management bears some psychological burden and the risk of under staging and under grading. Alternatively, High Intensity Focused Ultrasound (HIFU) therapy allows non-invasive partial ablation of detected PCa in a single session (focal therapy). Aim of this study was to evaluate clinical efficacy and side effects of a single focal HIFU session in low risk PCa patients.

Methods: 66 patients with localized prostate cancer who refused both definitive radical therapy and conservative management and had a strong desire to preserve potency were treated with focal HIFU. Men with strong obstructive symptoms, severe calcifications in the target area or severe prostatitis/abscess history were excluded. Treatments were performed with Ablatherm® integrated imaging.

Results: 53% of the patients had positive staging biopsies on the right, 47% on the left lobe. Initial PSA (PSAi = at diagnosis) was median 5.58 (1.02-10.43) and increased to a PSA at treatment (PSAtr) of median 6.01 (0.2-11.4) within 4 months (period of first diagnosis until HIFU treatment), corresponding to a median pretherapeutic PSA velocity of 1.29 ng spinal anesthesia in median 50 (27-75) min with 16 french urethral catheter in place. Applied HIFU lesions were median 138 (86-338). There were no significant therapy related intra-/postoperative side effects within the follow-up period of median 4.4 (0.25-9.85) years, according to CLAVIEN Score. Based on patient interviews 3 months after HIFU treatment potency was: worse (4.5%), equal (93.5%); better (0%). PDE-5 inhibitors were used by 74% of patients. Urinary continence was preserved in all cases, 3 patients used a safety pad before and after treatment. There were no rectal disorder, fistulae or rectal incontinence. Post-HIFU catheter time was 22.7% 5 days occurred. No late HIFU induced side effects were observed. PSA was reduced from median PSAtr (6.45) to a median Nadir of 1.45 ng/ml within median 12.5 weeks and showed a trend to relapse with a post-HIFU PSA velocity.

Conclusions: Focal HIFU - a non invasive single session therapy performed under spinal anesthesia - demonstrated high potency- and continence preservation while treating diagnosed prostate cancer lesions. There were no significant therapy related intra-/postoperative side effects within the follow-up period. Focal HIFU results in a significant deceleration of PSA velocity from pre-HIFU 1.29 to post-HIFU 0.12 ng/ml/year by treating only 25% of the prostatic volume. PCa diagnosis and consecutive watchful waiting and active surveillance bears an imminent psychological burden for the patient. The option of therapeutic efficacy, the wish to avoid therapeutic side effects and to preserve potency as well influences patients’ decision for focal therapy with HIFU.
High Intensity Focused Ultrasound in primary localized prostate cancer applicative evolution in 20 years of clinical practice

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Objectives: We report the long-term cancer control and morbidity of HIFU with neoadjuvant transurethral resection of the prostate (TURP), risk of metastatic induction and evolution of HIFU over a period of 20 years.

Methods: Monocentric Harlaching HIFU database was searched for patients with primary localized PCa (T1-2, N0, M0, PSA < 50 ng/ml) with follow-up >1 year; patients with previous long-term ADT, PCa therapy, or any PSA-influencing therapy were excluded. All patients were treated with Ablatherm® HIFU devices; HIFU retreatment was offered to patients with biopsy-confirmed residual or recurrent PCa. Evaluation was performed retrospectively, and by stratification according to cohort group, risk group (D’Amico criteria), PSA Nadir, and Gleason score. Phoenix definition was used for biochemical failure. Statistical analysis was performed using the Kaplan-Meier method, and univariate and multivariate analysis employing a Cox model.

Results: Of 704 study patients, 78.5% had intermediate- or high-risk disease. Mean (range) follow-up was 5.3(2–14) years. 2nd HIFU within the follow up was performed in 22%. Cancer-specific survival was 99%, metastasis-free survival was 95%, and 10-year salvage treatment-free rates were 98% in low-risk, 72% in intermediate-risk, and 68% in high-risk patients. Predicting factors for biochemical failure were PSA Nadir and Gleason score.

Conclusions: Long-term follow-up after HIFU therapy found a high overall rate of cancer-specific survival and a remarkable high salvage free rate in low-risk patients. Advances in technology and practice and the use of neoadjuvant TURP allow the treatment of patients with any prostate size and tissue morphology.
Local radical tumor ablation through combined transurethral resection and transrectal High Intensity Focused Ultrasound: A valid therapy to treat high risk prostate cancer?

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Objectives: Monocentric prospective long term cohort evaluation regarding therapeutic efficacy and side effects of combined transurethral prostate resection (TURP) combined with High Intensity focussed ultrasound (HIFU) ablation as “local radical ablation” of High Risk Prostate Cancer (hrPCa).

Methods: 480 hrPCa patients (T3-4 or initial PSA >20 or Gleason 8-10, N0, M0) were treated with TURP and consecutive transrectal complete HIFU Ablation with Ablatherm Integrated Imaging® (EDAP-TMS, Lyon-France) and followed prospectively up to 17 years. Disease progression (=therapy failure) was defined as: Last PSA>initial PSA (PSAi) or M +/- or onset of Salvage Therapy.

Results: In a high risk PCa patient cohort with a median follow up of 7 years (0.6-17.8) over all survival was 71.7%, metastasis free survival 91%, cancer specific survival 92.9% and progression free survival 63%. 30% needed adjuvant salvage ADT and 7% other salvage therapies in follow up after median 1.5 (0.1-12.5) years. Therapy induced side effects were: Stress Incontinence 7.9%, Obstruction: 3.7%, Fistula/Osteitis os pubis: 1.5%; UTI: 21.4%.

Conclusions: Local radical prostate cancer ablation through transurethral resection (TURP) and transrectal total High Intensity Focused Ultrasound (HIFU) with Ablatherm® is a valid therapy even in high risk prostate cancer patients.
Factors affecting the efficacy of the Magnetic Resonance guided Focused Ultrasound ablation for painful bone metastases: Results from a multicenter study in China

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Objectives: Bone is the third most common target organ that carcinomas metastasize to after lung and liver. Bone pain induced by cancer metastases contributes substantially to morbidity and mortality in patients with malignant carcinomas. Magnetic Resonance guided Focused Ultrasound (MRgFUS) is considered as one of the most promising therapeutic modality for tumor ablation. But, not all patients could obtain satisfied therapeutic efficacy. Some factors such as the number of bone metastasis, KPS and NPVR score can affects the results. The purpose of this non-randomized study was to evaluate the factors affecting the efficacy of the Magnetic Resonance guided Focused Ultrasound (MRgFUS) ablation for painful bone metastases treatment, and the safety and efficacy of this new non-invasive treatment modality.

Methods: One hundred and thirty one patients with painful bone metastases were screened for this study from June 2014 to September 2015. Seventy-one patients were finally enrolled in our study. All 71 patients underwent MRgFUS and none of them received radiotherapy or chemotherapy for pain palliation in the past two weeks. The Numerical Rating Scale for pain (NRS), the Brief Pain Inventory (BPI-QoL) score, the Karnofsky performance scale (KPS), morphine equivalent daily intake dose (MEDID), and the adverse events (AEs) were respectively recorded before and 1-week, 1-month, 2-month, 3-month after the treatment.

Results: 1) Seventy-one metastatic bone lesions in 71 patients were treated by MRgFUS and the treatment data was as follows: the mean treatment time was (83.94±27.87) minutes, the mean sonication number was (12.74±7.30) minutes. 2) Adverse events included: pain in therapy area in 3 patients and sciatica in 1 patient, which were obviously reduced after physiotherapy. 3) The NRS before treatment and at 1-week, 1-month, and 3-month after treatment was 6.70±1.82, 4.37±2.55, 3.96±2.83, 3.70±2.81 respectively. The NRS significantly decreased after treatment at all time points (P<0.01). 4) The BPI-QoL score before treatment and at 1-week, 1-month, and 3-month after treatment respectively was 5.70±2.09, 4.62±2.36, 4.42±2.60, and 4.42±2.70. The BPI-QoL score significantly decreased after the treatment at all time points (P<0.01). 5) After treatment, at 3-month, the mean ΔBPI-QoL score improvement in patients with solitary bone metastasis was statistically superior to that in patients with multiple bone metastases (P<0.05). 6) At 1-week and 1-month, the mean ΔBPI-QoL score improvement in patients with osteogenic bone metastasis was statistically superior to other two groups (P<0.05).

Conclusions: MRgFUS can be used as a non-invasive, safe, and effective method for treating painful bone metastases. The patients with solitary bone metastasis or osteogenic bone metastasis can achieve better therapeutic efficacy than those with multile bone metastasis or osteolytic bone metastasis.
Can MRgFUS achieve local control of bone metastases?

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Objectives: Magnetic Resonance Imaging guided Focused Ultrasound (MRgHIFU or MRgFUS) treatment of bone metastases is effective and safe for pain palliation. In a few circumstances, MRgFUS showed a partial or complete local control of the tumoral mass affecting bone. The aim of the work was to understand the efficacy of MRgFUS in terms of local tumor control of bone metastases and to explore this opportunity and the reasons for success or failure.

Methods: Patients with painful bone metastases were enrolled and submitted to MRgFUS (ExAblate 2100, InSightec Ltd, Israel), with imaging (CT/MRI) before and 3, 6 and 12 months after treatment. The primary endpoint was the number of lesions with partial or complete response at 3 months according to MD Anderson criteria.

Results: Out of 65 patients, 18 were lost and missed the 3-month imaging check. Forty-seven patients with 49 lesions were evaluated. The procedure was successful in terms of local control at 3 months in 24 lesions (49%) – complete in 11, partial in 13. A stable disease was observed in 20 lesions and a progression in 5. Lesions with osteolytic (or mixed) pattern, with complete accessibility of the margins to the ultrasound beam are perfect candidate for ablation with the intent to control the lesion. Results were statistically independent of histology (primary cancer), previous radiation therapy (59%), and size (up to 14 cm), though smaller lesions were generally more associated with complete or wide accessibility. Body mass index, age and sex of patients did not influence the outcome.

Conclusions: At present, MRgFUS for painful bone metastases may produce a local control of the lesion. In the future, patients with bone metastases should be selected for MRgFUS with three different intents, and the target should be clearly established before the treatment according to patient condition, prognosis, symptoms and to the features of the lesion: 1) palliation of pain; 2) palliation and local tumor control; or 3) ablation / local control.
Effect of heating duration on therapeutic ratio in MR-HIFU hyperthermia mediated drug delivery using thermosensitive liposomes in rabbit Vx2 tumors

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²University of Texas Southwestern Medical Center, Dallas, Texas, United States
³University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, United States

Objectives: Localized drug delivery using MR-HIFU hyperthermia to trigger the release of chemotherapeutic agents from thermosensitive liposomes (TSLs) has the potential to increase the therapeutic ratio between antitumor effect and systemic toxicity. For doxorubicin (DOX), which is active in many pediatric solid tumors, the late effects of cumulative dose to the heart are treatment-limiting. In this study, we investigate the effect of mild hyperthermia duration (10 vs. 40 minutes) on the ratio of DOX deposited in heated tumors compared to cardiac muscle, using a clinical formulation of TSL-DOX in a rabbit Vx2 tumor model.

Methods: Rabbits had Vx2 tumor cells injected into each thigh 12 days before therapy. For each rabbit, mild hyperthermia (10 or 40 minutes, 42°C) was delivered to a 10 mm diameter region in one tumor using a clinical MR-HIFU system (Sonalleve V2, Philips Healthcare) incorporated into a 3T MRI (Ingenia, Philips Healthcare). Feedback control of hyperthermia sonications was performed by modified software designed for mild hyperthermia, using MR thermometry data acquired in 6 slices every 3.2 seconds. During heating, TSL-DOX (Thermodox, Celsion Corporation) was administered intravenously at 2.5 mg/kg over 5-6 minutes. Rabbits were sacrificed and perfused with saline for tissue harvest 3 hours after the start of TSL-DOX infusion (Figure 1). Tissue DOX concentrations were quantified using liquid chromatography-mass spectrometry against a daunorubicin standard following homogenization in cell lysis buffer and extraction in silver nitrate.

Results: Vx2 tumors treated with 10 vs. 40 minutes of mild hyperthermia (n = 10 per group) had largest dimensions of 24.3 and 29.9 mm, and body temperatures of 36.6°C and 36.6°C before sonication. Heating quality in the two groups was similar: target region temperatures averaged 41.9 and 41.8°C, respectively, with T90 of 41.1 and 41.0°C, and T10 of 42.7 and 42.7°C. The average duration that target region voxels exceeded 40°C in the 10 and 40 min heating groups was 11.3 vs. 40.7 min.

In non-targeted organs (heart, lung, spleen, muscle) DOX concentrations demonstrated no significant differences between the two heating groups (ANOVA with selected Bonferroni post-tests). This indicates that systemic toxicity does not increase with heating duration (Figure 2).

In both heated and unheated tumors, tumor DOX concentrations were higher for 40 vs. 10 min (25.4 vs. 14.0 µg/g and 6.7 vs. 5.4 µg/g, Figure 3). Similarly, therapeutic ratio (DOX in tumor / DOX in heart) increased with heating duration (3.9 vs 2.2 times). Heated tumors had higher DOX concentrations and therapeutic ratios than unheated tumors (p = 0.03, 2-way ANOVA with Bonferroni). Therapeutic ratio and DOX concentration for heated tumors had a significant correlation with duration above 40°C.

Conclusions: Our results confirm the theory that longer heating duration increases doxorubicin deposition in the targeted tumor without increasing doxorubicin accumulation in critical structures such as the heart. This will have an impact on hyperthermia protocols used in clinical trials of MR-HIFU hyperthermia mediated drug delivery.
Figure 2. Doxorubicin biodistribution in rabbits following MR-HIFU hyperthermia mediated drug release.

Figure 3. Therapeutic ratio of doxorubicin concentration in tumor / heart in rabbits that underwent 10 or 40 minutes of MR-HIFU hyperthermia.
Technical and imaging parameters predicting successful Magnetic Resonance guided Focused Ultrasound treatment of painful osseous metastases

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Objectives: Magnetic Resonance guided Focused Ultrasound (MRgFUS) has proven to be effective in the treatment of painful osseous metastases. The results of the phase III randomized clinical trial comparing MRgFUS to sham treatment of bone metastases demonstrated a 64% response rate in the treatment group, compared to a 20% response rate in the sham arm (Fig. 1). We hypothesized that this intention to treat analysis included patients who were suboptimally treated. We reviewed images from the patient procedures to identify imaging features that indicated technically successful treatment, and assessed technical parameters for predictors of pain relief.

Methods: All images were anonymized such that the reviewer was blinded to the treatment outcome. Imaging features (size of tumor, osteolytic vs blastic tumor, location of tumor, T2 signal intensity, presence of edema around the tumor after treatment, devascularization of the periosteum around the tumor, tumor devascularization, etc.) and technical parameters of the treatments (number of sonications, total energy, average energy, sonication time, type of anesthesia etc.) were documented for each patient. After de-anonymization, imaging features and technical parameters were then correlated with successful pain relief (defined as at least a 2 point decline in pain score without significant increase in analgesic medication).

Results: We identified an imaging feature that we call the black band (BB), which represents devascularization of the periosteum around the tumor, as best seen on subtracted contrast enhanced T1 weighted fat saturated images obtained immediately after treatment (Fig. 2). The presence of the black band correlated with response to treatment (Odds Ratio of Complete or Partial Response with BB = 5.97; 95% CI: 1.08 – 33.0; p= 0.041; sensitivity: 96%, 95% CI: 88-99%; specificity: 22%, 95% CI: 9 – 40%). The presence of the BB after treatment predicted a successful treatment (positive predictive value = 72%, 95% CI: 61 – 81%).

We also identified a simple technical parameter, the Energy Density on Bone Surface (EDBS), which is the total energy delivered normalized by the bone surface area. EDBS was the only parameter that independently correlated with the likelihood of achieving complete or partial pain relief (OR of CR/PR with EDBS > 5 J/mm2 = 5.51; 95% CI: 1.70 – 17.8; sensitivity = 57%, 95% CI: 44- 69%; specificity = 81%, 95% CI: 61- 92%). The black band was significantly correlated with high EDBS (EDBSmean with BB was 5.7 ± 2.8 J/mm2; without BB was 2.7 ± 0.9 J/mm2, p<0.001).

Conclusions: With a technically successful treatment (BB+, EDBS > 5), the positive clinical response rate was 86%, higher than in the published report. Those sites that had previous experience using MRgFUS to treat bone metastases and that used deep anesthesia used the highest mean EDBS (6.8 ± 3.1 J/mm2, p=0.013), and also had the highest response rate (88%, p=0.009, Table 1). We also noted that the response rate (22%) after suboptimal treatments (BB-, EDBS < 5) was the same as in the sham arm of the trial. Utilizing this information will help optimize MRgFUS treatment of osseous metastases.
Table 1. Percentage of patients with complete or partial response.

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<th>Anesthesia</th>
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<tr>
<td></td>
<td>Not deep</td>
<td>Deep</td>
<td>Total</td>
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<tr>
<td>No prior experience</td>
<td>70%</td>
<td>36%</td>
<td>58%</td>
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<td></td>
<td>(4/11)</td>
<td>(14/20)</td>
<td>(18/31)</td>
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<tr>
<td>Prior experience</td>
<td>48%</td>
<td>88%</td>
<td>73%</td>
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<tr>
<td></td>
<td>(12/25)</td>
<td>(36/41)</td>
<td>(48/66)</td>
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<tr>
<td>Total</td>
<td>58%</td>
<td>77%</td>
<td>68%</td>
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<tr>
<td></td>
<td>(26/45)</td>
<td>(40/52)</td>
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Figure 1. Axial MR images of the right iliac bone showing a) T2 hyperintense permeative metastasis from lung cancer. The lesion had been treated with radiation and was still causing severe pain. b) Multiple sonications (green oval) result in deposition of thermal dose (blue) along the targeted bone contour. a) Change from baseline to 3 months in NRS score demonstrating a durable halving of pain scores in the treatment arm of the Phase III study.

Figure 2. a) Subtracted contrast enhanced image of the same patient as in Figure 1 shows lack of perfusion of the treated bone surface (black band), which is an immediate post-procedural indicator of successful ablation of the periosteal nerves and b) predicts subsequent pain relief. In this case in a), some of the cancer within the bone was also treated.
Technical aspects, feasibility, safety, and initial efficacy of osteoid osteoma ablation with MR-guided High Intensity Focused Ultrasound

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Objectives: Osteoid osteoma (OO) is a benign bone tumor that causes dull, but often debilitating pain, affecting mainly children and young adults. It is most commonly treated with computed tomography (CT)-guided radiofrequency ablation (RFA). However, RFA is invasive and CT guidance requires exposure to ionizing radiation. These shortcomings may be addressed through the use of Magnetic Resonance Imaging-guided High Intensity Focused Ultrasound (MR-HIFU), which allows non-invasive thermal ablation under real-time MRI guidance, with high spatial precision and without ionizing radiation. MR-HIFU may be used to reduce or eliminate pain through destruction of periosteal nerves and the OO nidus, without need for incisions or drilling. In an ongoing Phase I clinical trial, eight of the planned total of 12 children with symptomatic OOs have been treated at the Children’s National Medical Center (Washington D.C.). Herein we report on the technical aspects, feasibility, safety, and preliminary efficacy of OO MR-HIFU therapy.

Methods: Treatments were performed using the Sonalleve V2 clinical MR-HIFU system (Philips, Vantaa, Finland). The system includes a 256-element phased-array transducer, a positioning system with 5 degrees of freedom, and integrated MRI receive coils. Prior to therapy, patients were anesthetized or sedated, and positioned on the HIFU tabletop using gel pads, ultrasound gel, and degassed water for acoustic coupling. T2w and T1w MR images were acquired as a 3D stack, and subsequently used for treatment planning. Sonications of 16-48s duration were performed at a frequency of 1.2MHz, targeting regions 4-12 mm in diameter with acoustic powers of 20-160W. During sonications, real-time MRI temperature maps were acquired using a fast-field-echo pulse sequence and the proton resonance frequency shift method (3 orthogonal slices centered on the target region and an additional slice in the near field). Post-therapy, T1w contrast-enhanced MR images were acquired to report on ablated regions. While complete follow-up is still pending, we assess technical aspects of OO MR-HIFU therapy. Specifically, the following were evaluated: patient positioning, sonication parameters, tissue temperature and thermal dose, tissue perfusion changes, treatment times, and other technical data.

