# In vivo monitoring of ultrasound-mediated nanoparticle delivery in human colon cancer xenografts using magnetization-prepared rapid gradient echo (MPRAGE) imaging

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**Target Audience:** This abstract is intended to be viewed by scientists and clinicians interested in non-invasive monitoring of drug delivery using MRI.

**Purpose:** Passive delivery of therapeutics into tumors takes advantage of the enhanced permeability and retention effect (EPR)[1, 2]. Though this is an advantageous mechanism to utilize for cancer therapy, tumors are very heterogeneous and different regions (particularly the central area of metastatic tumors) do not show this effect[3]. This has led to multiple strategies to enhance tumor vessel permeability to aid in therapeutic delivery. Ultrasound (US)-mediated delivery of therapeutic-containing nanoparticles (NP) using microbubbles (MB) is a promising new imaging-guided approach that can potentially overcome the barriers to efficient, site specific drug delivery[4] (Figure 1). When these micron-sized, gas-filled MB interact with incident US, they expand and contract in a process known as acoustic cavitation. The cavitation of microbubbles can induce transient, enhanced permeability of blood vessels and is currently being explored as a mechanism for delivering therapeutics[5]. However, further optimization is required to prepare this strategy for clinical translation, including a method to quantify and monitor successful therapeutic delivery *in vivo*. Our goal was to investigate the feasibility of assessing US-mediated NP delivery into human colon cancer xenografts in mice by quantifying gadolinium-conjugated fluorescent NP accumulation using MRI.

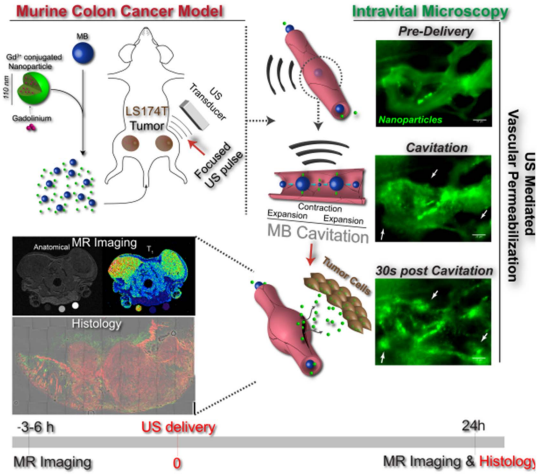


*Figure 1: Overview of US-mediated delivery approach. US-mediated MB cavitation can lead to site-specific NP delivery in targeted tissue.*

**Methods:** Human LS174T colon cancer tumor cells (2 x $10^6$) were subcutaneously injected into the right and left hindlimbs of two nu/nu mice and grown for 10 days. Mice were imaged at 3T using a GE MR750 scanner and a wrist RF coil. $T_1$ was quantified using three consecutive magnetization-prepared rapid gradient echo (MPRAGE) scans with inversion times TI=4000 ms, 1280 ms, and 150 ms. The resulting images were combined as described by Liu et al.[6], which has been demonstrated to provide fast 3D $T_1$ maps which are unaffected by inhomogeneous $B_0$ and $B_1$ fields. Mice were imaged before, and 24h post US-mediated NP delivery into one tumor (the other used as a control). Phantoms with different NP concentrations in water were imaged (Figure 2A) and 1/T1 was observed to linearly increase by 0.0103 s^-1 for every NP/pL. Assuming the NP had the same effect in tumors, the number of NP per unit volume was calculated. Ultrasound-mediated vascular permeabilization was initiated after i.v. administration of 150 µl of NPs ($5\times10^{11}$ NPs) and 350 µl ($3.5\times10^8$) MB at a rate of 100 µl per min (Figure 1). Tumor treatment consisted of 5 repetitive cycles, 1 min apart. The US focal beam was electronically steered across 6 locations, 1.5 mm apart at a pressure of 5.4 MPa. After imaging, the tissues were harvested to validate an increase in NP delivery using *ex vivo* tiled fluorescent microscopy.

