# MR imaging for the evaluation of boiling histotripsy treatment or thermal High Intensity Focused Ultrasound treatment in mouse lymphoma

Martijn Hoogenboom[1], Dylan Eikelenboom[2], Martijn H den Brok[2], Erik Dumont[3], Gosse J Adema[2], Arend Heerschap[1], and Jurgen J Futterrer[1,4]

[1]*Department of Radiology and Nuclear medicine, Radboud University Medical Center, Nijmegen, Gelderland, Netherlands,* [2]*Department of Tumor Immunology, Radboud University Medical Center, Nijmegen, Gelderland, Netherlands,* [3]*Image Guided Therapy, Pessac, France,* [4]*MIRA Institute for Biomedical Technology and Technical Medicine, University of Twente, Enschede, Overijssel, Netherlands*

Target audience: Researchers and clinicians who use high intensity focused ultrasound (HIFU) for tumor treatment.

Purpose: Thermal High intensity focused ultrasound (HIFU) is a widely known and clinically used ablation method. Besides thermal ablation, the interest for mechanical ablation of tumors is increasing. An example of mechanical ablation is boiling histotripsy, which is used for fragmentation of the tissue(1,2). The advantage of this technique is that the fragmented tissue can easily be absorbed as part of natural physiological healing responses. However, *in vivo* reactions of this treatment are not well understood and until now only ultrasound imaging is used for guidance of the treatment and the evaluation of the lesion. Due to the variety of MR imaging techniques, MR imaging might be a more viable option to evaluate the treated area. In this study a MR guided treatment set up is used to treat mouse lymphoma by boiling histotripsy or thermal HIFU ablation. Different MR imaging techniques are used for the evaluation of the lesion immediately after treatment.

| sequence | TR | TE | FA | voxel size mm |
|---|---|---|---|---|
| T1w dynamic contrast | 400 | 1.58 | 15 | 0.39×0.39×1.00 |
| T2w turbo spin echo | 2500 | 41 | 180 | 0.18×0.18×1.00 |
| PD turbo spin echo | 2500 | 14 | 180 | 0.18×0.18×1.01 |
| 3D vibe, flash | 10 | 1.57 | 25 | 0.26x0.26x0.26 |
| Echo planar imaging | 29.91 | 4.5 | 15 | 0.58×0.55×1.00 |

Methods: Twelve C57Bl/6n wild type mice were subcutaneously injected with EL4 lymphoma tumor cells at the right femur. After 10-12 days a round tumor with a tumor size of >8mm in diameter was reached. A 3MHz, 16 channel phased array HIFU system, focal spot size 0.5x0.5x2.0mm (Image Guided Therapy, Pessac, France) was placed in a 7T wide bore animal MR scanner (ClinScan, Bruker Biospin GmbH, Rheinstetten, Germany). An in house built surface coil was positioned around the tumor. Four mice were treated by a thermal ablation method: a four second continuous wave, acoustic output power of 50-52 Watt (i.e. 200 Joules), for each focal spot. 12 to 15 focal spots were positioned within one plane in the center of the tumor, each 1.5mm apart. A 40 second cooling time was used between the focal spots. Four mice were treated with boiling histotripsy: a pulsed wave method of 5ms pulses with an acoustic output power of 220-230Watt, pulse repetition frequency of 1Hz and 150 pulses (i.e. 165 Joules) per focal spot. A total of 22-25 spots (depending on tumor size) were focused 1mm apart in one plane. Four mice did not get any treatment. The ablation process was visualized using MR guided thermometry (EPI sequence, proton resonance frequency shift method -Siemens, Erlangen, Germany-). During thermal ablation 4 slices per 1.8 seconds were applied, and 7 slices per dynamic (3.1 sec) for boiling histotripsy. Immediately after treatment a 12 minute dynamic contrast enhanced (DCE) MR scan (2.4s/dynamic) was made with an injection of 0.2ml Gadovist (0.04mmol/ml). Before - , after treatment and after contrast injection, MR imaging was acquired (T2 weighted -T2w, Proton density weighted -PDw- and a T1 weighted 3D vibe). Directly after MR imaging the tumor was removed for pathologic evaluation (H&E staining). (Fig 1)


Figure 1: Flow chart of imaging and treatment.

Results: The average temperatures reached within the focal spot were 87.7°C (+/-12.4) and 44.4°C (+/-2.4) during thermal HIFU treatment or boiling histotripsy, respectively. Immediately after boiling histotripsy a hyper-intense lesion was visualized on both T2w and PDw imaging which correlated with the planned lesion size and the liquefied lesion size seen at the H&E stained pathological section (Fig. 2). Within the lesion, the tissue was completely fragmented and no cell structures remained intact. Immediately after thermal ablation, the MR images did not reveal clear lesions, but after contrast injection a clear dark lesion was shown at T1w 3D vibe imaging, as well as on T2w and PDw imaging. After both treatments, no contrast uptake was visualized within the lesion on DCE imaging. Some increase of the contrast agent was seen at the border of the lesions after both treatment, which correlate with small hemorrhages seen on the pathology coupes. The border of the boiling histotripsy lesion revealed a very sharp border of less than 200µm between liquefied lesion and vital tumor cells on the H&E sections.

Discussion/conclusion: T2w imaging is an accurate method for the evaluation of the treated area after boiling histotripsy. Based on T2w imaging the treatment can be continued as long as vital tumor tissue is visible. H&E slices revealed a sharp delineated lesion after boiling histotripsy with complete fragmentation of the cells. For thermal ablation, contrast imaging is required for visualization of the treated area. Alternatively, temperature measurement can be used to estimate the lesion size. The DCE scan revealed that there was no contrast uptake within the lesion, after both treatment methods, and only minor uptake was seen at the borders. This concludes that hemorrhaging due to treatment is limited, which was proven by the H&E sections. The T2w images revealed some increase of intensity due to the contrast agent, which suggested that there is still some T1 weighted within the image. Immediately after thermal HIFU ablation the tissue was not completely coagulated, however it could be expected that within days after treatment, the damaged cells will die. The use of other histopathological stainings will reveal more information about the created lesions. For further research a longer follow up is required to evaluate the treatment response.

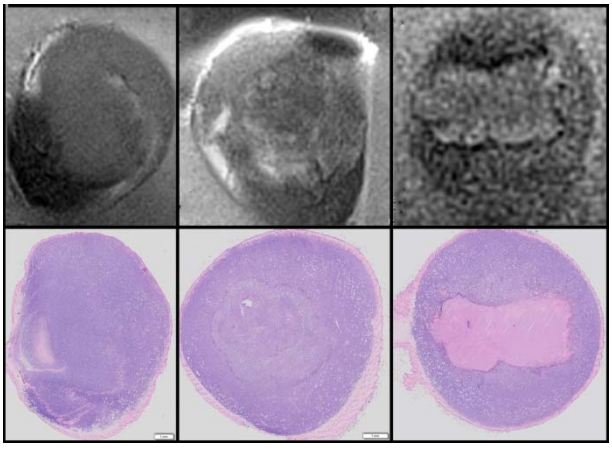

Figure 2: Top: T2w MR images, Bottom corresponding pathology (H&E stained section). Left: EL4 mouse tumor. Middle: EL4 mouse tumor after thermal ablation. Right: EL4 mouse tumor after boiling histotripsy treatment.

References:
1.Khokhlova TD et al. Proceedings of the National Academy of Sciences of the United States of America 2014. 2.Simon JC et al. Physics in medicine and biology 2012.