Results: Patient positioning on the HIFU table was feasible in all eight cases, with some modification to the shape of standard ultrasound standoff pads to provide acoustic coupling to extremities. Lesion locations included the tibia (n=3), femur (n=3), talus (n=1), and the great toe (n=1). Distance between OO nidus center and skin ranged 1.1-7.7cm, with estimated 43±16% power remaining at the bone surface due to soft tissue attenuation. Average acoustic power was 50±30W per sonication and total energy deposition per therapy session was on average 10±7kJ, resulting in complete lack of MR contrast enhancement of the OO nidus on T1w-imaging in six of eight patients, and partial enhancement in two patients. Immediately after the treatment, nonperfused volume extended 7±4 mm into the bone and 4±6mm into adjacent soft tissue. For seven of the eight patients, complete or nearly complete resolution of OO-related pain followed within days and all patients were able to cease using medication 28 days following treatment. Sonications, including cool-down times, took 30-77min, with 101-195min of MRI suite time required. The treatments required less than 4 hours of total anesthesia and/or sedation and they were well-tolerated without any serious adverse events.

Conclusions: With complete symptom resolution in all patients evaluated thus far, MR-HIFU has the potential to offer a fast, safe, completely noninvasive, and radiation-free treatment option for children with painful OO. Sonication parameters were chosen conservatively in this initial clinical trial, and overall treatment quality may be improved and its duration reduced through optimization of sonication power, duration, and frequency, as well as improved, or more specialized MRI equipment.
Bone thermal modelling for MRgFUS of osteoid osteoma — validation with human clinical data

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Objectives: MR-guided High Intensity Focused Ultrasound (MRgFUS) has recently been applied to patients with bone lesions such as osteoid osteomas (OO), which are painful benign tumours. While initial results are positive, there is much uncertainty about the ideal target location, effective thermal dose, overall bone heating and possible inadvertent injury. Simulation of MRgFUS heat transfer within and around bone can be used as a pre-treatment planning tool that could optimize and improve the safety and efficacy of this therapy.

Methods: To address the uncertainties of MRgFUS treatment, a computer simulator was developed. The simulator has three main components: 1) an automatic bone segmentation tool; 2) an acoustic propagation simulation to calculate the acoustic velocity distribution at the target; and 3) a heat distribution model, calculated by the Pennes Bioheat Equation, which simulates the temperature distribution at the target location. Concurrently, a clinical pilot study is being conducted, to treat OO patients with MRgFUS. The MR imaging and MRgFUS sonication data from the first four patients from this study was reprocessed with the simulator and the results were compared with the measured clinical thermal results.

Results: Automatically segmented femurs were compared to the manually segmented ones. The average volume overlap (VO) and sensitivity (S) between the automatic and manual segmentations were 92.2% and 96.3%, respectively. The calculations for VO and S are as follows: VO = 1-(FP+FN)/Vm and S = TP/Vm, were FP is the number of false positives, FN is the number of false negatives, TP is the number of true positives and Vm is the manually segmented volume. The average maximum temperature difference between the simulation and clinical data at a region near the sonication focus, after 20 seconds of sonication, was 1.4°C; after 40 seconds of cool down time, the difference was 7.3°C. The max temperature is defined to be the average of the hottest 9 voxels. The average distance between ablation centroids was 2.9 mm. Figure 1 represents the clinical MR thermometry distribution (left) and the simulated temperature distribution (right) from a representative sonication in one patient. Figure 2 represents the max temperature comparison over 60 seconds for this sonication.

Conclusions: The calculated segmentation validation metrics suggests that the automatically segmented data is comparable to the manually segmented data. The average difference between simulated and thermometry data at peak temperature suggests that the simulator can accurately predict the extent of ablation near the focus during sonication. However, the average cool down temperature difference suggests the simulation is consistently under-predicting the temperature near the focus after 40 seconds of cool down. The distance between ablation centroids suggests that the simulator can predict the site of ablation to within 3 image voxels. A robust MRgFUS bone thermal simulation platform has the potential to significantly improve efficiency, safety and outcomes of patients with bone lesions.

Figure 1. Comparison of MR thermometry (left) with simulated results (right)

Figure 2. Max temperature vs. time of simulated and MR thermometry treatment data
Non-invasive therapy for osteoid osteoma: Double site, long term, prospective development study with MR guided Focalized Ultrasound

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Objectives: Percutaneous or surgical therapy can lead to non-negligible side-effects and limited efficacy for paediatric population or young adults with a benign, self-limiting pathology such as osteoid osteoma. Over a period of 6-7 years we followed treated patients for at least 3-year for clinical, imaging and rehabilitation process. The aim of the study was to demonstrate that a completely non-invasive radiation-free focal ablation of osteoid lesions with MRgFUS can be a safe, effective and durable treatment option.

Methods: Patients were eligible for this dual-centre prospective development study if they had both clinical and radiological diagnosis of non-vertebral osteoid osteoma (typical nocturnal-worsening pain relieved with NSAIDs, pain score [VAS] >4, CT and MRI specific findings), and if they could safely undergo MRI, focalized US procedure and anesthetic. Patients received non-invasive therapy using MRI guided high-intensity Focused Ultrasound, delivered to all known osteoid lesions, identified on MRI, CT, or both. Feasibility, patient safety (adverse events), and clinical relevance profiles of MRgFUS were considered as primary outcomes; tumour control at imaging was considered as secondary outcome. Analyses were done on a per-protocol basis. This study is registered with ClinicalTrials.gov, number NCT02302651.

Results: Among 50 subjects recruited with both clinical and radiological diagnosis of OO, 45 underwent MRgFUS treatment and were included in the analysis. After intervention, no complications related to treatment or anaesthesia occurred and every patient lefted our hospital within 12-24 hours. Even during follow-up, no long-term complication related to treatment was reported. Clinical benefit was effective in all patients and overall VAS median value shifted from 8 (IQR 7-9) points before treatment to 0 at 1-week, 1-month, 6-month, 12-month, 24-month and 36-month follow-up. Similarly, VAS Scores median values for sleep disturbances, overall physical limitation, sport limitation and daily activities limitation dropped to 0 within the first month after treatment, and didn’t increase in subsequent controls. Quality of Life, assessed with FACT-BP Score, rose from a median value of 28 (22-34) before treatment to 55 at 1-week, 59 at 1-month, 60 at 6-month, 12-month, 24-month and 36-month follow-up. There was a complete response (a zero pain score one month after treatment, without recurrent symptoms, stable at follow up) in 80.00% of cases. 4 patients required a second line of intervention. Overall, patients who reached and maintained a stable 0 VAS score during our 3-year observation with MRgFUS treatment alone were 86.67%. At final

Conclusions: MRgFUS has shown complete absence of adverse events, persistent clinical efficacy and great tolerance profile. These strong features may induce a change in the current paradigm of OO management, making MRgFUS the treatment of choice, whilst CTgRFA role may shift to a second-line intervention.
Objectives: Osteoid osteoma (OO) is the most common benign bone tumor in children, often occurring in the long bones. Pain can be managed with medication but minimally-invasive CT-guided radiofrequency or laser ablation is the current, definitive, standard-of-care. However, non-target tissue injury is a concern as temperature cannot be measured with CT, and the treatment induces temperatures around 90°C that are maintained for several minutes. CT-guided radiofrequency or laser ablation includes risks from ionizing radiation, fracture, infection and transmitted thermal damage along the needle. Magnetic Resonance guided High Intensity Focused Ultrasound (MRgHIFU) is an alternative and has been used successfully for OO treatment. There is no mechanical penetration of the bone, reducing the chance of pathologic fractures. Also, procedures do not need to be conducted in a sterile environment reducing the chances for infection. We present our initial experience with OO MRgHIFU treatment in six pediatric patients.

Methods: MR provides excellent soft tissue contrast, which delineates the interface between bone and surrounding soft tissues as well as the highly vascularized core of the OO, known as the nidus (Figure 1). The nidus is targeted in order to kill cells that produce pain-inducing prostaglandins. Ten patients are being recruited to determine the impact of Focused Ultrasound treatment on bone pain, medication usage and health-related quality of life (HRQL) metrics, such as physical, emotional, social, and school functioning (determined through the use of age-appropriate and validated surveys (Pediatric Ouch, and PedsQL™)). An initial planning MRI ensures lesion accessibility/patient eligibility. MR thermometry (Figure 2) measures temperature in the target and surrounding tissue to ensure patient safety. As bone ablation is painful, patients are under general anesthesia for the treatment. Follow-up on days 1, 7, 14, 30, 90 and 180 following treatment record pain, HRQL, and drug usage. Clinical visits on days 30, 90 and 180 comprise a physical examination and a diagnostic MRI of the target lesion. Contrast enhanced MRI, indicate non-perfused tissues corresponding to the ablated tissue volume, which should be fully resolved by day 180 (Figure 1).

Results: This study is being conducted with the primary goal of assessing the pain response in MR-HIFU treatment of OO in pediatric patients. Between July 2014 and May 2016, 6 patients underwent 7 MR-HIFU procedures. Subject 2 underwent MR-HIFU twice due to an inadequate pain response after the first procedure. The average age of patients enrolled was 13 ± 4.1 years (range: 8 – 17) and weight was 52.2 ± 19.4 kg (range: 24 – 74). During this period, a total of 12 patients were referred for OO treatment. Reasons for exclusion from the study included acoustically inaccessible locations (proximity to nerve or growth plate) in 6 patients and patient refusal in 1. The overall technical success rate was 83% (5/6 subjects). Subject 4 could not be treated due to poor acoustic access to the lesion. Complete response (treatment success) was seen in of 80% (4/5) and partial response in 20% (1/5) of the treated subjects. Pain scores dropped from an average of 7.8 prior to treatment to 1.2 by day 7, as shown in Table 1. Prior to treatment, the time to fall asleep was reported as 30-60 minutes. Following MR-HIFU, this decreased to 7-38 minutes. There were no significant adverse events.
Table 1: Pain scores pre and post HIFU treatment

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Weight</th>
<th>Pre HIFU 1 Day</th>
<th>Post HIFU Day 1 Day 2 Day 7 Day 14 Day 30 Day 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIFU-OO-001</td>
<td>16</td>
<td>60</td>
<td>8 1 1</td>
<td>0 0 0</td>
</tr>
<tr>
<td>HIFU-OO-002</td>
<td>17</td>
<td>64</td>
<td>5 3 2</td>
<td>6 5 4 7</td>
</tr>
<tr>
<td>HIFU-OO-003</td>
<td>8</td>
<td>24</td>
<td>7 4 0</td>
<td>0 0 0</td>
</tr>
<tr>
<td>HIFU-OO-004</td>
<td>17</td>
<td>74</td>
<td>N/A N/A N/A</td>
<td>N/A N/A N/A</td>
</tr>
<tr>
<td>HIFU-OO-005</td>
<td>10</td>
<td>58</td>
<td>10 6 5 0</td>
<td>0 0 0</td>
</tr>
<tr>
<td>HIFU-OO-006</td>
<td>10</td>
<td>33</td>
<td>9 2 3 0</td>
<td>1 0 Pending</td>
</tr>
<tr>
<td>Average</td>
<td>13</td>
<td>52.2</td>
<td>7.8 3.2 2.2</td>
<td>1.2 1.2 0.8 1.8</td>
</tr>
<tr>
<td>SD</td>
<td>4.1</td>
<td>19.4</td>
<td>1.9 1.9 2.7</td>
<td>2.2 1.8 3.5</td>
</tr>
</tbody>
</table>

Pain scores pre and post HIFU treatment (at days 1, 2, 7, 14, 30 and 90) for all treated patients. Patient 4 could not be treated, and exited the study. Only the data from the first treatment of Patient 2 was used.

Conclusions: This study shows that noninvasive MRgHIFU treatment of OO can be used to successfully treat osteoid osteoma in pediatric patients. When the cortex is intact, MR-HIFU heats the bone surface. Treatment of OO lesions results from heat conduction through bone from the cortical surface. In intact bone, MR-HIFU heats the cortical surface with deeper areas being heated by conduction. Subjects 1, 3 and 5 had superficial lesions and responded very well to MRgHIFU therapy. Subject 2 had a large, sclerotic medullary lesion that presented a challenge for heat conduction through the bone and required a second session with a more aggressive MR-HIFU treatment. Further research is necessary to define the optimal treatment parameters and limitations of MRgHIFU. A prospective registry is currently being designed to compare MRgHIFU with other types of thermal ablation.

Figure 1 – Representative pre and post HIFU sagittal MRI of an osteoid osteoma lesion with gadolinium enhancement. The lesion is fully enhanced before HIFU treatment, and shows non-perfused regions following treatment.

Figure 2 – Representative MRI thermal imaging during HIFU treatment from a 30W, 20s exposure (axial and sagittal views). Temperatures in the nidus reached >70°C, indicating thermal ablation of the lesion.
TREATMENT OF BREAST FIBROADENOMA WITH HIGH INTENSITY FOCUSED ULTRASOUND: UPDATE OF FEASIBILITY STUDY IDE #G130252

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OBJECTIVES: Fibroadenomas are common, benign lesions of the breast. A minority of fibroadenomas will disappear without treatment, but most increase in size or remain unchanged. Current management of patients with fibroadenomas in the United States includes observation or surgical excision. Many patients find their fibroadenoma bothersome, and thus opt for surgical excision.

The objectives of this study are to evaluate the safety and feasibility of Ultrasound guided High Intensity Focused Ultrasound (USgHIFU) delivered by the Echopulse device (Theraclion, Paris) for treatment of breast fibroadenomas. General patient safety, cosmetic outcome, tumor response, patient experience, physician/operator experience, and device performance are being assessed.

METHODS: Twenty female patients diagnosed with palpable breast fibroadenomas 1cm or larger are being enrolled in a single arm study and will undergo treatment of their tumor utilizing a computer-driven, continuously cooled, extra-corporal HIFU probe mounted on an arm moved by motors, and guided in real-time with an integrated ultrasound imaging scanner. The integrated probe is positioned by the operator and the lesion is imaged. Treatment planning is automated and presented for review and approval on an integrated computer screen. Optimal energy per sonication is established for each patient by determining the minimal setting found to produce bubbles within the lesion as observed on real-time B-mode ultrasound. Patients will have tumors meeting the following criteria: Distance from the skin of ≤ 23 mm to the posterior border of the fibroadenoma, ≥ 5 mm from the anterior border of the fibroadenoma, and ≥ 11 mm from the focal point of the HIFU treatment. The chest wall must be more than 1 cm from the posterior margin of the tumor, and tumor volume must be between 0.3 cc and 10 cc.

Subjects are assessed immediately after treatment and at 3, 6, and 12 months.

RESULTS: The study remains open to accrual, the following are PRELIMINARY RESULTS, thus the denominators vary by data point: Enrollment at the time of the writing of the abstract is 16 patients. Six patients remain in follow-up. To date, there have been no grade 3 adverse events, nor any skin burns, persistent changes in skin appearance, nor other significant toxicities/morbidities observed in the patients treated. Mean patient-rated pain score during treatment on a scale from 0 to 100 was 17.8. One patient has reported persistent mild pain at 6 months follow-up (2 on a scale from 0 to 100). The most common toxicity observed was pain (reported by 9 of 16 patients). Preliminary patient satisfaction was 4.65 on a scale of 1-5 (5 = most satisfied), 10 of 11 patients reported they would undergo the procedure again, and 11/11 reported they would recommend the procedure to a friend or family member. Reduction in the size of the palpable mass was reported by both the patient and evaluating physician in almost all cases. Similar findings were found on ultrasound in the majority of cases. Cosmesis can be excellent, and unchanged from baseline in all cases to date.

CONCLUSIONS: To-date, USgHIFU, delivered by the Echopulse device for treatment of breast fibroadenomas in the IDE G130252 study has been well tolerated by patients, resulted in minimal toxicity, and appears to have been effective.
Long-term efficacy and tolerability of one or two US-guided HIFU treatment of breast fibroadenoma

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2University Medical Centre Ljubljana, Ljubljana, Slovenia

Objectives: Breast fibroadenoma (FA) is the most prevalent benign tumor, accounting for up to 70% of benign breast lesions.1,2 They affect females in the reproductive period with two peaks of incidence in the third and in the fifth decade of life. During the follow-up, a minority of FA decrease in size or disappear, more than half of them remain unchanged, and some of them significantly increase.3

Ultrasound (US)-guided high-intensity focused ultrasound (HIFU) is the only non-surgical and non-invasive procedure, where thermal destruction is achieved by precisely delivered energy to the target, without interrupting skin integrity. Recently, a multicentre study established that US-guided HIFU treatment of 51 FA resulted in 72.5% volume reduction at 1 year.4

The purpose of our study was to compare the long-term efficacy and tolerability of one or two HIFU treatments in patients with breast FA.

Methods: Twenty patients with 26 FA were selected for US-guided HIFU. The therapy was performed with the system EchoPulse (Theraclion, France) on an outpatient basis, in one or two sessions, under conscious sedation. FA volume was assessed before and followed up to 24 months after the last HIFU treatment. After each procedure, adverse events were evaluated. Written informed consent was acquired from all patients.

Results: In 19/26 FA (73.1 %) one HIFU was performed (group 1), whereas 7/26 FA (26.9 %) received second HIFU (group 2) 6-9 months (median, 7 months) after the first session. In group 1 and 2, FA volume decreased significantly at 1-month (p<0.001) and 3-month follow-up (p=0.005), respectively, and continued to reduce until 24-month follow-up (p<0.001 and p=0.003, respectively). At 24 months, mean volume reduction was 77.32 % in group 1 and 90.47 % in group 2 (p=0.025). Mild subcutaneous oedema was observed in 4 patients and skin irritation in 3 patients.

Conclusions: US-guided HIFU represents a promising non-invasive method with sustainable FA volume reduction and patient’s tolerability. Although one treatment is highly efficient, the volume reduction can be increased with second treatment.

References
High intensity Focused Ultrasound in the treatment of breast fibroadenomata: Results of the HIFU-F trial

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Objectives: Breast fibroadenomata (FAD) are the most common breast lumps in women and are treated conservatively unless they are symptomatic in which case surgical excision is usually recommended. High intensity Focused Ultrasound (HIFU) is a non-invasive ablative technique that can be used to treat FAD but is associated with prolonged treatment times. In the HIFU-F trial, we evaluated the change in volume over time with circumferential HIFU treatment of FAD and compared this to no treatment (control).

Methods: Patients aged 18 years or older, diagnosed with symptomatic, palpable FAD visible on ultrasound (US) were recruited. Patients were treated using the US-guided Echopulse device (Theraclion, Malakoff, France) under local anaesthesia. Primary outcome measures included: reduction in symptoms, reduction in treatment time compared to whole lesion ablation; feasibility to achieve a 50% reduction in volume after six months and decrease in volume compared to an observation only group (control). This study received ethical approval (REC 13/LO/1221).

Results: HIFU treatment was performed on 20 patients (mean age 30.3 years, SD 7.5 years). Six out of eight patients who experienced pre-treatment pain had complete resolution of their symptoms six months post-treatment. All short-term complications (e.g. ecchymosis and erythema) completely resolved within the first month post-treatment without the need for intervention. Hyperpigmentation was found at three months in six patients and persisted at six months in four patients although it was asymptomatic. Circumferential ablation significantly reduced the mean treatment time by 37.5% (SD 20.1) compared to whole lesion ablation. US demonstrated a significant mean reduction in FAD volume of 43.5% (SD 38.8%) (P=0.016) in the HIFU group and this was also significantly greater than the 4.6% (SD 46.0%) reduction in volume observed in the control group at six months (P=0.002).

Conclusions: Circumferential HIFU ablation of FAD is feasible, with a significant reduction in volume compared to control patients. It provides a simple, non-invasive, outpatient – based alternative management option for FAD.
Harmonic motion imaging for characterization and Focused Ultrasound ablation monitoring of post-surgical human breast tumors

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Objectives: Each year in the United States, over 60,000 women are diagnosed with breast cancer. High-Intensity Focused Ultrasound (HIFU) holds promise as a non-invasive and targeted therapeutic technique for breast cancer patients. To facilitate its widespread translation to the clinic, however, there is still a need for a real-time and cost-effective device that can reliably monitor HIFU ablation. Harmonic Motion Imaging for Focused Ultrasound (HMIFU) is an all-ultrasound radiation-force-based technique, which can be used for real-time HIFU ablation monitoring by tracking relative stiffness change at the treated area without interrupting HIFU.

Methods: Specimen collection and handling of post-surgical breast tissues were approved by the Institutional Review Board (IRB) board of Columbia University and informed consent was obtained from all enrolled patients. HMIFU was performed in 10 normal, 10 malignant tumor (invasive ductal carcinoma, namely IDC) and one benign tumor (fibroadenoma, namely FA) specimens. The HMIFU setup consists of a 93-element, 4.5-MHz HIFU transducer and a confocally-aligned 64-element, 2.5-MHz phased array imaging probe, which is connected to an ultrasound imaging research system. All HIFU elements were synchronously excited by a 25 Hz amplitude-modulated signal to vibrate the tissue at 50 Hz. A GPU-based fast image reconstruction method was used to monitor lesion development in real-time.