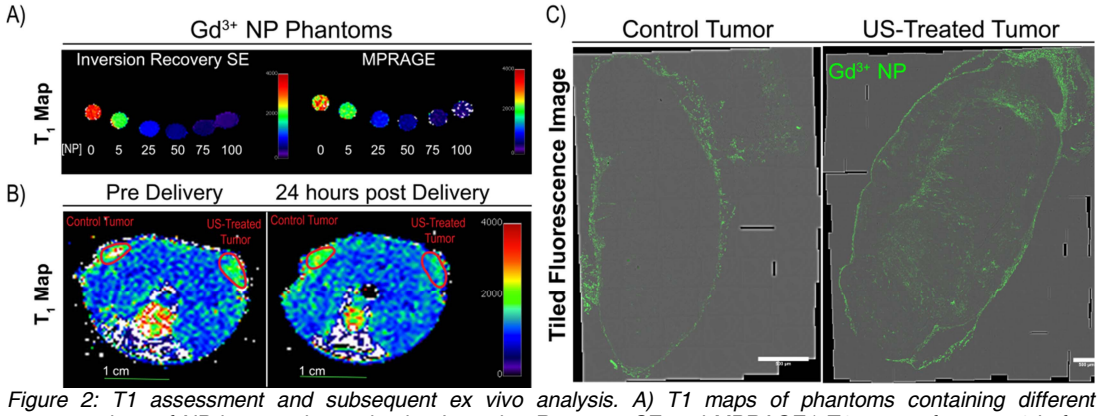


*Figure 2: T1 assessment and subsequent ex vivo analysis. A) T1 maps of phantoms containing different concentrations of NP in water imaged using Inversion Recovery SE and MPRAGE.) T1 maps of mouse 1 before (left) and 24 hours after (right) US-mediated delivery of NP using MPRAGE. C) Ex vivo analysis of NP (green) accumulation within control (left: passive delivery) and US-treated tumors (right) in mouse 1. Scale bar = 500 µm.*

**Results:** A comparison of inversion recovery spin echo (SE) and MPRAGE resulted in good correlation ($R^2$ = 0.996) between methods (Figure 2 A). After US-mediated delivery in mouse 1, $T_1$ of the tumor decreased from a weighted average of 1.71s to 1.62s (an estimated increase of $4.73\text{x}10^8$ NP; Figure 2B) compared to control tumor which decreased from 2.05s to 2.00s (+$5.59\text{x}10^7$ NP). In mouse 2, US-mediated delivery resulted in a drop in $T_1$ from 1.78s to 1.62s (+$3.47\text{x}10^8$ NP) compared to the control tumor (1.91s to 1.87s: +$1.51\text{x}10^8$ NP). *Ex vivo* analysis of fluorescent NP tumor accumulation showed that there was an increase in the US-treated tumor (mouse 1: 2.5 fold increase in mean fluorescence intensity, mouse 2: 1.7 fold increase) compared to control tumors with only passive accumulation (Figure 2C).

**Discussion:** In this early proof of concept study we show that US-mediated delivery of fluorescent, $Gd^{3+}$-labeled NP into human colon cancer xenografts resulted in a more pronounced decrease in $T_1$ compared to passive, EPR-mediated accumulation. This decrease in $T_1$ in treated tumors corresponded to an increase in accumulation of NP within the tumors. We also show that using the MPRAGE sequence, we can obtain similar $T_1$ values to inversion recovery SE, with the added advantage that volumetric quantitative measures of $T_1$ can be obtained in reasonable scan times.

**Conclusion:** Volumetric assessment of US-mediated delivery of $Gd^{3+}$-conjugated therapeutics using MRI could be utilized to non-invasively quantify and monitor drug delivery efficiency within a targeted tissue and to spatially map regions where successful delivery has occurred.

**References**: **1. Prabhakar, U., et al. (2013) *Cancer Research.* 73, 2412-2417. 2. Matsumura, Y., et al. (1986) *Cancer Research.* 46, 6387-6392. 3. Fang, J., et al. (2011) *Advanced Drug Delivery Reviews.* 63, 136-151. 4. Tzu-Yin, W., et al. (2014) *Current pharmaceutical biotechnology.* 14, 743-52. 5. Hernot, S., et al. (2008) *Advanced Drug Delivery Reviews.* 60, 1153-1166. 6. Liu, J. V., et al. (2011) *NeuroImage.* 56, 1154-1163.**