Results: For real-time monitoring, the displacement map and lesion map were streamed on the computer screen at a display frame rate of 2.4 Hz during treatment without interruption. Following a 2D raster scan, 3D HMI displacement maps were reconstructed representing the relative stiffness of the tissue (Figure 1). The mean HMI displacement within the ROI decreased by 60% from 24.73±10.97 µm to 9.83±6.46 µm in normal breast tissue (n = 10, p = 0.0048), decreased by 58% from 12.77±3.50 µm to 5.35±2.74 µm (n = 10, p = 0.045) in IDC and decreased by 20% from 2.56 µm to 2.06 µm (n = 1) in FA. There were statistically significant differences between before and after HMIFU ablation in both the normal and tumor specimens.

Conclusions: HMIFU is shown to be capable of differentiating relative stiffness between normal and abnormal breast tissues prior to treatment. HMIFU can also successfully monitor thermal lesions formation in breast tissue in real-time.

Figure 1 Gross pathology and 3D HMI displacement images of normal breast tissue (A-C), IDC (D-F) and FA (G-I) before and after HMIFU ablation. The brighter the color is indicates the higher HMI displacement and lower relative stiffness, and vice versa.
MRgFUS for desmoid tumors within the thigh: Early clinical experiences

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Objectives: Desmoid tumors are benign but locally aggressive tumors derived from fibroblasts. Surgery, chemotherapy, and radiation therapy have historically been the mainstay of treatment but recurrence is common and side effects can result in significant morbidity. Desmoid tumors of the thigh are particularly difficult to treat with conventional approaches and have a higher recurrence rate. MRgFUS has increasingly been used for management of desmoid tumors because of the favorable side effect profile. In this case series we highlight our experiences performing treatments in the thigh, including strategies for optimizing ablation size and safety.

Methods: Since December 2014, 14 MRgFUS treatments for desmoid tumors were performed at our institution in seven patients. 9 of these treatments were completed in three patients with large tumors within the posterior thigh. The first was a 7 year-old boy who had previously been treated with surgical resection, intraoperative radiation, along with courses of vinblastine/methotrexate and sorafenib. The second was a 21 year-old woman who had previously taken sulindac and celecoxib but had no other therapy. The third patient was a 14 year-old girl with no prior treatment. Treatment efficacy was evaluated by calculation of post-contrast ablation volume immediately following treatment and in diagnostic studies between treatments. Side effects and complications were also documented for each treatment.

Results: Case 1: Pre-treatment tumor volume 770cc with 75% non-enhancing volume following the initial treatment. However, first treatment was complicated by a third degree burn along the far-field skin. Despite the complication, the family and referring clinicians wished to proceed with additional treatments. Enhanced safety measures were implemented to protect the far-field skin including water bags and fiber-optic temperature probes, as well as interval skin checks. The patient had four subsequent treatments over 14 months, without complication, with non-perfused volume of 90% most recently.

Case 2: Pretreatment tumor volume 740cc. Notably, the sciatic nerve courses along the tumor’s anterior margin. Left lateral decubitus position was used to minimize the amount of energy through the sciatic nerve. Enhanced safety measures as above. First treatment resulted in a relatively low non-perfused volume of 30%, likely related to a prominent fascial band. Second treatment resulted in 75-80% ablation of the target.

Case 3: Pretreatment tumor volume was approximately 440cc. The sciatic nerve was encased by the anteromedial portion of the mass. Left lateral decubitus position and enhanced safety measures again used. First treatment resulted in a relatively low non-perfused volume of 30%, likely related to low energies. Second treatment resulted in 70-80% ablation.

Conclusions: MRgFUS is an effective treatment for desmoid tumors with a favorable side effect profile, allowing for repeated treatments if necessary. Ablation size and safety can be improved by use of far-field coupling devices, careful patient positioning, and optimized sonication planning.
Anatomical distribution of pediatric sarcoma and neuroblastoma: Targetability with MR-HIFU

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\textbf{Objectives: } Not released for publication
\textbf{Methods: } Not released for publication
\textbf{Results: } Not released for publication
\textbf{Conclusions: } Not released for publication
A prospective study on the efficacy of single High Intensity Focused Ultrasound treatment of patients with benign symptomatic thyroid nodule

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Objectives: Benign thyroid nodules are prevalent among the general population and some nodules may exhibit growth over time leading to compression symptoms or cosmetic concerns. Although surgery remains the treatment of choice for symptomatic thyroid nodule, it is associated with a 2%–10% risk of complications and requires a general anesthesia. The aims of the present study were to assess the efficacy of a single treatment of High Intensity Focused Ultrasound (HIFU) in reducing benign thyroid nodule volume and to evaluate the changes in health-related quality of life (HRQL) following a single HIFU treatment.

Methods: After obtaining IRB approval, consecutive patients with symptomatic thyroid nodule were assessed for eligibility. Inclusions were nodule(s): 1) without signs of malignancy (i.e. no suspicious clinical and ultrasonic features and benign cytology on fine needle aspiration); 2) measuring ≥10 mm on ultrasound (USG) in three orthogonal dimensions; and 3) menable to HIFU. Exclusions were nodule(s): 1) measuring<40mm (by largest dimension); and 2) located <2mm from trachea, esophagus or recurrent laryngeal nerve (where ablation might pose thermal injury). Eligible patients were offered a choice of HIFU treatment, active observation or surgical resection. HIFU treatment was conducted with the USG-guided Echopulse (Theraclion SA, France). Primary outcome was a change in index thyroid nodule volume 6 months after HIFU. To have 80% power and 95% confidence interval (two-sided) to detect minimal important difference of 20%, 20 patients were needed. Assuming a 10% incomplete and withdrawal rate, 22 patients were required. Thyroid volume (mL) was assessed at baseline (i.e. before ablation) (Figure 1), 1-week, 3-month and 6-month (Figure 2) while HRQL was assessed by the Chinese version of the SF-12v2 at baseline and 6-month. The HRQL measured by eight domain scores and two summary scores (physical and mental component summary) was then compared between those who received HIFU and those who chose active observation (controls) at 6-month.

Results: Over this period, 22 (52.4%) chose to receive a single course of HIFU (HIFU group) while the other 20 patients chose to have active observation (controls). Among the HIFU group, the majority were females (90.9%) and the majority (77.3%) had their index nodule as the dominant nodule in a multinodular goiter. The mean base index nodule volume was 6.48 ± 4.34mL (range: 0.92 - 16.76mL). At 6-month following HIFU, the treated nodule volume decreased to 1.38 ± 1.31 mL (n=22, p<0.001). The average extent of nodule reduction was 71.58 ± 11.81% (range: 54.45 – 90.09%). However, there was no significant correlation between extent of 6-month volume reduction and basal volume (r=0.096, p=0.806), total treatment time (r=0.503, p=0.168), total energy delivered (r=0.122, p=0.0755) or mean delivered energy per treated volume tissue (r=0.150, p=0.700). Compared with controls, the HIFU group achieved a significantly greater improvement in four quality-of-life domains (17.57 ± 6.46, p=0.009 for role physical; 18.01 ± 6.78, p=0.011 for bodily pain; 24.77 ± 8.15, p=0.004 for general health; 32.61 ± 8.93 for social functioning), and physical component summary of the SF-12v2 ((7.89 ± 2.83, p=0.001).

Conclusions: USG-guided HIFU ablation is not only an effective and safe treatment option for patients with benign symptomatic thyroid nodules but has the potential of improving the HRQL of patients who do not wish to undergo surgical resection.
The effect of different treatment regimens with US-guided HIFU on thyroid nodule volume

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Objectives: Thyroid nodules can be detected by ultrasound (US) with a prevalence of 19-67%. Although 95% of them are benign, 1/3 show continuous growth and should be treated because of compression symptoms or cosmetic concerns. Surgery is still the main therapeutic strategy, in spite of the fact that it carries 2–10% risk of complications.

US-guided High-Intensity Focused Ultrasound (HIFU) is a non-invasive thermo-ablative method, developed to reduce thyroid nodule size. The purpose of our work was to compare the long-term efficacy and safety of a single and repeated HIFU treatment of benign solid thyroid nodules.

Methods: Twenty euthyroid patients (mean age, 44.5 years) with benign solitary or dominant thyroid nodule were treated with US-guided HIFU system (EchoPulse, Theraclion, France) under conscious sedation. Twelve patients (group 1) received one treatment and 8 patients (group 2) repeated the treatment after 3 months of follow-up. US volume measurement was performed at baseline, 3 and 12 months after the final treatment. Adverse events were evaluated. Written informed consent was acquired from all patients.

Results: The baseline nodule volume and the energy applied per nodule volume did not differ significantly between group 1 and group 2 (5.04 ± 2.70 ml and 4.83 ± 2.74 ml, respectively; 3.5 ± 1.4 kJ/mL and 4.1 ± 1.6 kJ/mL, respectively). At 12-month follow-up the mean nodule volume decreased significantly in both groups (2.35 ± 2.44 ml, p=0.003, and 2.63 ± 1.85 ml, p=0.017, respectively) with a maximal volume reduction of 95.4% and 66%, respectively. The mean percent of volume reduction at M3 after the first HIFU and at M12 after the final HIFU differed significantly between group 1 and 2 (47.4%±20.8 vs 24.2%±15.8, p=0.02 at M3, and 55.5% ± 28.4 vs 46% ± 22, p=0.011 at M12). After the first treatment transient subcutaneous oedema and mild skin redness were observed in 2 patients and after the second treatment, one patient developed Horner syndrome, which resolved 6 months later.

Conclusions: In solid benign thyroid nodules, the effect of one and two consecutive HIFU treatments is comparable. Larger studies are needed to explain the different thyroid nodule susceptibility to HIFU ablation.
Prostate cancer HIFU — novelty or innovation?

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Objectives: Not released for publication

Methods: Not released for publication

Results: Not released for publication

Conclusions: Not released for publication
MR-HIFU ablation of osteoid osteoma: Experience in a pediatric tertiary care center

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Objectives: To describe our experience with Magnetic Resonance-guided High Intensity Focused Ultrasound (MR-HIFU) ablation of painful Osteoid Osteoma (OO) in children.

Methods: Nine children with OO (7M, 2F; 16±6 years) underwent MR-HIFU ablation through an FDA and IRB-approved safety and feasibility clinical trial using the Sonalleve V2 MR-HIFU system. Treatment feasibility, patient safety, and clinical response were evaluated in all patients over 28 days. Additional follow-up was performed to evaluate longer-term safety and durability of clinical response.

Results: MR-HIFU therapy was feasible in all nine patients without any serious treatment-related adverse events. Eight out of 9 patients reported complete response in terms of pain resolution and cessation of medication usage and one out of 9 patients reported partial response. All treatments were performed on an outpatient basis without overnight admission.

Conclusions: These findings show that MR-HIFU ablation of OO is feasible and safe in pediatric patients and offers clinical response rates similar to those seen with radiofrequency ablation, the standard of care therapy at most US hospitals. However, the completely noninvasive and radiation-free nature of MR-HIFU is advantageous over RFA, particularly in children and adolescents for whom collateral damage and radiation exposure may cause long-term morbidity.
Noninvasive thrombolysis using microtripsy in a porcine deep vein thrombosis model

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Objectives: Histotripsy is a novel therapeutic technique that uses ultrasound generated from outside the body to create controlled cavitation in a target tissue, and fractionates it into acellular debris. We have developed a new histotripsy approach, termed microtripsy, to improve targeting accuracy and to avoid collateral tissue damage. This in vivo study evaluates the efficacy and safety of microtripsy thrombolysis in a deep vein thrombosis (DVT) model.

Methods: Acute thrombi were formed in the left femoral veins of pigs (~35 kg) by occluding the vessel using two balloon catheters and infusing with thrombin. Guided by ultrasound imaging, microtripsy thrombolysis treatment was conducted in 14 pigs. 10 pigs were euthanized on the same day (acute) and 4 at 2 weeks (subacute). To evaluate the vessel damage, 30-min free-flow treatment (no thrombus) in the right femoral vein was also conducted in 8 acute pigs.

Results: Blood flow was restored or significantly increased after treatment in 13 out of the 14 pigs (Figure 1). One treatment was not effective due to a technical issue with clot formation. The flow channels reopened by microtripsy had a diameter up to 64% of the vessel diameter (~6 mm). The average treatment time was 16 minute per cm-long thrombus. Minor hemolysis was observed in both thrombolysis and free-flow treatments. Histology showed no vessel damage and only microscopic hemorrhage outside the veins for the free-flow treatments with nothing abnormal observed for the subacute treatments (Figure 2).

Conclusions: Microtripsy is a safe and effective treatment for DVT in a porcine model. Further studies are warranted to study the role of this promising noninvasive thrombolytic method in human subjects.
Objectives: Hypertension represents a critical health challenge for millions of people and despite the availability of numerous pharmaceutical agents, approximately 10% of the patient population who are currently taking three or more medications continue to have high blood pressure and are identified with resistant hypertension. Renal sympathetic nerves are key in initiating and maintaining systemic hypertension. Many studies have demonstrated that performing renal denervation (RD) using both catheter-based and Focused Ultrasound have achieved significant decreases in blood pressure. Mixed clinical trial results [N Engl J Med 2014;370(15)] and the lack of an acute metric that demonstrates successful RD highlight the need to find alternative technologies and monitoring techniques that can consistently result in RD.

This work evaluates using MRgFUS as a RD technique in a normotensive rat model. Real-time MR monitoring, acute invasive blood pressure probe measurements and histological results all demonstrate the potential of using MRgFUS to control resistant hypertension.

Methods: In this pilot study, both male and female (n=6 treated/5 sham) normotensive Sprague-Dawley rats underwent bilateral RD with MRgFUS. All procedures were performed in a 3T MRI scanner (Siemens 3T Trio) using a small animal MRgFUS system (f = 3 MHz, Image Guided Therapy). Sonications were applied along the length of both renal arteries (8 points/animal, 2W, 20s) and monitored in real-time with 3D MR thermometry. Pre- and post-RD procedure T1 maps were obtained evaluating the entire insonified area. In 5 animals (36 total sonications), mean arterial pressure (MAP) was monitored continuously using an invasive fiberoptic blood pressure probe (SA instruments) inserted in the tail artery. One month post-RD procedure, animals were euthanized and kidney medulla norepinephrine concentration, a proven, robust metric of successful denervation, was obtained using an ELISA (Rocky Mountain Diagnostics). Histological analysis was performed on both renal arteries and surrounding tissues and kidney function, as measured by blood urea nitrogen and urinary albumin secretion, was evaluated.

Results: Acute blood pressure response: A transient decrease in MAP (> 5%) was observed during 11 of the 36 monitored sonications (~30%). The normalized MAP measurements obtained during these 11 sonications are seen in Figure 1. In all animals the effect was dependent on sonication location. In addition, the change in MAP occurred several seconds after the sonication start time indicating it was an accumulated effect. Treatment efficacy: Kidney medulla norepinephrine concentration was reduced by 36% (p=0.05) one-month post ablation when compared to the sham animals, indicating RD was successfully performed. Efficacy was further confirmed by the presence of inflammatory cells, pyknotic nuclei as well as degraded nerve architecture in the histological results (Figure 2). Treatment safety: Comparison of the treated and sham RD animal’s blood urea nitrogen and urinary albumin/creatinine ratio resulted in no significant changes indicating kidney function was not affected by MRgFUS renal denervation. In addition, mean T1 values in both kidneys did not significantly change during the MRgFUS RD procedure. The peak mean temperature rise measured by real-time MR thermometry in the back muscle (Figure 3) located in the near field of all animals ranged from 15.5 to 25.0°C, confirming energy delivery and targeting accuracy.

Conclusions: Controlling resistant hypertension with renal denervation using Focused Ultrasound has been demonstrated in this and other studies to be a safe and potentially efficacious procedure. This work indicates that an acute, systemic response that is a function of sonication position can be detected during MRgFUS ablation of the renal sympathetic nerves. Future work will further evaluate this response in a hypertensive animal model and assess the effect of ultrasound parameters.
Normalized mean arterial pressure response for 11 sonications measured in 5 animals. Thick lines indicate the 20-second sonication time.

H&E stain of ablated renal nerve region with inflammatory cells, pyknotic nuclei and degraded nerve fiber indicated. Scale bar = 50 μm.

Coronal MR thermometry image showing the temperature rise measured in the back muscles (hollow arrows) during a single sonication. The white arrow indicates the spine.
Trans esophageal HIFU for cardiac ablation: First experiment in non-human primate

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Objectives: Atrial fibrillation and ventricular tachycardia are currently treated with catheter ablation using radiofrequency or cryoenergy. These endocardiac approaches are invasive and not fully satisfactory as the treatments are often incomplete and can be associated with serious side effects. The esophagus offers an excellent acoustic window to the heart and transesophageal HIFU were proposed as an alternative strategy. The present work describes the first attempt to perform thermal ablation with trans esophageal HIFU in the heart of a non-human primate.

Methods: For that purpose, an endoscope integrating a 5MHz 64-element commercial transesophageal echocardiography (TEE) probe and a HIFU transducer was built. Anatomical configuration and numerical simulations (Constanciel 2013) allowed setting the features of the HIFU transducer: 8 elements truncated at 14 mm, 3 MHz operating frequency and 40mm focal length. The focus could be steered electronically over a 15 to 55 mm range from the transducer. Circulation of water at 5°C inside the endoscope ensured cooling of the front face of the HIFU transducer and acoustic coupling through the inflation of a latex balloon. The probe was tested \textit{in vivo} in a 30kg-baboon after preliminary TEE and CT-scanner demonstrated the acoustic access from the esophagus to the heart in this animal model. HIFU were delivered to the interatrial septum, the inferior wall of the left atrium and the upper posterior wall of the left ventricle (LV). A multi channel amplifier allowed delivering a focal intensity (Ispta) of 3000W/cm\textsuperscript{2} applied 4 times for 16s at each selected locations. B-mode, shear-wave (SWE) and passive elastography were performed before and after HIFU with an ultrafast scanner. MR imaging (T1 mapping and contrast) was performed one-day post HIFU. Animal experimentation approved under agreement 04938.04.

Results: The endoscope was successfully inserted in the esophagus of the baboon. The inflation of the latex balloon allowed acoustic transmission from the esophagus to the heart and visualizing satisfactorily the cardiac structures by TEE. HIFU could be delivered at the locations identified on the CT scans even though the heart was slightly shifted in the chest due to different positions of the animal during CT and HIFU procedures. The ECG was not impacted by the treatments. No change in echogenicity of treated zones could be evidenced after HIFU. SWE showed a stiffening of the LV myocardial wall after ablation (two-fold increase of the myocardial stiffness). Increase of stiffness could be observed similarly in the beating heart on passive elastogram. MRI of the atrial wall for detecting thermal ablation could not be achieved. A hypo signal zone could be evidenced on T1 maps in the lateral wall of the LV where one treatment was performed. Contrast MRI did not show local fibrosis in the heart. Endoscopic examination did not reveal esophageal damage. The baboon recovered properly from the protocol and no significant side effect could be evidenced for one month after treatment.

Conclusions: This experiment was a first attempt to perform thermal ablation in the heart with a trans esophageal probe \textit{in vivo} in a non-human primate. While the procedure was safe and lesions seemed to be induced in the heart, developments will be necessary in order to 1- Deliver more power locally by HIFU, to compensate for motion and heat sink by blood circulation and to generate more pronounced lesions, 2- Improve the performance of monitoring techniques for the atrium (sensitivity and resolution) and for the ventricle (difficult to generate shearwave farther than 50 mm from the esophagus).
Ex vivo and in vivo non-invasive ultrasound-based cardiac pacing

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Objectives: Currently, no non-invasive cardiac pacing device acceptable for prolonged use in conscious patients exists. The main approach is invasive, employing intravascular catheters, which has associated risks. Focused ultrasound can be used to perform remote pacing using reversibility of electromechanical coupling of cardiomyocytes. This technique might be useful in the short term in the clinical settings in various conditions: temporary pacing for bradycardia or any clinical condition with risks of asystole; terminating or examining the inducibility of tachyarrhythmia; screening and optimization of cardiac resynchronization therapy. Here we described an extracorporeal cardiac stimulation device and study its efficiency and safety.

Methods: Ex vivo acoustic stimulation threshold was determined performing 756 sonications in 10 pig beating hearts. In vivo non-invasive stimulation was performed using 314 sonications in 4 anesthetized pigs. The animals were injected with ultrasound contrast agents. Experiments were performed using a Focused Ultrasound device (256 elements, 13/13 cm aperture/focal, operating at 1 MHz) under MR-guidance. At the end of each in vivo experiment, a navigated delayed inversion-recovery 3D Flash sequence was performed. Masson’s staining was performed to assess acute damages screening from acoustic stimulation of both ex vivo and in vivo experiments.

Results: Using HIFU it was possible to perform ventricular continuous pacing (A) or to induce ventricular tachycardia (B). Consecutive stimulations of different heart chambers with a single ultrasonic probe was shown, allowing to modify the resulting atrio-ventricular delay (C-D). The results of the 756 stimulation sites performed in the right atrium, and the left and right ventricles in 10 ex vivo beating hearts from pigs were processed to determine stimulation threshold for pulse duration ranging from 30 µs to 10 ms. Two different pressure thresholds were highlighted: one around 4MPa peak negative for HIFU pulse durations above 1 ms and one around 6 MPa peak negative for HIFU pulses ranging from 50 µs to 1 ms (E). The same setup was used in vivo in 4 pigs to show clinical potential (F). Electrophysiological changes were also confirmed by arterial pressure modifications (G). The maximal peak negative pressure was estimated to be around 2 MPa at focus during in vivo experiments, due to the limited acoustic window. At this pressure level, stimulation of the LV was observed but with an insufficient success rate. Using ultrasound contrast agents, consistent cardiac stimulation was achievable for up to 1 hour sessions in 4 different animals. No damage was observed in the 4 animals.

Conclusions: To the best of our knowledge, this study is the first ex vivo and in vivo proof of feasibility of controlled non-invasive ultrasound-based cardiac stimulation in large animals. Preliminary safety results showed that this novel technology offers good prospects for clinical developments.
Restoring perfusion after critical hindlimb ischemia using pulsed Focused Ultrasound and mesenchymal stem cell in aged mouse

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Objectives: Critical limb ischemia (CLI) is associated with a 5 year mortality rate in excess of 70% with limited effective therapies. The goal of this study was to determine if pulsed Focused Ultrasound (pFUS) would enhance homing of mesenchymal stromal cells (MSC) to CLI in an aged mouse model and reestablish perfusion compared to pFUS or MSC alone.

Methods: CLI model was created by cauterizing the external iliac artery (EIA) on C3H mice (age = 10-12 months). Laser Doppler perfusion imaging (LDPI) was performed to confirm surgery and subsequently performed for weekly for 7 weeks post surgery. At 14 days post surgery, mice were divided into 4 groups: saline (n=8), pFUS (n=8), MSC (n=8), and MSC+pFUS (n=17). Mice received either 3 consecutive days of saline, pFUS, MSC, or MSC+pFUS starting on day 14 post surgery. Laser Doppler imaging revealed differences in perfusion compared to saline, pFUS, or MSC alone. At 7 weeks post EIA, 36 mice were euthanized and tissues were harvested for histology and fluorescence microscopy.

Results: LDPI demonstrated significant (p<0.01) differences between (MSC+ pFUS) versus saline, pFUS, and MSC groups when treatment was delayed 2 weeks after CLI (Figure 1). Perfusion significantly increased with the MSC+pFUS treatment out to 7 weeks compared to other cohorts. Histological examination of muscle revealed significant increase (p<0.05) in CD31+ cells and vascular density treated with MSC+pFUS compared to other groups (Fig 1b,1c). Mice were euthanized on day 15-post surgery and human cells were counted in CLI muscle to determine if pFUS would enhance homing of infused cells in CLI model. Following IV MSC alone, MSC detected in the ischemic limb 101.0±67.4 compared to mice that received pFUS+MSC, MSC in the ischemic limb was 306±175 (p<0.01 for MSC alone vs. pFUS+MSC). We also observed ~4-6 fold increases (p<0.05) in human VEGF and IL10 expression in MSC+pFUS compared to MSC alone groups.

Conclusions: This study demonstrates that pFUS enhanced homing of IV MSC to targeted muscle resulting in reperfusion and neovascularization in CLI model compared to MSC alone. pFUS preconditioning effects in the ischemic muscle stimulated local molecular changes in the tissue microenvironment that when combined with MSC infusion increased stem cell numbers and potency by producing increased amount anti-inflammatory faction that lead to increased perfusion compared to MSC alone. The ability of pFUS to modulate the molecular microenvironment in chronic diseased tissue opens the possibilities for enhancing cellular therapies in regenerative medicine and potentially improves clinical outcomes in patients suffering from CLI.

Figure 1 contains the results from the LDPI studies at 7 weeks post surgical ligation of the external iliac artery demonstrating clear differences in perfusion in the lower extremities for the saline control, pFUS and MSC alone groups versus the (pFUS+MSC)x3 group. Figure 1A is a graph of the ratio of measured perfusion in the ischemic limb/contralateral limb. The only group that showed an increase in perfusion starting 2 weeks after intervention with pFUS with or without MSC or MSC alone is the cohort of mice that received both treatments. The pFUS+MSCx3 group of mice had approximately 60% recovery of perfusion to the hind limb compared to the other cohorts of animals. Figure 1B contains representative sections from the hamstring muscle stained for CD31+ endothelial cells (brown cells) clearly showing an increase in vascular density in the pFUS+MSCx3 group of mice compared to the other groups (Fig 1C).
A novel ultrasound guided therapeutic system for hypertension treatment

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Objectives: Hypertension affects 1.2 billion people worldwide. A novel method to address this need is ablation of the renal (kidney) sympathetic nerves which run along the renal arteries (“RDN”). In contrast to catheter-based RDN, which relies on intravascular delivery energy through the wall of the artery to ablate renal nerves, the Kona Medical Surround Sound™ system delivers non-invasive Focused Ultrasound energy from outside the body.

Methods: Surround Sound® System is a fully-integrated, self-contained system which can be used in virtually any exam room in a hospital, office or clinic, and does not require a catheterization lab. The system administers focused therapeutic ultrasound to the renal nerve complex using a custom therapeutic transducer array with a unique shape consisting of 205 individually phased elements that can provide more than 800W peak acoustic power. The clinical dose includes 14 lesions delivered over a 2.8 minute period. A separate ultrasound imaging probe is used to identify the renal artery position and an optical tracking system transforms the target location position via the imaging array to a motor that steers the therapy array to the selected target. A fully automated image tracking algorithm corrects for target motion while interleaved imaging and therapy pulses provide monitoring during the entire treatment. Beamforming and target motion tracking utilize fast, GPU computation to incorporate robotic, closed-loop motion control for 3D energy delivery, focusing, and positioning in real-time.

Results: 69 patients with uncontrolled hypertension were treated in the first 3 clinical trials (WAVE I, II and III) using the Kona Surround Sound™ system. 64 out of 69 subjects have completed at least one year follow up with an average systolic and diastolic blood pressure reduction of 23.8 mmHg/10.3 mmHg at one year. No devices related unanticipated serious adverse events occurred. This data suggests that externally delivered ultrasound appears to be safe with an encouraging efficacy signal. A blinded, randomized, sham-controlled clinical trial (Wave IV; 132 patients) is currently enrolling at leading health care institutions in the United Kingdom, Czech Republic, Germany, New Zealand, and Poland. 81 eligible study participants have been treated to date.

Conclusions: The Surround Sound system, which is developed to provide a one-time, non-invasive procedure to treat hypertension, has the potential to greatly reduce cost, lower risk, and improve access to millions of hypertension patients worldwide who are not adequately controlled by drug therapy. The initial clinical data have shown that ablation of renal nerves using Surround Sound system can result in profound and lasting reduction in blood pressure. The technology platform in which the system targets specific tissue while automatically tracking movement and continuously enabling therapy delivery through a custom shaped high power therapeutic phased array, could be easily adapted and used in other clinical applications.
Ultrasound ablation enhances nanoparticle accumulation and survival in mammary carcinoma models

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Objectives: Magnetic Resonance-guided Focused Ultrasound (MRgFUS) ablation is a noninvasive method for treating solid tumors. However, ablation of the entirety of the tumor may not be possible due to constraints imposed by surrounding tissue, resulting in a small rim of viable tumor following thermal therapy. Therefore, augmentation of MRgFUS with chemotherapeutics such as liposomal doxorubicin may be necessary. These formulations reduce systemic toxicity by encapsulating non-bioactive copper-doxorubicin crystals which dissociate in the low pH of the tumor microenvironment. Our ultimate goal is to use Focused Ultrasound (US) within curative protocols in the mouse and in human medicine.

Methods: All studies were approved by the UC Davis Institutional Animal Care and Use committee. Tumors were generated in FVB/n mice expressing an activated form of ErbB2/neu, a system modeling human HER2 amplified breast cancer. These tumors were then transplanted into the mammary fat pads of wild type FVB/n mice. The results were validated in another mammary carcinoma model (4T1 in BALB/c mice).

US ablation was accomplished under Magnetic Resonance (MR) guidance via either a 20 s insonation at a single point or a 60 s insonation in a circular pattern (16 element annular array with a 3 MHz central frequency, 3.1 MPa peak negative pressure (PNP)). Both protocols induced temperatures >65°C and CEM43>5000. Hyperthermia (41°C) was induced using a 192-element transducer with 128 therapeutic elements operated with a center frequency of 1.5 MHz and 1.1 MPa PNP, under US guidance and a needle thermocouple for temperature monitoring.

Temperature-sensitive and long-circulating doxorubicin liposomal formulations (Dox-TSL and Dox-LCL, respectively) were evaluated in combination with each ablation protocol. Accumulation of 64Cu-labelled liposomes was assessed with positron emission tomography (PET) and autoradiography following each of the ablation protocols.

Results: Ablation enhanced accumulation of 64Cu-LCL in the remaining rim of viable tumor (Fig. 1), with a 5-fold increase in nanoparticle and 50-fold increase in local drug concentration. With the combination of a 5 mm circular ablation region and Dox-LCL, tumors were eliminated in all mice and recurrence was not observed within 180 days. Integrating this sphere of ablation with Dox-TSL achieved a durable response with 4 treatments in 75% of mice treated. Alternatively, preapplication of hyperthermia to enhance release of Dox-TSL prior to the application of the spherical region of ablation produced durable response in 100% of mice within 4 treatments.

Conclusions: In conclusion, initial results for the combination of hyperthermia, ablation and liposomal doxorubicin in a fully immunocompetent aggressive syngeneic model demonstrated multiple opportunities to achieve a durable response in local disease.
Targeted drug delivery with cyclodextrin-based nanocarriers and Focused Ultrasound triggering

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Objectives: The Nanoporation project set out to explore specific solutions to overcome the current challenges of targeted drug delivery (TDD) to tumours using Magnetic Resonance Imaging guided Focused Ultrasound (MRgFUS) to cavitate microbubbles (MBs) for increasing cell permeability and to open ‘drug nano-capsules’ to release proven active anticancer drugs directly to the tumour site with reduction of systemic drug dosage needed for the desired therapeutic effect. The work reported here aimed to develop novel nanocarriers for existing anticancer drugs, by establishment of human cancer cell models to evaluate the carriers’ encapsulation efficiency in vitro and in vivo, by using animal models and a clinical MRgFUS system to investigate the carrier-drug vehicles’ in vivo distribution and localised drug release / cellular drug uptake.

Methods: A novel γ-cyclodextrin (γ-CD) based carrier for encapsulation of doxorubicin (DOX) was synthesised and fully characterised. The encapsulation efficiency was assessed under various temperatures and pH levels by both chemical analysis and in vitro human cancer cell modeling with KB and HCT116 cells. A computer-controlled high-throughput in vitro FUS device facilitating exposure to High Intensity FUS fields in a standard 96-well plate was designed and applied. It allows maximum flexibility in choosing experimental parameters in combination with carrier-DOX inclusion. SonoVue® MBs was used to investigate TDD in cell monolayers. An extensive in vitro study had been carried out to investigate both mechanical and thermal effects of ultrasound. An unique MRgFUS system compatible animal house were designed and built to allow ex vivo and in vivo experiments by using small rodents. Ex vivo and in vivo trials were carried out with a clinically approved ExAblate MRgFUS system (InSightec, Israel) to establish a safe and efficient clinical TDD protocol on small rodents.

Results: The desired γ-CD based carrier greatly reduced DOX’s toxicity and the carrier-DOX inclusion was highly stable under physiological temperature conditions as well as under a wide range of acidic conditions (pH 1.0 ~ 7.0); the encapsulated DOX is slowly released under hyperthermic conditions (up to 50 °C). In the presence of MBs, application of FUS with low mechanical indexes, under which no thermal effect was observed, enhanced the drug uptake into tumour cells for both encapsulated and free DOX. Optimal setups of MR parameters and FUS parameters were identified ex vivo and in vivo, allowing application of MRgFUS treatments to 4 live mice bearing tumours (human colorectal carcinoma, up to 1059.71 mm3) under anaesthesia with full recovery.

Conclusions: The study demonstrated the possibility of translation of the constructed γ-CD derivative to potential clinical use as a delivery vehicle for DOX using combined thermal and mechanical release mechanisms by clinically applicable MRgFUS—triggered TDD with the potential for cancer therapy, it provides better understanding of the mechanism of and potential for this novel delivery approach: MRI-guided, ultrasound-mediated, site-specific drug delivery assisted by MB contrast agents. The chemical modifications and in vitro, ex vivo and in vivo preclinical studies discussed here represent only a glimpse into the future of clinical application. It is likely that this novel technology will enter the clinical arena in the near future, based on the ever-increasing scientific contributions from researchers.
AFM cell surface morphology of KB cells after sonication with and without MBs

MRgFUS compatible small animal house for ex vivo and in vivo study

MRgFUS ex vivo study
Objectives: Thermosensitive liposomes (TSLs) have been reported to accumulate and deliver an effective therapeutic to tumours with the application of hyperthermia. However there is limited understanding of the effect of pharmacokinetics, the time frames and the duration of hyperthermia applications. For instance, the timing between drug injection and application of focused ultrasound (FUS) needs to be optimised considering nanoparticle biodistribution in the tumour and drug release. Near-Infrared Fluorescence (NIRF) imaging offers an easy and sensitive method to follow the biodistribution of dye-labelled theranostic nanoparticles and drug release in real time and could act as a useful preclinical surrogate for other forms of imaging to help understand the mechanism of drug kinetics in the tumours. In the present study we examined the biodistribution of novel dual MRI and NIRF-labelled thermosensitive liposomes (image guided iTSLs), and we modulate their distribution with hyperthermia induced by a high intensity focused ultrasound transducer (FUS).

Methods: We have synthesized lipids that can be used for imaging in mice by MRI and NIRF. We have prepared iTSLs with the optimum concentration of imaging lipids for thermally triggered release and we developed them in combination with a FUS treatments regimen (thermal dosing). Labeling of liposomes for imaging can provide substantial information of the mechanism of tumor uptake (post injection) and provide insight on the reasons why this uptake is more enhanced in FUS treated tumors. We prepared iTSLs to encapsulate the anticancer drug topotecan (Hycamtin®), a chemotherapeutic agent which when released in vivo can be monitored by its intrinsic drug fluorescence. We have optimized drug encapsulation for maximum fluorescence signal difference (before /after release) for both in vitro and in vivo. FUS (TIPS Phillips) was applied using temperature feedback via subcutaneously placed fine-wire thermocouples to maintain hyperthermic temperatures. NIRF imaging was performed using multispectral analysis bioimaging (Maestro EX) following the emission of the NIRF lipid and topotecan. FUS was applied using imaging as guidance. FUS was applied 30 min post injection and the tumors were monitored. In a separate group of animals FUS was applied at 30 and 90 min post injection. MRI imaging confirmed observations obtained with NIRF imaging.

Results: iTSL tumour accumulation was detected using NIRF imaging immediately after liposome administration, this is due to the enhanced permeability and retention effect. Mice were bearing tumours at both flanks allowing one tumour to act for control (no FUS treatment). FUS-induced hyperthermia (3 min at 42 °C, 30 min post i.v.) greatly enhanced liposomal uptake as seen by imaging. A co-localised, enhancement of topotecan fluorescence emission was also observed immediately after application of hyperthermia indicating rapid thermally triggered drug release within the area of the tumour. Topotecan is used as an anticancer drug model and its intrinsic fluorescent properties allow to easily follow thermal drug release. The phenomena of increased iTSL accumulation and concomitant topotecan release appeared to be amplified by a second mild hyperthermia treatment applied 1 hour after the first. NIRF imaging detected the signal coming from the liposomes (NIRF incorporated lipid) indicating that liposomes accumulated in tumours post injection. This accumulation was substantially enhanced after each application of FUS. Topotecan fluorescent appeared transient in the tumour indicating phenomena of cell uptake and DNA binding. MR imaging also confirmed enhanced iTSLs uptake due to the FUS treatments in the same animals that were imaged using NIRF imaging.

Conclusions: In this study we have formulated a theranostic novel dual MRI/NIRF labeled thermosensitive liposome (iTSL) that encapsulates the anti-cancer agent topotecan and enables real time/diagnostic imaging, making use of pre-clinically and clinically relevant imaging modalities. Image-guidance in turn enables the application of brief, moderate intensity FUS treatments that greatly increase iTSL tumor uptake and set up the possibility for substantial FUS triggered topotecan release within the tumor volume. FUS hyperthermia applied during the half-life of the iTSL in plasma promotes iTSL accumulation in the
tumors and takes advantage of the high concentration of the liposomal drug in plasma seen immediately after injection. Should these effects be translated to the clinic, this suggests substantial benefits to cancer patients and improvements of the standard of the chemotherapy treatments for both primary and metastatic tumors.
Near infrared fluorescence imaging for Focused-Ultrasound mediated local drug delivery of doxorubicin

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Objectives: High-intensity Focused Ultrasound (FUS) is valuable clinical technique for non-invasive thermal tissue ablation. Its capability for localised drug delivery has also been investigated recently, with application to cancer therapies. The ability of thermosensitive liposomes (TSLs) to accumulate and deliver a therapeutic drug to tumours under application of hyperthermia has been well reported but there is limited understanding of the time frames and kinetics involved. For instance, the optimal delay (if any) between injection of the therapeutic and application of heat. Near-Infrared Fluorescence (NIRF) imaging offers a sensitive method to follow the biodistribution of dye-labelled theranostic nanoparticles in near real time and acts as a useful preclinical surrogate for other forms of imaging (such as PET or MRI).

Methods: Our iTSLs (imageable TSL) containing doxorubicin (0.8 mg/mL) were formulated with different molar ratios of key lipids, and a dye-labelled lipid - XL750-DSA. In vitro studies were performed to characterise the temperature-triggered doxorubixin release by measuring changes in its intrinsic fluorescence. In vivo experiments were performed on SHO mice bearing subcutaneous MDA-MB-231 tumours on both flanks. For bioimaging experiments, iTSLs were injected i.v. with and without any hyperthermia induced by FUS (TIPS, Philips) and controlled by three thermocouples placed around the tumour. TSLs and doxorubicin were respectively monitored by recording the emitted fluorescence in the NIR and the blue regions of the spectrum. Finally, tumour sizes were measured by calliper for 3-4 weeks following treatment.

Results: In vitro release studies showed minimal leakage of the drug at 37°C over a 10 min period, while the release was almost immediate when the temperature was raised to 41°C. In vivo, a first hyperthermia event (3 min at 43°C) was applied on one of the two tumours before iTSLs administration. After 30 min, the iTSLs were injected i.v. (tail vein) and their tumour accumulation was monitored by tracking the NIRF signal. A second hyperthermia treatment (3 min at 42 oC) was then applied 45 min later in order to release the doxorubicin. As a consequence of this regimen, the mice showed a very significant NIRF intensity increase in the treated area. At the same time, doxorubicin release was observed but only for a short period of time. The combination of this protocol & doxorubicin-iTSLs showed a significant impact on tumour growth, including tumour eradication in some cases.

Conclusions: NIR fluorophores carried by the iTSLs allowed the monitoring of the liposomes’ biodistribution over a period of several weeks post-injection in our mouse model. Mild hyperthermia applications on the tumour greatly favoured nanoparticle uptake. The hyperthermia also had the effect of releasing encapsulated doxorubicin, therefore improving the drug delivery to the tumour. Finally, a strong correlation between thermal dosing and tumour targeting was observed with an increase of the NIRF signal in the heated area with a subsequent enhanced therapeutic effect.
Focused Ultrasound 2016

Histology (H&E). The tumours were excised 48 hours after the treatment. Four micrometer H&E slices of MDA-MB-231 tumors untreated and treated. The arrows indicate the large necrotic areas only found when the combination of drug and hyperthermia was applied.

Treatment efficacy: Mice were injected i.v. with F5-XL750 (6mg/kg) and were treated twice by FUS hyperthermia applied to the right side.
Interleaved mapping of temperature and longitudinal relaxation rate monitor drug delivery from temperature sensitive liposomes during MR-HIFU induced hyperthermia

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Objectives: Not released for publication
Methods: Not released for publication
Results: Not released for publication
Conclusions: Not released for publication
Vascular permeability changes following doxorubicin release from temperature sensitive liposomes are detected by dynamic contrast enhanced MRI

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Objectives: Thermally sensitive liposomal formulations of chemotherapeutics offer reduced systemic toxicity while achieving lethal concentrations at the tumor site via localized heating. The substantial local release of drug into the tumor vasculature can cause immediate changes in vascular permeability. Our goals in this study were to assess the rapid extravasation of doxorubicin from the vasculature and to examine the feasibility of dynamic contrast enhanced (DCE) MRI as a method to assess doxorubicin release from temperature sensitive liposomes.

Methods: All studies were approved by the UC Davis Institutional Animal Care and Use committee. Female FVB mice bearing bilateral NDL tumors had one tumor insonified (center frequency of 1.5 MHz, 1.1 MPa peak negative pressure (PNP)) to 42 °C. Tumor temperature was maintained for 5 minutes upon which time 6 mg/kg doxorubicin encapsulated in temperature sensitive liposomes was injected intravenously. The tumor temperature was kept at 42 °C for an additional 20 minutes. Control groups consisted of hyperthermia only, drug only, and no treatment controls.

Changes in permeability of the tumor vasculature following treatment were assessed with DCE-MRI. A T1w gradient echo (TE/TR/FA: 2.7ms/100ms/30°; FOV = 4 x 2 cm, matrix = 160 x 80; 11 slices; 140 repetitions) was acquired with gadolinium contrast (Prohance, 0.3 μmol/g) injected as a bolus. Regions of interest (ROIs) were drawn over the treated and the contralateral tumors and intensity data was extracted as a function of time. The signal intensity was converted to contrast agent concentration using the R1 of each tumor and the relaxivity of Prohance. The concentration versus time curve was used as input to a two-compartment pharmacokinetic model. Kinetic parameters were derived from the fit.

Results: We found that treatment with drug + hyperthermia resulted in a decrease of Ktrans from 0.486 min-1 to 0.223 min-1 while tumors treated with hyperthermia exhibited an increased Ktrans to 0.925 min-1 in the absence of drug (Fig. 1). The apparent decrease in permeability in tumors receiving drug + hyperthermia was observed immediately following treatment but was no longer significant at 48 hours. Hematoxylin & eosin (H&E) stained sections revealed hemorrhage in tumors treated with drug + hyperthermia suggesting immediate gross changes in tumor vasculature resulting from intravascular release of doxorubicin in hyperthermic tumors. H&E stained sections of tumors treated with hyperthermia alone appeared identical to tumors receiving no treatment.

Conclusions: DCE MRI offers promise for evaluating local release of doxorubicin form liposomal carriers via the drug’s ability to alter vascular permeability. Moreover, DCE MRI can potentially be utilized as a non-invasive technique to evaluate chemotherapeutic release in vivo of new temperature sensitive liposomal drug formulations.

H&E of an (A) insonified tumor and its (B) untreated contralateral 2 hours post treatment. (C) Contrast agent versus time curves of the above tumors. (D) Comparison of tumors treated with hyperthermia + drug to tumors treated with hyperthermia alone.
Objectives: Magnetic Resonance guided Focused Ultrasound (MRgFUS) ablation is a noninvasive method to deliver a precise, and lethal, thermal dose to solid tumors. During thermal ablation, tissue temperature typically exceeds 50-60 °C and results in rapid coagulative necrosis. Such ablation often results in “heat fixing” of the tissue which is thought to restrict the diffusion of molecules into and out of the ablated region. In this study we used gadoteridol (MW 558D) to evaluate the extent to which small molecules could be delivered to tissue following thermal ablation.

Methods: All studies were approved by the UC Davis Institutional Animal Care and Use committee. MRgFUS ablation of syngeneic murine mammary carcinoma (FVB/n mice expressing an activated form of ErbB2/neu) was accomplished via a 60 s insonation in a circular pattern (16 element annular array with a 3 MHz central frequency, 3.1 MPa PNP). This ablation protocol resulted in temperatures >65°C and CEM43>5000.

Animals were injected with intraperitoneal gadoteridol either immediately before or after treatment (0.05 mmol/kg) or with intravenous gadoteridol before or after treatment. Mice were then imaged with a T1w scan (TE/TR = 12.5/750 ms, FOV = 3.2 cm × 3.2 cm, MTX = 256 × 256, ST/SI = 1/1 mm, and 9 slices) at 0.5, 1.5, 3, 6, 20, and 48 hours following ablation. The ablated region and quadriceps were then manually segmented and the tumor-to-muscle (T/M) ratio measured. In addition, two control groups of mice were also imaged with T1w MRI, a set treated with circle pattern MRgFUS and but no gadoteridol and a set not treated with ultrasound, but injected with intraperitoneal gadoteridol.

Results: Immediately following ablation, the T1w signal of the ablated volume increased, beginning with the rim. The T1w T/M signal ratio progressively increased until 1.5 hours post-ablation where it peaked (Fig. 1). The T/M ratio remained elevated compared to pre-ablation values for up to 6 hours post-ablation. Control animals treated with the same ablation protocol without gadoteridol injection did not exhibit an increase in T1w signal post-ablation. At 20 and 48 hours post-ablation, the T1w signal had decreased to pre-ablation levels and re-injection of gadoteridol at these time points had no effect on tumor T1w signal. Thus, the small molecule gadoteridol accumulated within the tumor, specifically within the ablated volume, for several hours following ablation.

Conclusions: The results suggest that there is a window of time post-ablation when small molecule drugs can be successfully delivered to ablated tissue.
Transcutaneous boiling histotripsy ablation of the kidney in an in vivo porcine model

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Objectives: Boiling histotripsy (BH) is a pulsed Focused Ultrasound (FUS) technique that uses milliseconds-long pulses at low duty cycle to mechanically ablate tissue. Our group is developing BH as a non-invasive treatment for renal masses. The aim of this study was to evaluate the feasibility of transcutaneous BH ablation of renal tissue in an in vivo porcine model.

Methods: Pigs (wt = 37-40 kg, n=6) were anesthetized and placed in the lateral position. A 1.5 MHz FUS transducer was submerged in a bath of degassed water, with 10% glycerol, coupled to the abdomen. Transcutaneous BH (1-10 ms pulses, duty cycle 1%) was applied transcutaneously under ultrasound (US) image guidance, targeting the lower pole of n=12 kidneys. Volumetric lesions (n=25) containing cortex, medulla, and renal sinus were created by delivering 5-30 pulses/focus to a grid of foci spaced 1 mm apart. Following BH, US assessment of the treated regions was conducted. Necropsy was then performed and the kidneys were collected for gross, histologic, and ultrastructural assessment.

Results: BH was feasible in 11/12 kidneys, with one kidney being inaccessible due to positioning entirely above the costal margin. During sonications hyperechoic bubbles were visible at the focus with treatment producing loss of tissue echotexture consistent with BH. Post-treatment US revealed well defined hypoechoic cavities consistent with BH treatment effects. At necropsy no gross evidence of collateral thermal damage was appreciated within the beam path. On gross inspection, small clots were seen within the collecting system in 8/11 kidneys with regions of petechial hemorrhage surrounding a centrally located homogenized volume (Figure) of parenchyma. Histologically, all BH exposures produced completely homogenized cortex sharply demarcated from histologically normal untreated tissue. In the medulla, blood was noted within the collecting ducts with homogenized tissue in the central portion of the targeted volume at higher dose exposures. Ultrastructural evaluation showed fully ablated lesions containing erythrocytes and echinocytes; a few intracellular organelles (e.g. mitochondria) were observed in vessels and in intracellular spaces surrounding the lesion. Within the wall of the collecting system, focal petechial hemorrhage with disaggregation of collagen bundles was visualized without disruption of the wall.

Conclusions: These data indicate that in vivo transcutaneous BH treatment of the kidney is feasible in a large animal model and represents the successful application of transcutaneous histotripsy in different renal tissues. Studies are ongoing to optimize treatment parameters with the goal of clinical BH renal ablation.
Molecular changes that potentiate mesenchymal stem cells to further improve acute kidney injury

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Objectives: Pulsed Focused Ultrasound (pFUS) enhances homing of IV-infused mesenchymal stem cells (MSC) to kidneys during cisplatin (CIS)-induced acute kidney injury (AKI). pFUS serves as a neo-adjuvant to MSC therapy where sonicating kidneys with IV MSC leads to better AKI outcomes than MSC therapy alone. Nearly twice as many MSC home to pFUS-treated AKI kidneys, but >10 times as much interleukin (IL)-10 is produced by MSC that home to pFUS-treated kidneys. This result suggests that pFUS sonications modify the renal microenvironment to increase potency of MSC that home to pFUS-treated kidneys. Interferon-g (IFNg) increases MSC potency and is upregulated in kidneys by pFUS. This study investigates pFUS-induced IFNg expression in kidneys as an activator of MSCs that improves their therapeutic efficacy during AKI.

Methods: C3H or IFNg-ko mice received CIS (15 mg/kg ip), kidney pFUS (4 MPa; 5% duty cycle) and/or MSC (106 human MSC). IV MSC injections were performed 3-4 hr post-pFUS. siRNA was used to knockdown IL-10 in some experiments. Experimental groups included AKI only, AKI+pFUS, AKI+MSC, AKI+pFUS+MSC, and healthy mice. Mice received CIS on Day(D)0 and pFUS/MSC on D1. Mice were euthanized on D4. One-way analysis of variance used Bonferroni post-hoc with p-values <0.05 considered significant.

Results: Following pFUS to the kidneys of IFNg ko mice, more MSC homed to sonicated kidneys (~2 fold) but did not lead to improved AKI [serum blood urea nitrogen (BUN) or serum Creatinine (SCR)] outcomes compared to mice that received MSC injections alone. Levels of BUN and SCR, as well as expression of kidney injury molecule 1 (KIM1), were all significantly reduced by MSC treatment alone, but not further reduced by combination pFUS/MSC treatment like was previously observed in wild-type mice. Furthermore, the production of IL-10 by MSC seen in wild-type mice treated with pFUS+MSC was absent in IFNg-ko mice. Lastly, the improved AKI outcomes using pFUS+MSC in wild-type mice were erased when mice were given MSC that had been pretreated with siRNA to knockdown IL-10.

Conclusions: pFUS creates a molecular zip code in AKI kidneys that enhances MSC homing. While MSC infusions alone improve AKI in IFNg KO mice, pFUS+MSCs did not replicate the additional improvements in outcomes seen in wild-type mice. While pFUS-independent mechanisms of AKI repair by MSCs do not require renal IFNg, the pFUS-dependent mechanism that yields improved recovery does depend on preconditioning of the parenchyma. IFNg upregulated by pFUS mediates the neoadjuvant function of pFUS. Furthermore, the IFNg upregulated by pFUS serves to enhance MSC function by increasing MSC production of IL10, which has previously been shown to improve AKI. These data demonstrate that an IFNg/IL10 molecular axis is activated by pFUS and critical for improved AKI outcomes. Moreover, these results provide justification in incorporating pFUS as a treatment to improve MSC therapy during AKI, which often has limited clinical therapeutic options.

MSC better improve AKI with pFUS to increase renal IFNg and MSC IL10 expression. MSCs with reduced IL10 or IFNg-deficient mice to not see AKI benefit from pFUS+MSC.
Use of focused beams of special structure for pushing and trapping kidney stones with acoustic radiation force

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Objectives: Focused ultrasound not only causes lesions in biological tissue, but can effectively push the medium in the focal region. This effect, associated with the ability of ultrasound to remotely deliver and transform momentum to an absorbing or scattering target, is called radiation force. Until recently, therapeutic applications of this effect did not exist, although it has been employed in ultrasound imaging, e.g. shear wave elasticity imaging. Previously, our team developed a technology to reposition kidney stones with radiation force. In a clinical trial, the technology was used to transcutaneously facilitate passage of small stones and to relieve pain by dislodging larger obstructing stones. While successful, the trial revealed a need for optimization of the ultrasound beam structure, frequency, and intensity to make it more effective.

Methods: Two aspects of the kidney stone repositioning were studied. First, we investigated the optimal ultrasonic beam diameter vs. stone size. Numerical modeling was performed to calculate the force acting on an elastic sphere in liquid from a focused beam. Simulations were performed for a given acoustic power, changing the ratio of the beam width to the diameter of the stone. The second study investigated the feasibility of trapping and moving a stone in a direction transverse to the beam axis. Toward this goal, vortex beams were considered that produce a ring-like intensity distribution in the focal region with zero on-axis intensity. Theoretical modeling was performed using a previously developed algorithm. Acoustic trapping and manipulation of kidney stones in water was investigated using both a single-element transducer (combined with a sector-shaped phase plate) and sector arrays with operating frequencies 0.3–1.5 MHz. Human stones approximately 3–5 mm, as well as glass and aluminum beads, were displaced in a water bath.

Results: The numerical modeling indicated that pushing a kidney stone is strongest when the beam width is slightly wider than the stone diameter. This can be explained by more effective generation of shear waves inside the stone resulting from their effective coupling with the acoustic waves in liquid at the stone edges. During exposures with the vortex beams, stones could be drawn to the beam axis and then controllably translated along the surface in any direction transverse to the beam. The phase between sector elements could be used to control the vortex size and thus adjusted for trapping different-sized stones.

Conclusions: The intensity distribution in the focal region significantly influences the ability to manipulate kidney stones using radiation force. To achieve the strongest force with a predetermined acoustic power, it is necessary to select a mode in which the focal waist diameter slightly exceeds the stone diameter. For transverse confinement, it is convenient to use vortex beams radiated by sector arrays or single-element sources with an attached phase plate. In such an approach, the beam diameter can be controlled by choosing a proper phase distribution on the source surface.
**Modulated Focused Ultrasound for treatment of de-myelinating axons in multiple sclerosis lesions — pilot animal studies**

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**Objectives:** Multiple sclerosis (MS) is a debilitating disease of the central nervous system whose symptoms arise from de-myelination of axons within brain and spinal cord tissue. De-myelination can lead to loss of central and peripheral function, including vision and muscular problems. There currently exists no cure for multiple sclerosis. In this exploratory study, we seek to induce re-myelination of axons de-myelinated by an MS model using pulsed Focused Ultrasound (pFU). There exists a rich history targeting the use of ultrasound to temporarily and non-destructively activate central neural circuits. Our own work has shown that pFU can induce detectable electroencephalography (EEG) signals in the brain. Recent work by Gibson et al. (2014) who showed increased myelin thickness of neurons that had been activated by laser light in an optogenetic mouse model. It seems plausible, therefore, that ultrasound induced neural activation could affect myelin growth and thickness.

**Methods:** We hypothesize that pFU activation of axons within MS lesions in a rodent model will decrease de-myelination and increase re-myelination. To test this, we selected the cuprizone model for MS, which causes de-myelination in the corpus callosum of the mouse brain and is reversible, allowing us to assess the effects of ultrasound during the phase of disease progression and during the phase of disease recovery. Rodents undergoing the MS model received five consecutive days of pFU therapy and underwent EEG monitoring. We applied pFU at two different time points, first during the demyelination process and second (in separate groups of animals) during recovery from demyelination. The pFU therapy protocols were inspired by the optogenetic stimulation protocols of Gibson et al (2014), and consisted of 30 seconds of pFU application followed by 90 seconds of rest, repeated 15 times. We tested three different pFU transducers with center frequencies of 0.65 MHz, 1.09 MHz, and 2.0 MHz, all with Ispta of 1.2 W/cm²*s. For each of those frequencies, we verified that each pFU protocol could activate brain. We collected Magnetic Resonance (MR) images at three time points and collected the brains for histological analysis at the end of the study.

**Results:** Initial results show that mice undergoing the MS model exhibit different shapes in their pFU-induced EEG signals than normal healthy mice. Further results of the therapeutic pFU treatment following analysis of MR and histological results will be presented.

**Conclusions:** If successful, this non-invasive therapy may lead to rapid advancements in the treatment of MS and other de-myelinating neurological disorders.
Noninvasive peripheral nerve stimulation via Focused Ultrasound \textit{in vivo}

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Objectives: The leading technique to treat peripheral neurological disorders is currently through implantation of electrodes along the peripheral nerve and stimulating the nerve with electrical current. Recently, Focused Ultrasound (FUS) has been shown to elicit modulation of both brain and peripheral neurons. While the effects of the FUS brain stimulation experiments have been shown \textit{in vivo}, the majority of the peripheral nerve stimulation studies have been \textit{in vitro}, or only investigating the effects of FUS stimulation on the nerve itself. Thus far, there have been no studies determining if non-invasive stimulation of peripheral nerves with FUS can elicit physiological effects \textit{in vivo}.

Methods: Peripheral nerve stimulation was conducted on both hind limbs of 13 mice under general anesthesia (pentobarbital 65mg/kg). Both the imaging and stimulation transducers were mounted interchangeably on a 3-DOF positioning system with 1-mm resolution. Targeting of the sciatic nerve was achieved using a 1282element imaging probe with a center frequency of 18.5 MHz. A single element transducer (3.57 MHz center frequency, 90% duty cycle, 1 kHz PRF, 5-10ms sonication duration, 3.65 x 0.45 mm focal area) was used for stimulation of the sciatic nerve. Evoked EMG activity in the Tibialis Anterior muscle was recorded via two stainless steel electrodes sampling at 2kHz. Gross pathology was used to assess safety of the procedure. Behavioral testing occurred an hour before, and 24 hours after FUS stimulation. Electrophysiology experiments were used as positive controls with electrodes directly stimulating the sciatic nerve while recording EMG from the Tibialis Anterior muscle. Negative controls were conducted via administration of 5mg/kg lidocaine to the stimulation region as well as clipping the nerves downstream from stimulation.

Results: Stimulation of the sciatic nerve was successful eliciting both muscle movement and EMG activity. Two distinct responses to stimulation were observed, a fast and slow activation (2.1 ±2.6ms and 18.8±5.5ms average delay following onset of FUS stimulation). The fast activation was accompanied by an average higher peak-to-peak EMG response than the slow responses (0.86±0.87, 0.19±0.63 mV respectively). EMG spike duration for both responses were similar and not significantly different (6.9±2.6, 7.1±2.2 ms respectively). Administration of lidocaine IM to the target region eliminated EMG responses from FUS stimulation. An hour following lidocaine administration EMG responses were detected again with FUS stimulation of the nerve. Electrical stimulation resulted in similar EMG spike durations to FUS stimulation, although at significantly higher peak-to-peak responses (7.3±1.6 mV average). Gross pathology and behavioral testing revealed no significant damage (no red blood cell extravasation, significant difference in distance traveled, rotations or gap distance) with FUS stimulation at the powers used for stimulation.

Conclusions: In this study, we show physiological effects of \textit{in vivo} FUS stimulation of peripheral nerves in the mouse for the first time. FUS stimulation can safely elicit muscle activation with no short-term behavioral effects. Positive controls verified EMG responses similar to that of electrical stimulation while negative controls verified FUS was stimulating the nerves and not the muscle tissue. These results support the further investigation of FUS-based techniques for the treatment of peripheral neurological disorders.
The mechanosensitive TRPC1 channel is activated by pulsed Focused Ultrasound to induce stem cell homing

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Objectives: Stem cell therapies are promising regenerative medicine approaches. Pulsed Focused Ultrasound (pFUS) induces microenvironmental changes in normal and diseased tissues that enhance homing and efficacy of intravenously-infused mesenchymal stromal cells (MSC) to further improve disease outcomes. pFUS/tissue interactions that induce molecular changes is unclear. Mouse muscle and kidneys were sonicated at increasing powers while passive cavitation and tissue displacement were measured. Tissue was measured for cyclooxygenase-2 (COX2) to correlate with ultrasound effects, as COX2 signaling is critical for MSC homing. To test that mechanotransduction causes the necessary molecular changes, inhibitors of mechanosensitive channels were given before pFUS as well as pFUS to TRPC-knockout mice, as TRPC can function as a mechanosensitive receptor.

Methods: C3H or TRPC-ko mice received hamstring or kidney pFUS from a VIFU 2000 system. pFUS was at 1 MHz, 5 Hz pulse repetition frequency, 5% duty cycle, and varying transducer output powers (ranging from 10-80W). A built-in hydrophone passively detected cavitation. Mice were euthanized 4hr post-pFUS and COX2 expression was measured by ELISA. GdCl3 (0.04mmoles/kg) or ruthenium red (RR) (0.01mmoles/kg) was given by intravenous injection before pFUS. One-way analysis of variance using Bonferroni post-hoc with p-values <0.05 were considered significant.

Results: Statistically significant increases in COX2 were measured at 20, 40, 60, and 80W compared to untreated muscle. Mean tissue displacement correlated with COX2 expression. Statistically significant increases in cavitation were only observed at 60 and 80W. At 40W, pFUS-induced COX2 increases and MSC homing were blocked in TRPC-ko mice or when Gd or RR were administered to wild-type mice before sonication.

Conclusions: Mechanical pFUS forces interact with the mechanostretch receptor TRPC1 to drive molecular changes in tissue that are critical to stem cell homing processes. We have previously determined that COX2 expression is an acceptable proxy for molecular outcomes. At lower powers (20 and 40 W), cavitation from the sonications was not detectable, suggesting that cavitation-independent mechanical forces (i.e., acoustic radiation forces) drive COX2 expression and thus cell homing. At higher powers (60 and 80 W), cavitation was detectable and COX2 expression was elevated compared to sonications at 20 and 40 W. It is unclear whether cavitation at these elevated powers drives the additional COX2 expression, or if it is the result of increased acoustic radiation forces at higher powers. Regardless, 40 W was maximum power we previously determined not to cause detectable tissue damage and from the point of view of pFUS use in regenerative medicine, would be the maximum power for pFUS-induced MSC homing is blocked by inhibiting mechano-stretch receptors or in TRPC-ko mice
Low-intensity ultrasound prolongs lifetimes of transplanted mesenchymal stem cells

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Objectives: Mesenchymal stem cells (MSC) are currently the most clinically applicable stem cell type for transplantation. Transplanted MSCs act as “drug-pumps” by continually releasing cytokines, chemokines, and trophic factors (CCTF) that reduce host inflammation and stimulate endogenous regeneration mechanisms. MSCs have shown promise for a wide range of diseases from ischemic events to graft-versus-host disease. While MSCs are considered immune privileged and evade immune surveillance to some extent, they typically do not engraft into host tissue and usually die within 3-10 days post-transplantation. Efforts to extend the lifetimes of MSCs previously have involved chemical or genetic manipulation of MSCs prior to transplantation, but these approaches are not readily translatable. We have found that pulsed Focused Ultrasound interacts with host tissues to upregulate a variety of anti-apoptotic, pro-mitotic factors that could increase the lifespan of transplanted MSCs. In this study we tested the hypothesis that the application of daily unfocused therapeutic ultrasound

Methods: 106 human LMSCs were intramuscularly transplanted into each hamstring of C3H mice. MSCs were transfected with renilla luciferase using lentiviral vectors and expressed under the EF1 promoter. Beginning on the day of implantation (D1), mice received daily TUS sonication to one hamstring (1 MHz US, 2 W/cm², 10% duty cycle [1ms “on”/9ms “off”], 10 min total exposure time). After TUS, mice were subjected to bioluminescence imaging for 10s after receiving an intraperitoneal injection of D-luciferin (150mg/kg). Mice were euthanized on D6. Temperature measurements were obtained in different group of mice using an implanted thermocouple.

Results: TUS exposures result in a combination of mechanical and thermal effects. TUS raised hamstring temperature from 36 to 41 during the 10 min exposure. Total bioluminescence in treated and control legs decayed each successive day, but decay was slower in the TUS-treated hamstrings. Accordingly, by D6, bioluminescence was nearly undetectable in untreated legs, while still imageable in TUS-treated legs (Figure). The number of MSCs that remained in treated legs was nearly 10 times greater than in contralateral muscle.

Conclusions: The application of TUS to skeletal muscle containing transplanted MSCs extends the resident time of live MSCs in tissue by slowing MSC death/clearance rates. This has profound implications for a wide range of MSC therapies because TUS is noninvasive, safe, and inexpensive. Therefore, such a routine and uninvolved procedure to extend the survival of transplanted MSCs could easily and dramatically improve clinical cell therapies, extending the functional lifetimes of transplanted cells and reducing the number of transplantations/injections required for disease treatment.
**Red blood cells as ultrasound-triggered drug delivery vehicles: Activation via perfluorocarbon nanodroplets, targeting and photoacoustic imaging**

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**Objectives:** Red blood cells (RBC), when modified to operate as drug carriers, combine the biocompatibility of a patient’s own cells, long circulation time, large volume to sequester the drug, and ability to accumulate at the target sites (e.g., tumor vasculature) via molecular targeting. Recently point-of-care devices for RBC drug encapsulation have received CE approval mark in Europe. However, triggered release of the drug at the desired site is still complicated; therefore, we present in this study ultrasound-triggered release of entrapped materials from RBCs, by the preparation of RBC decorated with superheated perfluorocarbon (PFC) nanodroplets, and subsequent rupture of RBC membrane with contents release.

**Methods:** Fluorescent dyes, calcein and indocyanine green (ICG) and magnetic nanoparticles were loaded inside RBCs via hypotonic loading. After incubation in hypotonic media, the loaded RBCs were resealed by PIGPA buffer (adenine, glucose, inosine and pyruvate). Unincorporated material was removed by centrifugation (1000g, 5 min). Nanoparticles made of decafluorobutane and dodecafluoropentane were prepared by sonication followed by the repeated Nuclepore filtration under elevated pressure; they were stabilized with a positively charged lipid shell, made of PEG stearate, DSPC and distearoyl trimethylammonium propane. Nanoparticles were attached externally to the membranes of dye-loaded RBCs. For molecular targeting, RBCs were decorated with biotin or alphaVbeta3-specific peptide cyclo-(RGDfK), via insertion of the molecules anchored to the cell membrane via a PEG spacer. To characterize the efficacy of ligand attachment, fluorescein-PEG-lipid was used as a model. Magnetic sensitivity of iron oxide-loaded RBCs was confirmed with a N52 neodymium magnet. Peptide-mediated targeting was confirmed in a solid phase binding system, where RGDfK-PEG-DSPE RBCs were incubated with polystyrene dishes carrying recombinant alphaVbeta3, or control albumin surface. Photoacoustic imaging of ICG-loaded RBCs was performed with a custom-built apparatus (Verasonics Vantage mated with a SpectraPhysics laser). Ultrasound-triggered contents release was monitored by fluorescence microscopy: dye-loaded RBCs carrying perfluorocarbon nanodroplets were subjected to insonation.

**Results:** Fluorescent dyes were loaded into RBCs with good stability (minor leakage over many hours). Fluorescent PFC nanodroplets demonstrated stable adherence to the RBC membrane. Upon a single ten-cycle 10 MHz ultrasound pulse, dye leaked from the RBC within a fraction of a second. Specific adhesion (~65-fold over control) of targeted RBCs to alphaVbeta3-coated surface was observed. Magnetic targeting demonstrated efficient pulling of magnetic RBCs in a tube and phantom, confirmed by photoacoustic imaging of ICG-loaded RBCs. RBC-nanodroplet complexes remained in the bloodstream for at least two hours following intravenous injection, as determined with Cr-51-radiolabeling.

**Conclusions:** Red blood cells decorated with perfluorocarbon nanodroplets rapidly release entrapped fluorescent dye after a short ultrasound pulse. As a drug carrier, modified RBCs stay in the bloodstream for extended time; they can be targeted by molecular or magnetic means, and monitored by photoacoustic imaging. Overall, PFC-RBCs may be an ideal drug carrier system for ultrasound-triggered drug delivery.
HIFU for targeted antibiotic delivery and therapy of chronic wounds and osteomyelitis

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Objectives: Chronic non-healing wound infections require long duration antibiotic therapy, and are associated with significant morbidity and healthcare costs. This results in both systemic and local infection to deeper tissues (e.g. bones), thereby requiring long duration treatment (generally >6 weeks), resection of tissues, and emergence of drug resistance. Novel approaches for efficient, readily-translatable targeted and localized antimicrobial delivery, as well as targeted ablation of pathogens are a critical clinical need. The objectives of this study were to: 1) develop low temperature-sensitive liposomes (LTSLs) containing an antimicrobial agent (ciprofloxacin) for induced release at mild hyperthermia (~42°C), 2) characterize in vitro ciprofloxacin release, and efficacy against Staphylococcus aureus plankton and biofilms, and 3) determine the feasibility of localized ciprofloxacin delivery in combination with MR-HIFU hyperthermia in a rat model.

Methods: LTSLs were loaded actively with ciprofloxacin, and their efficacy was determined using a disc diffusion method, MBEC biofilm device, and scanning electron microscopy (SEM). Ciprofloxacin release from LTSLs was assessed in a physiologic buffer (serum and PBS), and in vivo in a rat model using MR-HIFU by fluorescence spectroscopy.

Results: Results indicated that > 95% loading of ciprofloxacin was achieved. Further, < 5% was released from the LTSL within 15 min at baseline (25°C) and body (37°C) temperature, while > 95% was released at 42°C. Sonication of rat muscles resulted in accurate and homogeneous temperature control within the heated ROI (42.0 ± 0.2 °C), with a 90th percentile (T90) and 10th percentile (T10) of 43.2 ± 0.3 °C and 41.0 ± 0.3 °C respectively. Precise hyperthermia exposures in the thigh of rats using MR-HIFU during IV administration of the LTSLs resulted in a 4-fold greater local concentration of ciprofloxacin compared to controls (free ciprofloxacin + MR-HIFU or LTSL alone). The biodistribution of ciprofloxacin in unheated tissues was fairly similar between treatment groups. Triggered release at 42°C from LTSL achieved significantly greater S. aureus killing and induced membrane deformation and changes in biofilm matrix compared to free ciprofloxacin or LTSL at 37°C.

Conclusions: This technique has potential as a method to deliver high concentration antimicrobials to chronic wounds.
Effectiveness of MR-guided Focused Ultrasound treatment for the tender points in patients with painful bone metastasis or with chronic osteoarthritic pain

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Objectives: MR-guided Focused Ultrasound surgery (MRgFUS) has been recently demonstrated as a promising tool for pain alleviation of painful bone metastases (pBM) and lumbar facet joint pain. The mechanisms of pain alleviation are believed to be most likely local denervation of the nociceptors at the ablated area using the Focused Ultrasound. However, few previous studies have suggested an assessment method to estimate the denervation. Peripheral nociceptive sensitization resulting in bone metastatic pain and osteoarthritic pain is thought to make pain on pressure (tenderness) at the lesion sites too sensitive. Therefore, our hypothesis is that MRgFUS treatment of the sensitized tender points could improve the refractory chronic pain in patients with bone metastasis or osteoarthritic (OA). The aim of the present study is to clarify changes in the tenderness and the worst pain intensity at the lesion treated with MRgFUS.

Methods: We have conducted MRgFUS treatments using ExAblate® system (InSightec Ltd, Haifa, Israel) for 8 patients (mean age; 64 years old) with pBM, 9 patients (72 y.o.) with chronic lumbar facet joint osteoarthritic pain (LFP), and 11 patients (78 y.o.) with chronic knee osteoarthritic pain (KP). Patients with the worst pain intensity (worst) ≥ 4 on the Numerical Rating Scale (NRS) and tenderness at the lesion site were enrolled. Treatments were applied to the bone surface of the most painful tender point of the metastasis in pBM group, the dorsal area of these joints which selected on the basis of 70% reduction of pain with diagnostic block in LFP group, the bone surface around osteophyte of medial femorotibial joint tenderness in KP group. We assessed pressure pain threshold (PPT) using an electronic pressure algometer to evaluate tenderness at the lesion sites, and the subjective local worst pain intensity with NRS. PPT is defined as the minimal amount of pressure where a sensation of pressure first change to pain. We evaluated changes in the PPT on the treated site and at the contralateral non-treated site as a control and NRS before and 1 week, 1 month and 3 months after the treatments.

Results: PPTs at the treated sites were significantly increased from 107 kPa [40-432] (median [min-max]) at the baseline to 271 kPa [94-534] at the final follow-up in pBM group, 280 kPa [66-427] to 462 kPa [207-818] in OALFJP group, and 156 kPa [50-249] to 246 kPa [114-6-427] in OAKP group. At the control sites, the PPTs were significantly higher than at the treated sites and there was no significant difference of PPTs between before and after the treatment in all groups. The NRS were significantly decreased from 6.5 [4-8] to 1 [0-3] in pBM group, 8 [4-9] to 1 [0-7] in LFP group, and 6 [5-9] and 3 [1-6] in KP group. The treatment responder, defined as reduction of 2 or more in the NRS without increase analgesic intake in pBM, and a 50% or greater decrease in the NRS in OA group, were all patients in pBM group, 14 of 20 patients in OA group. In OA group, the PPTs at the treated sites were significantly increased from 223 kPa [50-427] to 326 kPa [184-731] at 1 week after the treatment in responders, but there were no significant differences in non-responders.

Conclusions: These results illustrate the excellent pain-relieving effects of MRgFUS treatment for the tender points in patients with painful bone metastasis or with chronic osteoarthritic pain. Significant increase of PPTs speculated successful denervation effect on the nociceptive nerve terminals of the treated area. PPTs measurement is a useful tool for quantitative evaluation after MRgFUS and might be a useful parameter for assessing a treatment's effect.
Effectiveness and safety of High Intensity Focused Ultrasound guided by Magnetic Resonance imaging (MRgFUS) in the treatment of osteoarticular lumbar spinal pain originating from the facet joints

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Objectives: The objective of our study is to evaluate the effectiveness and safety of MRgFUS in the treatment of osteoarticular lumbar spinal pain originating from the facet joints segment. Also to assess whether MRgFUS as a treatment for chronic osteoarticular lumbar pain originating from facet joints increase the quality of life of patients.

Methods: Seven patients (3 males and 4 females) with chronic lumbar facet joint pain who did not respond to medical therapies were treated by MRgFUS rhizolysis, after obtaining informed consent. The response to treatment was assessed using a validated pain numerical rating scale (NRS), Oswestry disability questionnaire (ODQ), and the EuroQol (EQ-5D) health state score immediately after treatment and at 1, 3, 6 and 12 months from the treatment.

Results: No major complications were observed. The mean NRS pain score pre-treatment was 6.5 (average) and 8.4 (worst). A decrease in the NRS average pain score of 3.0 (53.8 %) and the NRS worst pain score of 4.3 (48.8 %) was found at 12 months. A 41.7 % improvement in the ODQ and a 49.0 % improvement in the QOL were also observed at 12 months.

Conclusions: MRgFUS represent a valid tool and enriches the therapeutic options for chronic back pain caused by facet joint osteoarthritis. This is a new non invasive technique that potentially prevents complications derived from needle punctures, under real-time thermal and anatomic image control provided by MRI.
Passive acoustic mapping and MR thermometry for real-time multi-modality imaging of Focused Ultrasound ablation

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Objectives: MRI provides outstanding soft-tissue contrast and ability to map temperature which are ideal for monitoring of Focused Ultrasound therapy. However, at the high intensities required for ablation acoustic cavitation may be nucleated, leading to an enhancement in ultrasound-mediated heating. The likelihood of cavitation may also vary significantly between adjacent tissue regions, which has frustrated efforts to control ablation over large volumes. As the onset of cavitation is accompanied by characteristic acoustic emissions, simultaneous acoustic mapping during treatment can provide critical information that may used for control of ultrasound output to achieve ablation of entire tumors. Previous work has shown that such monitoring is feasible within an MR scanner using post-processed ultrasound data. The objective of the current study was to optimize ultrasound data acquisition and processing to allow rapid real-time display of acoustic mapping data alongside MR thermometry.

Methods: An ultrasound imaging array was aligned with the focus of a 1.5MHz HIFU transducer (Figure 1). The two components were clamped together, immersed in degassed water, and connected to a MR-compatible positioning stage. Rabbits with subcutaneous VX2 tumors were anaesthetized and placed on a platform with a cutout for ultrasound propagation and acoustically coupled using a bag filled with degassed water. A receive-only MR surface coil was placed in the air gap between the water and animal platform. The entire setup was placed within a clinical 3T MR scanner (GE Signa Excite). The HIFU transducer was then driven from a waveform generator via a power amplifier. During sonication the MR scanner performed temperature mapping while an ultrasound research platform (Verasonics) performed B-mode and passive acoustic mapping (PAM) using the array. Temperature maps, B-mode and passive acoustic images were displayed in real time. The process was repeated in rabbits injected with phase-shift nanoemulsions (PSNE) to promote cavitation or saline as a control under a range of ultrasound conditions.

Results: Real time passive acoustic mapping was successfully implemented using a rapid frequency-domain beamformer and employed simultaneously with MR thermometry for imaging of tumor ablation. Illustrative results are shown in Figure 2. Over 50s (40s sonication, 10s baseline and cooling time) 477 frames of ultrasound data were acquired, processed and immediately displayed at an average frame rate of 10 fps. MR temperature maps were also acquired and displayed every 2.3s (0.4 fps). Comparison of the two modalities (Figure 2a) shows accelerated temperature rise following the onset of cavitation (30s) and decline thereafter as the amplitude of cavitation dropped (40s), perhaps due to depletion of cavitation nuclei or changes in tissue structure. MR temperature maps (Figure 2b) and passive acoustic maps (Figure 2c) show that heating and cavitation activity were both well localized to the intended focal region. Extraction of tumors after sonication showed lesions were successfully produced in the tumors.

Conclusions: These results show that rapid, real-time passive acoustic imaging coupled with PSNE for nucleating cavitation locally may be applied simultaneously with MR thermometry for enhancing Focused Ultrasound ablation. Future work will apply the method for optimization and control of PSNE enhanced ablation of whole tumors.

(a) Passive acoustic mapping (PAM) and MR-derived temperature rise over time. (b) MRI temperature map and (c) acoustic map for cavitation-enhanced focal heating.
**Acoustic characterization of a clinical MR-guided HIFU system inside and outside MR scanner**

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**Objectives:** With the rapid advancement of MR-guided HIFU clinical applications, calibration of HIFU pressure fields is an urgent need to ensure consistent and safe treatment outcomes. Few studies have been reported on the characterization of acoustic pressure fields of clinical MR-guided HIFU outside the MR scanner. To date, no study has been reported on acoustic field calibration inside the MR scanner, owing to combined effects of strong magnetic field and limited space in the MR bore that makes it difficult to perform the measurements inside the MR scanner. In this study, we present a method of acoustic field calibration and results for a clinical MR-HIFU system both inside and outside a clinical MR scanner.

**Methods:** For hydrophone measurement outside the MR scanner (Ingenia 1.5T, Philips), the HIFU patient table with an integrated 256-element transducer (Sonalleve V2, Philips) was moved outside the magnet room. A fiber-optic hydrophone (HFO-690, Onda) attached to a 3D motor stage was used for pressure field measurements in a deionized and degassed water tank placed above the transducer. A Matlab® program was developed to control the 3D stage, capture the hydrophone signals, and synchronize the data acquisition with the HIFU pulses. For measurements inside the MRI, an MRI-compatible water tank, a 20-meter long optical fiber was used for the hydrophone measurements and it was visualized in MR imaging (Fig. 1). The HIFU transducer was moved in 3D space while keeping the optical fiber fixed. The HIFU transducer was operated at a frequency of 1.2 MHz, a pulse length of 40 cycles and pulse repetition frequency of 10 Hz. The nominal acoustic power was varied from 50-650 W. Hydrophone measurements were performed in planes parallel and perpendicular to the beam path. The acoustic parameters such as peak positive/negative pressures (p+, p-), temporal and pulse average intensities (ITA, IPA) at the HIFU focus, and the full-widths of the pressure distribution along and perpendicular to the beam path were calculated.

**Results:** Outside MRI Scanner: The HIFU focus was identified approximately 6.8 cm away from membrane (when the transducer was at the homing position and without steering) by scanning the hydrophone in planes parallel and perpendicular to the beam path (Fig. 2; left-hand side). The acoustic parameters p+ (3.4 - 65.8 MPa), p- (3.1 - 13.2 MPa) (Fig. 3), ITA (0.06 - 6.2 W/cm²), IPA (78.5-20159 W/cm²) at the focus were measured with respect to nominal acoustical power (50-650W). The FWHM of pressure profiles at the focus was 1.0 mm in the lateral direction and 9.5 mm in the axial direction (Fig. 2; right-hand side).

Inside MRI scanner: The acoustic pressure measurements inside MR scanner were found consistent with the outside measurements. The motor step-size of the HIFU transducer was 0.1 mm which allowed high spatial resolution scanning of the acoustic field inside the MR scanner.

**Conclusions:** In conclusion, HIFU pressure field measurements both outside and inside an MRI scanner provides a systemic method for ensuring safe and consistent treatment in a clinical MR-guided HIFU system. Moreover, the proposed inside MR scanner calibration method allowed characterization of the acoustic fields of a clinical MR-guided HIFU system without the need of moving the HIFU table outside of MR scanner, which is not feasible for most clinical systems.

Figure 1. The location of fiber tip inside the water tank in the MR scanner was identified precisely with the help of MR imaging and the HIFU transducer was moved to the corresponding location for the acoustic field calibration.
Figure 2. Results of pressure field measurements in YZ (along) (top left) and XY (perpendicular) (bottom left) planes to beam paths and right hand side plots are the normalized pressures with respect to distances to find the FWHM of the pressure distribution.

Figure 3. Peak positive and negative pressures at the HIFU transducer focus with respect to acoustic power between 50-650 W.
Preservation of endometrium after Magnetic Resonance Imaging-guided High-Intensity Focused ultrasound (MR-HIFU) ablation for uterine fibroids of submucosal type

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Objectives: To evaluate the endometrial integrity after MR-HIFU ablation for submucosal uterine fibroids based on MRI findings and to know the risk factors associated with endometrial injury

Methods: A total of 117 submucosal uterine fibroids (diameter: 5.9±3.0 cm, 1.0-12.9 cm) in 101 women (age: 43.6±4.4 year, 33-55 year) treated with volumetric MR-HIFU ablation between November 2010 and December 2015 were retrospectively analyzed. Endometrial integrity was assessed with contrast-enhanced T1WI of immediate (n=101), 3-month (n=62), and 12-month (n=15) follow-up. Endometrial injury was classified into one of grade 0 (continuous involved endometrial lining), grade 1 (pin-point, full-thickness discontinuity of involved endometrium), grade 2 (between grade 1 and 3) and grade 3 (full-thickness discontinuity of involved endometrium over 1cm in size). Potential risk factors for endometrial injury (age, duration from LMP, history of full term delivery, GnRH agonist pretreatment, FIGO grading of endometrial protrusion, T2 signal intensity and perfusion degree of fibroid, and average acoustic power) were assessed with a generalized estimating equation (GEE) analysis.

Results: Among 117 fibroids, endometrial injury was of grade 0 in 66 (56.4%), grade 1 in 29 (24.8%), grade 2 in 16 (13.7%), and grade 3 in 5 (4.3%) at immediate follow-up. Among 37 fibroids of which endometrium was injured and underwent follow-up studies, 30 (81.1%) showed improvements at 3- and/or 12-month follow-up. Among 14 fibroids in which endometrium was injured and followed-up till 12 months, 12 (85.7%) were completely normalized. GEE analysis revealed that the degree of endometrial protrusion were significantly associated with severer endometrial injury (p<0.0001, B=-0.687).

Conclusions: After MR-HIFU ablation of submucosal uterine fibroids, endometrium is preserved or minimally injured in majority of the cases. Injured endometrium which is more frequently encountered after treating intracavitary fibroids usually recovers spontaneously.
Responses of uterine fibroids to Gonadotropin-Releasing Hormone agonist (GnRHa) as a pretreatment of MR-guided High-Intensity Focused Ultrasound (MR-HIFU) ablation

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Objectives: To evaluate changes in MR findings of uterine fibroids after using GnRHa as a pretreatment of MR-HIFU ablation and to know the influencing factors for favorable changes to MR-HIFU ablation

Methods: From August 2012 to March 2016, 60 women (age: 43.6±5.7 years, 26-54 years) with 127 uterine fibroids (diameter, 5.9±3.0cm; volume, 121.1±162.3mL) underwent GnRHa injections as a pretreatment of MR-HIFU ablation (1-4 cycles; 5 women with 10 fibroids, 8 women with 17 fibroids, 40 women with 87 fibroids, and 7 women with 13 fibroids, respectively) and retrospectively analyzed. Fibroid volumes and T2 signal intensity ratios relative to skeletal muscle were compared between before and 2-3 weeks after the last GnRHa injection. Potential factors (age, BMI, history of full term delivery, multiplicity of fibroids, number of GnRHa injections, volume, T2 signal intensity ratio, and semiquantitative perfusion MR parameters of fibroid) were evaluated for the association with greater volume and T2 signal intensity changes. All statistical tests were performed using with model analyses.

Results: While fibroid volume significantly reduced after GnRHa therapy (volume reduction ratio = 74.8±25.3%, p<0.001), T2 signal intensity ratios did not show a significant difference (T2 signal change ratio = 102.0±32.2%, p=0.257). Increased number of GnRH injection (B=-0.067, p=0.028), greater T2 signal intensity ratio (B=-0.014, p=0.028) and greater wash-in rate (B=0.0006, p=0.012) were revealed to be independently significant for greater volume reduction of fibroids. However, no factor turned out to be associated with T2 signal intensity change.

Conclusions: After using GnRHa as a pretreatment of MR-HIFU ablation, uterine fibroids with higher T2 signal intensity and/or greater wash-in rate in semiquantitative perfusion MRI show greater volume reduction, of which effect is strengthened with an increased number of GnRHa cycles. T2 signal intensity does not show a significant change after GnRHa therapy.
Targeted vessel ablation with MR-HIFU for the treatment of type 3 uterine fibroids

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Objectives: Type 3 uterine fibroids are considered highly perfused and/or to have a high extracellular fluid content and are known to have poor treatment outcome in terms of non-perfused volume (NPV), volume reduction, symptom relief and an increased risk of adverse events. Type 3 fibroids have therefore been excluded from HIFU treatment at our institution for the last two years. However, a novel approach using higher acoustic power to precisely target the feeding vessels might result in vessel occlusion rendering the bulk tissue less perfused and more susceptible to ‘lower’ powers, which in turn might lead to better treatment outcomes. Therefore, the aim of this research is the feasibility and safety of high power targeted vessel ablation of type 3 fibroids with MR-HIFU.

Methods: In this study 10 patients with a type 3 fibroid will be included. Screening MR scans include a T2w-scan to determine fibroid type, a dynamic contrast enhanced (DCE) series to assess perfusion, and a time-spatial labeling inversion pulse (TimeSLIP) sequence to visualize the vasculature without using a contrast agent. Areas of blood inflow are determined based on the DCE and TimeSLIP and are marked. The TimeSLIP is repeated prior to treatment, spatially matched with the screening DCE and TimeSLIP sequence, and used for planning of the high power (450 W) sonications. A Philips Sonalleve V2 system with skin cooling (water temperature ~ 15°C) is used. After sonicating the inflow areas with high power cells the bulk volume is treated with ‘low power’ sonications (£300W). NPV is assessed on contrast-enhanced T1w-images. Follow-up MRI is performed after three months. As a preparation a patient with a type 2 fibroid was treated in a similar manner except that all sonication were at a power £300W. Philips software was used to determine areas with a high wash-in rate (WIR) indicating the areas of early inflow and these regions were treated.

Results: The WIR map of the type 2 fibroid showed areas of higher inflow (Figure 1). These regions were targeted with 12/14mm sonications (maximum power of 230W, 42-55s). The resulting NPV was larger than the planned treatment volume (PTV) (Figure 2).

So far, one type 3 fibroid has been treated. Two inflow areas were determined (Figure 3) and seven treatment cells (4mm) were planned in each of these areas. Sonications were completed successfully. Hereafter, 8/12mm volumetric feedback cells (max. 250W) were used. Two NPVs corresponding to the two high-power clusters were observed post treatment (Figure 4). Small NPVs (2-3mm) could be observed in the bulk tissue volume. However, no confluent NPV similar in volume to the PTV could be observed. Three-month follow-up has not yet been performed. No adverse events occurred during treatment.

Conclusions: The results of the type 2 fibroid treatment using MR-HIFU showed the potential of targeted vessel ablation. A similar strategy was used for type 3 fibroids. Based on the first treatment the high power sonications might result in a non-perfused volume in type 3 fibroids. More data are expected soon and are needed to draw conclusions on the feasibility and safety of this approach.
Left: coronal view Right: sagittal view of the treatment cells overlaid on the contrast-enhanced T1w-image showing the NPV (dark region). The NPV is much larger than the planned treatment volume most likely due to vessel occlusion by the sonications.

Dynamic contrast enhanced image showing the fibroid in the red circle and one of the targeted blood-inflow areas in the white circle.

Contrast enhanced T1w-image showing the two non-perfused volumes created by the two clusters of high power sonications.
A non-contrast method for evaluating non-perfused volume after MRgFUS treatment of uterine fibroids

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Objectives: The efficacy of MRgFUS treatment of uterine fibroids is evaluated post-operatively by measuring the non-perfused volume (NPV) on post-contrast images as an estimate of the ablated region. NPV is not a direct measurement of ablation but rather of non-perfused tissue. This may lead to an underestimate of the ultimately ablated volume, as some cell death may be delayed, or to an overestimate of the treatment effect, for example, in cases where temporary vasoconstriction contributes to the non-perfused volume (Figure 1a,b). In addition, this can be confusing in cases where non-targeted tissue appears non-perfused; since there is no thermal dose predicted in these regions, it may be unclear whether the tissue is actually ablated.

A hypointense contour, which we call the Black-Line Volume (BLV), is present around the margin of fibroids on non-contrast images obtained at follow-up (figure 1c). Our aim was to quantitatively compare the BLV to the NPV.

Methods: Five patients with follow-up imaging after treatment were included in this study. Four of them had a second treatment within 7 to 14 days either because the fibroid was too large for a single treatment, or it appeared incompletely ablated. Patient 2 had no additional treatment. In three of the retreated patients, a reperfused volume was noted on follow-up images in regions that the NPV indicated as non-perfused at the end of the first treatment. The NPVs obtained at the end of each treatment (NPV1 and NPV2) and the reperfused volumes were measured and compared by multiplying slice-by-slice measurements of the manually contoured area by the slice thickness. Using the same methodology, the BLV was calculated using EPI thermometry magnitude images acquired during each sonication in follow-up images.

Results: The results are provided in Table 1. In these patients, the BLV contour is not present prior to treatment, but is reliably visualized on follow-up imaging obtained 7-43 days after treatment. Quantitation of the BLV demonstrates that it is very similar to the NPV. In cases where the BLV was less than NPV1, the discrepancy is explained by reperfusion (Figure 1d).

Table 1. Comparison of non-perfused volume after first fibroid treatment (NPV1) and black line volume (BLV) observed on follow-up imaging

<table>
<thead>
<tr>
<th>Patient</th>
<th>NPV1 (cc)</th>
<th>Days to Follow Up Imaging</th>
<th>BLV on Follow Up (cc)</th>
<th>Reperfused Volume on Follow Up (cc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>412</td>
<td>14</td>
<td>419</td>
<td>none</td>
</tr>
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<td>2</td>
<td>42</td>
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<tr>
<td>5</td>
<td>85</td>
<td>14</td>
<td>82</td>
<td>5</td>
</tr>
</tbody>
</table>

In three cases, at the end of a second treatment, portions of the fibroid that were part of NPV1 were seen to have reperfused. In these cases, BLV consistently identified the reperfused volume, which was outside of the BLV.

Conclusions: These results show that BLV correlates well with the ablated region, and the non-perfused volume. In cases in which NPV1 overestimated the truly ablated volume, as defined during the follow-up imaging, BLV was a better predictor because it excluded the reperfused volume. Thus, BLV may prove to be a powerful tool to disclose inadequately treated regions during the planning session of a second treatment, allowing more tailored targeting of the area to ablate and preventing under-treatment.

We are currently assessing how soon after the initial treatment the BLV becomes visible. If further research demonstrates a BLV at the end of the first treatment, then it may be used to
identify untreated parts of the fibroid and guide further, more complete treatment without contrast administration.

Figure 1. Comparison of a) NPV1 and b) NPV2 demonstrates an area of reperfusion (green outline). The BLV contour (c, pink), matches the NPV2 contour (d, pink), and excludes the reperfused contour (green) in Patient 3.
Portable Ultrasound-guided High Intensity Focused Ultrasound with 3D electronic steering and targeting forecast function: 2-Year prospective clinical trial for uterine fibroids

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Objectives: Not released for publication
Methods: Not released for publication
Results: Not released for publication
Conclusions: Not released for publication
**Preliminary clinical evaluation of Magnetic Resonance guided Focused Ultrasound Surgery (MRgFUS) combined with GnRH-a analog therapy in the treatment of diffuse adenomyosis**

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**Objectives:** Adenomyosis is the common gynecologic disorder that in the presence of endometrial glands and stroma deep within the myometrium. Treatment of diffuse Adenomyosis has always been difficult, especially for patients who desire subsequent pregnancy. GnRH-a is a ovarian suppression drug that has been effectively used for Adenomyosis, while as long time use will result in symptoms of low estrogen, it is not recommended for use more than 6 months. In previous reports, symptoms recurred after stopping the drugs, therefore GnRH-a is often used as an adjuvant prior to or after the surgery. MRgFUS is safe and feasible for the treatment of focal Adenomyosis, while it showed limited efficacy in the treatment of diffuse Adenomyosis. In this study, the preliminary clinical effects of combined MRgFUS and GnRH-a therapy in the treatment of diffuse Adenomyosis will be evaluated, and the fertility and pregnancy outcomes for patients who desired pregnancy will be investigated.

**Methods:** 9 patients with diffuse Adenomyosis were treated in Harbin MRgFUS Center between April 2015 and May 2016. Including, 7 patients (1 patient has fertility requirements) received MRgFUS treatments only, while the rest 2 patients (1 patient has fertility requirements) received 3 months of GnRH-a prior to MRgFUS treatment. The treatment response was evaluated by the symptom severity score (SSS, 0-100), visual analogue scale score for assessment of pain (VAS, 0-10) and Pictorial Blood Loss Assessment Chart (PBAC). The questionnaires were evaluated on treatment day as well as before, 3 and 6 months after treatment. Uterine volume reduction was recorded before and 6 months after the treatments.

**Results:** During the 6-month follow up, 89% (8/9) of the patients showed significant symptomatic relief (based on SSS and PBAC) and 67% (6/9) of the patients experienced pain reduction (based on VAS). The statistic uterine volume reduction was 14.5±1.2% in patients treated with MRgFUS only. On the contrary, uterine volume reduction of 20.3±1.8% was observed in patients treated with MRgFUS combined with GnRH-a. 1 patient underwent single session of MRgFUS treatment had relapse 6M after MRgFUS and enlarged uterus was found on FU and MR exam. Up to now, the 2 patients that desired fertility are still not pregnant, this study will extend the follow up period to 24 months.

**Conclusions:** MRgFUS combined with GnRH-a can potentially prevent recurrences after solely GnRH-a analog therapy, and can also enhance the treatment efficacy of MRgFUS for diffuse Adenomyosis. MRgFUS is capable of preserving the functionality of myometrial structures, hence may reduce the length of duration before pregnancy. MRgFUS is a very promising technique for Adenomyosis patients who desired fertility, while its short and long term impacts in the fertility and pregnancy outcomes are still required.
Endoluminal ultrasound applicators for thermal ablation of pancreatic cancer under MR-guidance: Preliminary investigations in an in vivo porcine model

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Objectives: While conventional treatments remain limited, thermal ablation has been demonstrated to provide pain palliation and survival benefit to patients with advanced pancreatic cancer. In light of the anatomical challenges that limit safe and effective thermal ablation to the pancreas, endoluminal ultrasound may serve as a minimally invasive option for treating pancreatic tumors adjacent to the gastrointestinal (GI) tract, and could be compatible with MR-guidance for target confirmation and real-time thermometry. This study’s objective was to perform a preliminary investigation of endoluminal placement and heating performance of prototype ultrasound applicators and temperature monitoring capabilities in MR-guided in vivo porcine pancreas ablations.

Methods: A family of MR-compatible endoluminal applicators were fabricated and characterized using radiation force balance and hydrophone measurements. Each applicator possessed a distinct two-element transducer array (~3.2 MHz) covered by a cooling balloon at the tip of a flexible catheter assembly, as shown in Figure 1. Three transducer geometries were considered: 10x10 mm (planar), 8x10 mm with 20 mm radius of curvature (ROC) along the short dimension (lightly-focused curvilinear), and 9.3x11.4 mm with 25 mm ROC along the long axis (strongly-focused curvilinear). In each in vivo porcine experiment, an applicator was orally introduced and positioned in the stomach adjacent to the splenic lobe of the pancreas. Sonication were performed through the luminal wall into the pancreas for ablation, at durations ranging from 3-16 minutes and applied intensities of 5-7 W/cm². MR-guidance techniques were developed and evaluated for anatomical target identification, tracking/placement of the applicator, and PRF-based MR temperature imaging (MRTI), implemented in the real-time RTHawk software environment.

Results: Placement of the endoluminal applicators in the stomach adjacent to pancreatic tissue, as demonstrated in Figure 2, was achieved in all porcine studies (n=5). In vivo MRTI-guided heating trials demonstrated capability of ~15-20 °C temperature elevation in pancreatic tissue at 1-2 cm depths using the planar and lightly-curvilinear applicators (6-16 mins, 5-7 W/cm²). Strongly-focused curvilinear applicators were capable of reaching higher temperature elevations of ~25-35 °C at 2-3 cm depths and more spatially localized lesions in shorter treatment durations (3-5 mins, ~5 W/cm²), as shown in Figure 3. Dimensions of thermal lesions in excised pancreas ranged from 9-28 mm, 3-10 mm, and 5-10 mm in length, width, and depth, respectively, as verified through histopathological analysis of tissue sections (Figure 4). Multiple-baseline reconstruction and respiratory-gated acquisition were effective in suppressing motion artifacts and improving temperature precision for multi-slice MRTI in the in vivo studies.

Conclusions: This study demonstrates the technical feasibility of generating volumetric ablation in porcine pancreatic tissue using endoluminal ultrasound applicators positioned in the GI tract, with integrated MR-guidance for target identification, device positioning/alignment, and MRTI treatment monitoring. Future applicator development will involve incorporating endoscopic steering capabilities to enhance control over endoluminal placement, fixation, and coupling to the gastric wall to improve positional accuracy and luminal sparing.

Prototype endoluminal ultrasound applicator assembly.
T2-weighted MRI image showing placement of endoluminal applicator in porcine stomach and alignment for treatment of pancreatic tissue.

MRTI temperature map for in vivo porcine pancreatic ablation using an endoluminal ultrasound applicator with strongly focused curvilinear elements, at 6 W/cm² applied intensity.

H&E staining of a section of excised pancreatic tissue following endoluminal ablation, showing regions of coagulative necrosis (CN) and the transition zone (TZ) to untreated tissue (UT).
A randomized, sham-controlled trial of transcranial MR guided Focused Ultrasound thalamotomy trial for the treatment of tremor-dominant, idiopathic Parkinson’s disease

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Objectives: Traditional stereotactic RF thalamotomy has been used with success in medication refractory tremor-dominant Parkinson’s disease. Recently, transcranial MR guided Focused Ultrasound (MRgFUS) has been used to successfully perform thalamotomy for essential tremor. We designed a double blinded, randomized controlled trial to investigate the effectiveness of MRgFUS thalamotomy in tremor-dominant PD.

Methods: Patients with medication refractory, tremor-dominant Parkinson’s disease were enrolled in the two center study and randomized 1:2 to receive either a sham procedure or treatment. After the 3 month blinded phase, the sham group was offered treatment. Outcome was measured with blinded CRST and UPDRS ratings. The primary outcome compared improvement in hand tremor between the treatment and sham procedure at 3 months. Secondary outcomes were measured with UPDRS and hand tremor at 12 months. Safety was assessed with MRI, adverse events, and comprehensive Neurocognitive assessment.

Results: Twenty-seven patients were enrolled and six were randomized to a sham procedure. For the primary outcome assessment, there was a mean 50% improvement in hand tremor from MRgFUS thalamotomy at 3 months compared to a 22% improvement from the sham procedures (p=0.088). The 1yr tremor scores for all 19 patients treated with 1yr follow up data (blinded and unblinded) showed a reduction in tremor scores of 40.6% (p=0.0154) and a mean reduction in medicated UPDRS motor scores of 3.7 (32%, p=0.0326). Sham patients had a notable placebo effect with a mean 21.5% improvement in tremor scores at 3 months. Twenty seven patients completed the primary analysis, 19 patients completed the 12 months assessment, 3 patients opted for DBS, 3 were lost to follow up, 1 patient opted for no treatment, and 1 is pending 12m evaluation.

Conclusions: Transcranial MRgFUS demonstrates a trend towards improvement in hand tremor, and a clinically significant reduction in mean UPDRS. A significant placebo response was noted in the randomized trial.
MR Image-guided delivery of non-viral miRNA-34a gene vectors via Focused Ultrasound inhibits tumor growth in a mouse glioma model

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Objectives: Glioblastoma (GBM) is remarkably difficult to treat with systemically administered therapeutics because the blood brain/blood tumor barrier (BBB/BTB) blocks their diffusion from the bloodstream, while the bioadhesive and nanoporous ECM hinders their tissue dispersion. In addition, GBM is characterized by genetic complexity, molecular adaptability, and multiple deregulated pathways, all of which markedly limit conventional therapies. Here, we address these obstacles by combining Focused Ultrasound (FUS)-mediated disruption of the BBB/BTB with brain-penetrating non-viral gene vectors (DNA-BPN) bearing the plasmid for miRNA-34a, a miRNA that is downregulated in human GBM and predicted to regulate multiple tumor suppressive pathways.

Methods: U87 malignant glioma cells were injected into the brains of athymic nude mice. Five days later, anesthetized mice were coupled to an MRI compatible 1.14 MHz FUS system. T1 contrast MR imaging was used to visualize and target tumors. miR-34a plasmid-bearing DNA-BPN that had been densely PEGylated to permit brain penetration were i.v. co-injected with microbubbles. FUS was applied to tumors with a 0.5% duty cycle for 2 minutes at either 0.4 or 0.5 MPa peak negative pressure (PNP). Immediately after sonication, additional contrast MR images confirmed BBB/BTB opening. Untreated mice and mice that were i.v. injected with miRNA-34a BPNs and MBs without FUS application served as controls. At 7 days and 15 days after treatment, corresponding to 12 and 20 days after tumor implantation, tumor volumes were determined from 7T MR images.

Results: Figure 1A shows representative pre- and post-FUS T1-weighted contrast MR images when using PNP of 0.4 and 0.5 MPa. Enhanced signal in the post-FUS images indicates increased leakage of gadolinium contrast agent across the vasculature due to FUS-mediated BBB/BTB disruption. Note that BBB/BTB disruption appears to be further enhanced at 0.5 MPa when compared to 0.4 MPa. Figure 1B shows representative 7T images of U87 tumors for each group taken at 12 and 20 days post tumor implantation. These images were used to quantify post-treatment tumor volume (Figure 1C). At Day 20, mice treated with 0.5 MPa FUS + MB + miRNA-34a BPN (n=3) showed a marked and statistically significant (p<0.05) decrease in tumor volume when compared to the untreated (n=5), miRNA-34a BPN+MB (n=4), and 0.4 MPa FUS + MB + miRNA-34a BPN (n=4) groups. For all groups, T2* MRI (not shown) confirmed that there was no leakage of blood products across the BBB/BTB.

Conclusions: We show that a single MR image-guided treatment, entailing the FUS (1.14 MHz; 0.5 MPa) mediated delivery of brain-penetrating non-viral miR-34a gene carriers across the BBB/BTB, can significantly inhibit glioma growth in mice. To the best of our knowledge, this represents the first time that therapeutic miRNA plasmid has been complexed into a non-viral gene nanocarrier and delivered using Focused Ultrasound for treatment of gliomas. Ongoing studies are centered on optimizing miRNA-34a expression levels, verifying changes in miRNA-34a oncogenic target protein expression, and assessing tumor cell apoptosis and proliferation.
Pulsed ultrasound non-destructively expands the extracellular and perivascular spaces of the brain

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Objectives: Diffusion within the extracellular and perivascular spaces of the brain plays an important role in biological processes, therapeutic delivery, and clearance mechanisms within the central nervous system. Recently, ultrasound has been used to enhance the dispersion of locally administered molecules and particles within the brain, but ultrasound-mediated effects on the brain parenchyma remain poorly understood. We combined an electron microscopy-based ultrastructural analysis with high-resolution tracking of non-adhesive nanoparticles in order to probe changes in the extracellular and perivascular spaces of the brain following a non-destructive pulsed ultrasound regimen known to alter diffusivity in other tissues.

Methods: Freshly obtained rat brain neocortical slices underwent sham treatment or pulsed, low intensity ultrasound for 5 minutes at 1 MHz. Samples from each brain slice were examined with transmission electron microscopy (TEM), and image processing was used to measure the area of the extracellular and perivascular spaces in each micrograph. In a second cohort, non-adhesive nanoparticles were injected into the brain slice following sham or ultrasound treatment, and the particle movements were tracked with confocal microscopy. A linear mixed-effects models approach was used to compare the sham- and ultrasound-treated groups. Additionally, a tetrazolium (TTC) assay and hematoxylin and eosin (H&E) staining were used to assess the effect of pulsed ultrasound on brain slice viability.

Results: TEM revealed intact cells and blood vessels and evidence of enlarged extracellular and perivascular spaces in ultrasound-treated brain slices (Figure 1). Sonication produced geographically heterogeneous effects. On average, enlarged perivascular regions accounted for 3.7% of the field of view in ultrasound-treated slices vs. 1.5% in sham-treated slices (Figure 2). Enlarged extracellular spaces within the brain parenchyma, accounted for 2.1% of the field of view in ultrasound-treated slices vs. 1.1% in sham-treated slices. Additionally, ultrasound significantly increased the diffusion rate of 100 nm, 200 nm, and 500 nm nanoparticles that were injected into the brain slices, while 2000 nm particles were unaffected (Figure 3A). In ultrasound-treated slices, 91.6% of the 100 nm particles, 20.7% of the 200 nm particles, 13.8% of the 500 nm particles, and 0% of the 2000 nm particles exhibited diffusive motion (Figure 3B). Notably, sham- and ultrasound-treated brain slices were stained red following tetrazolium incubation, and H&E staining of sham- and pulsed ultrasound-treated slices appeared similar, without evidence of damage (Figure 4).

Conclusions: Our results show that the application of ultrasound has a substantial effect on the structure of the brain parenchyma, specifically the extracellular and perivascular spaces. Transcranial ultrasound is gaining widespread use at the clinical level, and has been applied in patients for the creation of focal ablative lesions as well as opening of the Blood-Brain Barrier. As the technology advances, larger regions of the brain are likely to undergo sonication. Notably, our findings show that ultrasound may also affect the extracellular and perivascular spaces of the brain. These results support further investigations into the impact of ultrasound-induced structural changes on diffusion, clearance, and physiologic processes of the CNS in vivo, and may lay the groundwork for novel applications of Focused Ultrasound for the treatment of intracranial neoplasms as well as other neurological disorders.
Figure 2. (A) A representative transmission electron micrograph (magnification 3200x). (B) A mask depicting the perivascular (PVS) and extracellular (ECS) space. (C) The percentage of the total area comprised of ECS and PVS in each field of view.

Figure 3. Movement of nanoparticles in rat brain slices. (A) Ensemble-averaged mean square displacements (MSD) as a function of time for 100 nm, 200 nm, 500 nm, and 2000 nm non-adhesive nanoparticles. (B) Distributions of individual particle MSDs.

Figure 4. TTC staining following exposure to sham treatment (A), 2 W/cm² (pulsed) for 5 min (B), or 2 W/cm² (continuous) for 30 min (C) (scale bar=2.5 mm). (D-E) Low magnification H&E (scale bar=600 µm). (G-I) High magnification H&E (scale bar = 120 µm).
Neurorestoration of the nigrostriatal pathway through multiple treatments with FUS-facilitated brain drug delivery

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Objectives: Current Central Nervous System (CNS) drug delivery techniques are confined to either targeted but invasive or to non-targeted and non-invasive. Focused Ultrasound (FUS) coupled with the systemic administration of microbubbles has been proven to open the Blood-Brain Barrier (BBB) locally, transiently and non-invasively, thus facilitating the diffusion of neurotrophic factors. IV injection of Neurturin, a member of the glial derived neurotrophic factors (GDNF) family has been demonstrated to activate the downstream signaling pathway following BBB opening. The promising previous findings set the milestone for investigating the effect of Neurturin on the depleted dopaminergic neurons in vivo. The aim of the current study was thus to investigate the neurorestorative effects of single and triple delivery sessions of the neurotrophic factor Neurturin in a Parkinsonian mouse model.

Methods: For this study a single control group was employed and three groups per treatment cascade; single and triple. Wild type mice (12 months old) were infused with sub-acute dosages of MPTP causing apoptotic degeneration in the nigrostriatal pathway. After stabilization of the MPTP lesions and the decontamination period, the treatment groups were sonicated on the left hemisphere targeting twice the Caudate Putamen region (CPu) and once the Substantia Nigra region (SN) while one received an IV injection of 0.5mg Neurturin. The survival period after the last treatment was equal to 28 days allowing the neurotrophic factor to develop its restorative effects followed by coronal sectioning for tissue processing. The brain slices of both the SN and the CPu were stained for tyrosine hydroxylase positive cells (TH+) with a custom protocol. Brain sections were imaged to count the TH+ nerve cell bodies on the SN while the dendritic and terminal areas were quantified by a custom MATLAB algorithm by computing the percentage of the relative difference (RD) between the two hemispheres as for each mouse the contralateral side was compared to the ipsilateral side to eliminate across-mice variation in the number of nuclei and projections.

Results: Comparison between the neuronal cells on the contralateral side and the ipsilateral side revealed an increase in the cell number on the treated side only for the group that received Neurturin with FUS. The RD was found to be significantly higher in the SN region for the groups that received Neurturin, both in the cases of single and multiple treatments. An increase in RD for the group that received multiple treatments with Neurturin compared to the single administration group was not observed. On the other hand, significant increase in the RD was only found in the case of the triple-treatment group in the CPu region.

Conclusions: The increased RD at the SN region revealed the immense potential of the current treatment and the necessity of expanding the study into multiple administrations of Neurturin. The comparable findings in dendritic density at the SN region between the two treatment regimens pointed towards the direction of examining the treatment effect at the CPu region. The significantly increased CPu terminal density could be explained as the restoration of the neuronal processes through collateral sprouting. Finally, despite the increase in neuronal cells on the ipsilateral side, no statistical significance was observed. This finding is in accordance with our knowledge of Neurturin restoring impaired neurons and not regenerating them. The presented findings are essential considering the therapeutic effect of multiple treatments with FUS enhanced drug delivery in patients.
Figure 1. Substantia Nigra: (a) Atlas 3D representation of the nigrostriatal pathway and the involved structures, Substantia Nigra (SN) and Caudoputamen (CPu). (b) Coronal section at a cross-section of the SN in increasing magnification. (c) Fluorescent im
Magnetic Resonance guided Focused Ultrasound Surgery (MRgFUS) treatment of osteoid osteoma: A prospective development study

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Objectives: Osteoid osteoma represents approximately 10% of all benign bone tumors; its etiology is unknown and it typically affects children and young adults. Although it doesn’t have a specific location, it mostly affects the appendicular skeleton, especially the proximal femur. OO consists of a well-vascularised nidus, surrounded by osteoblasts and a periosteal reaction. Clinical presentation is localized dull pain, not influenced by physical exercise or trauma, tends to worsen at night or at rest, and is typically alleviated by NSAIDs. Although CTgRFA represents the standard treatment, MRgFUS has already proved to be a valid alternative using high power ultrasound waves, focused on a single point, to generate heat. When focal temperature is raised above 56°C for more than 1sec coagulation occurs, moreover the mechanical stress itself contributes to determine necrosis. MR imaging is used for the planning, the targeting, the monitoring of the procedure and, finally, to assess the technical success.

Methods: This prospective IRB approved study involved 29 consecutive patients with clinical and imaging diagnosis of Osteoid Osteoma; all patients underwent MRgFUS ablation (ExAblate, InSightec; Discovery 750 MR unit, GE). Lesions located in vertebral body were excluded; prior RFA or surgery was not considered an exclusion criteria. Patients received therapy using MRgFUS, delivered toward the nidus, identified on MRI and/or CT. Primary endpoints were adverse events (serious and otherwise) and pain relief assessed using questionnaires on Visual Analog Pain Score (VAS) and daily intake of Non-steroidal drugs (NSAIDs). Patient’s follow-up, including clinical and imaging examinations, was established at 1, 12 and 24 months. As secondary endpoint, imaging examinations (CT and CE-MRI; Gd-BOPTA, Bracco) were used to evaluate inflammatory status after treatment and bone remodeling.

Results: 29 patients (female 8; male, 21; mean age 23) were recruited for MRgFUS treatment; all safely completed the procedure. The treatment was well tolerated by all patients and no adverse events were recorded after and during 12-24 months follow-up period. A mean number of 4 ± 1.8 sonications with mean energy of 894 ± 209 J was necessary to complete the treatment. Three patients underwent staged treatment (1 post-RFA, 1 post surgery, 1 intrarticular position). Complete clinical response was found in 27/29 (93% CI 6–18) patients in term of pain absence and no intake of NSAIDs. There was a statistically significant difference (p=0,001) between baseline (7,9 ± 1,4) and follow-up values (0,7 ± 0,1) for pain severity, according to VAS. Two patients (0.6%) reported pain recurrence requiring both RFA. Imaging evaluation with CE-MRI demonstrated edema and hyperemia decrease in every lesion associated with complete response. At CT, bone remodeling was evident in all complete responders (27/29); in 15/27 (55%) patients, nidus fading was demonstrated.

Conclusions: Our study has shown that MRgFUS is a safe and effective procedure that could be adopted for the treatment of Osteoid Osteoma. This application is non-invasive and is carried out in a single session. Pain relief is achieved on the following day after treatment. Our results also indicated a positive trend to bone restoration as shown by radiologic imaging at the follow-up.
Long-term effects of Blood-Brain Barrier opening with pulsed Focused Ultrasound and microbubbles

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Objectives: The objective of this study is to use advanced Magnetic Resonance Imaging (MRI) techniques to characterize the long-term effects of pulsed Focused Ultrasound (pFUS) and microbubbles (MB) in the rat brain. We evaluated the effects of single and repeated Blood-Brain Barrier (BBB) disruptions (D) on morphology in the rat striatum and hippocampus as monitored by MRI and histology over 12 weeks.

Methods: Female Sprague Dawley rats (n=6/group) received either pFUS+MB once (Group A) or six weekly exposures (Group B) to the striatum and the contralateral hippocampus along with 100μl of intravenous MB (OptisonTM, GE Healthcare). Prior to the first sonication rats received 3 daily doses of 300mg/kg 5-Bromo-2′-deoxy-uridine (BrdU, Sigma Aldrich) intraperitoneally to label proliferating (neurogenesis) cells in vivo. 0.3-0.5MPa acoustic pressures were applied in 10ms burst length and 1% duty cycle (9 focal points, 120sec/9 focal points – striatum, 120sec/4 focal points – hippocampus) using a single-element spherical FUS transducer (center frequency 589.636kHz; FUS Instruments). T2w, T2*w and Gadofosveset (Gd)-enhanced T1w images were obtained by 3.0T MRI (Philips) and T2w, T2*w, diffusion tensor imaging (DTI) was performed by 9.4T MRI (Bruker). Animals were euthanized 6 or 12 weeks after the first pFUS treatment. Histological evaluation of brain and tracking of BrdU tagged cells was performed and compared to untreated contralateral brain.

Results: In groups A and B, contrast enhancement on T1w images was detected post sonication in the striatum and the hippocampus. Gd-extravasation, T2 and T2* abnormalities were not seen in the brain 1 day post pFUS+MB at 9.4T MRI. Approximately 50% of Group A and 100% of Group B rats had hypointense voxels appeared on T2*w 3T MRI 2-3 weeks post pFUS+MB (Figure 1a) consistent with microhemorrhages or influx of metallophagocytic cells from the spleen within the parenchyma. Fractional anisotropic (FA) changes in white matter fiber structure- and gray matter-abnormalities on DTI MRI were detected in regions with the T2* abnormalities suggestive of increased astrogliosis and transient axonal damage along with increased cell density. Group B also demonstrated ventriculomegaly and meningeal abnormalities (Figure 1a, 1b). Histologically, increase in numbers of Nissl positive cells along with activated microglia and BrdU labeled cells were detected in both cortex and hippocampus in greater amounts in Group B compared to Group A rats, consistent with multiple episodes of induced sterile inflammatory response (SIR) within the parenchyma.

Conclusions: We have observed a complex graded molecular and cellular SIR with increase in the brain up to 24hrs after pFUS+MB. However, little has been reported on using advanced imaging techniques at high magnetic field strengths on the long-term effects of single and repeated pFUS+MB in the brain. Based on changes in FA, DTI MRI demonstrated low degree of structural injury at the location of both single and multiple sonication exposures. Multiple pFUS+MB exposures resulted in increased ventriculomegaly and numbers of activated microglia, Nissl positive cells and BrdU in both striatum and hippocampus. Increased numbers of BrdU+ cells would be consistent with the stimulation of neurogenesis indicative of increased damage secondary to repeated SIR episodes in the parenchyma.

Figure 1. (a) T1wGd, T2w, T2*w MRI (3.0T), and (b) T2*w and DTI MRI (9.4T) of a representative brain after 6 weekly sonications
Activatable nanodelivery combined with CpG-ODN and anti-PD-1 achieves a complete response in directly-treated and contralateral tumors in a murine breast cancer model

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Objectives: Activatable nanotherapeutics are attractive since the toxicity of chemotherapeutics can be constrained to a small region; combining such a strategy with immunotherapy is the goal of this study. We have previously shown that administration of CpG-ODN as an adjuvant, together with local release of doxorubicin from temperature sensitive liposomes (TSL) resulted in regression of directly-treated tumors, suppressed growth of contralateral tumors and reduced chemotherapeutic-mediated toxicity in a murine breast cancer model.

Methods: The following is the protocol explored for the addition of anti-PD1: immune intact FVB/n mice with bilateral invasive neu deletion syngeneic transplanted tumors were treated with a combination of anti-PD1 (aPD-1, 200 µg, i.p.) and intratumoral administration of CpG-ODN (100 µg, i.t.) on days 0, 7, 14 and 0, 3, 7, 10, 17 and 24, respectively. Doxorubicin TSL were prepared from DPPC:MPPC:DSPE-PEG2k, 86:10:4 in the presence of copper (II) gluconate and triethanolamine at 0.2 mg-drug/mg-lipid and administrated i.v. at 6 mg doxorubicin/kg body weight on days 10, 17, 24. The formation of a complex between doxorubicin and copper was created to enhance the circulation and stability of TSL and to reduce systemic toxicity. To trigger drug release, hyperthermia was induced in the primary tumor with ultrasound (peak ultrasound pressure of 1.1 MPa at a frequency of 1.5 MHz) at 42°C for 5 min prior to and 20 min post drug injection with a variable duty cycle. Immediately afterwards, 100 µg of CpG-ODN 1826 was administered intratumorally to the insonified tumor.

Results: Upon treatment with this combination of locally-released doxorubicin, local administration of CpG-ODN and systemic aPD-1, 100% of treated and contralateral tumors regressed by at least 80%; further, all of the directly-treated tumors and 50% of the contralateral tumors were eliminated without recurrence. Thus, a 50% complete response rate was achieved, with tumor regression observed immediately after the incorporation of the doxorubicin treatment. By contrast, administration of CpG-ODN and systemic aPD-1 alone resulted in regression of 66% of treated and contralateral tumors.

Conclusions: We demonstrate for the first time that blocking of the programmed death-1 (PD-1) pathway in conjunction with immunogenic cell death induced by CpG-ODN and activatable nanodelivery of doxorubicin can generate curative responses in both primary and contralateral tumors. Increases in cytotoxic CD8+ T lymphocytes and a reduction in regulatory T cells and myeloid-derived suppressor cells were observed in both directly treated and contralateral tumors. This combinatorial approach was curative for directly-treated tumors and overall survival was significantly extended, however, the contralateral tumor returned in all treated mice.
Towards FUS lung cancer ablation: Aspects of MR guidance in flooded lung

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Objectives: One-Lung flooding (OLF) enables HIFU application under sonographic guidance in lung. Additionally MRgFUS is superior for non-invasive ablation under thermal dose monitoring. FUS treatment of lung tumours requires complete gas-liquid exchange in the treated lung section, which will likely affect also imaging modalities. MRI is seldom used for diagnosis and staging of thoracic tumours because of low proton density with short T2* and field inhomogeneities in ventilated lung. So far no data is accessible for MR imaging of lung in flooded condition and therefore its use for FUS guidance on lung tumours. This study was aimed to investigate in vivo OLF in MR environment and to evaluate the capabilities of MR imaging on lung tumours (NSCLC).

Methods: OLF was performed on six pigs (female 35-60 kg) in 3T MR (Prisma, Siemens AG, Germany). After narcosis, mechanical ventilation was performed with an ICU respirator (Servo 900, Siemens AG, Germany) through a 39 Ch double-lumen tube (Mallinckrodt, Ireland). After ventilation with FIO2 = 1.0 for 30 min the left lung wing was flooded with isotonic saline (0.9%@ 35°C). Ventilation of the right lung was maintained for 60-90 min, followed by re-ventilation for 30 min. MR imaging was performed with spine and body array coils in lateral position using T2 HASTE, and T1 weighted GRE sequences. As a cancerous ex vivo model, MR imaging was performed on ten human lung lobes containing lung cancer (NSCLC). Lobes where flooded immediately after resection with saline (35°C) and imaged using GRE and T2 HASTE sequences in 1.5 T MR (Achieva, Philips, NL).

Results: OLF was successfully performed in MR environment on all animals (6/6). Flooded lung appears hyperintense in T2 weighted images (Fig. 1) and hypointense in T1w. Bronchial wall and vascular structures in flooded lung clearly appear as hypointense structures in T2 weighted image at high level of detail. GRE phase images show a homogenous phase in bronchial and alveolar tissue of the flooded lung wing. NSCLC tissue appear in flooded lung as strongly hyperintense lesions in T1 (Fig. 2) and hypointense in T2 weighted images with clear demarcation from lung parenchyma.

Conclusions: This study shows that in vivo OLF is operable in MR environment, all animals survived the procedure. The absence of susceptibility changes in GRE phase images suggests a complete gas to saline exchange in the flooded lung wing. For FUS guidance a demarcation of tumor to parenchymal structures is essential. In T1w images the lung tumour appears bright hyperintens to surrounding lung in all tumours. Further in T2w images a good demarcation between bronchus and parenchyma can be used for anatomic orientation. It can be concluded that MR imaging based on general fast GRE and TSE sequences is sufficient for FUS guidance on lung cancer during OLF. Further research investigating nodule size detection limits and the use of PRFS thermometry in flooded lung is required.

In-vivo T2w (84ms/900ms) image of porcine lung, right ventilated, left flooded lung.

Ex-vivo T1w GRE (5 ms/244 ms) image of flooded human lung lobe with hyperintense NSCLC-Adenocarcinoma