Evaluation of the novel SPIO GEH121333 for monitoring changes in tumor vascularity and vascular permeability after anti-angiogenic treatment using susceptibility contrast and T1-mapping

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Target audience: This work is targeted to preclinical and clinical researchers investigating imaging biomarkers for anti-angiogenic therapy.

Purpose: In this study we are using the novel, preclinical-phase iron oxide particles GEH121333 (GE Global Research, Niskayuna, NY, USA, supplied through GE Healthcare AS, Oslo, Norway) for monitoring vascular response to bevacizumab treatment in ovarian xenografts. GEH121333 are particles with a relatively large diameter and a high r1/r2 rate[1], which makes them suitable for both T1 and T2 contrast. Changes in T2 and T2* relaxation rate can be used for calculation of a theoretically derived marker for the blood vessel density $Q=\Delta R2/(\Delta R2^{*2/3})^{2}$ and decreased tissue T1 values after clearance of iron particles from the blood pool will give information about the tumor vessel permeability. The aim of this pilot study was to investigate the potential of the GEH121333 particles for detection of changes in blood vessel density and permeability after anti-angiogenic treatment and to evaluate the clearance from the tumor after injection.

Methods: All experimental procedures involving animals were approved by the institutional ethics committee and were in accordance with national, regional and institutional guidelines. Xenografts of the human ovarian cancer cell line TOV-21G were grown on the hind leg of athymic mice (n=7). MRI was performed on a 7T Bruker Biospec with an 86mm volume resonator for RF transmission and a quadrature mouse brain surface coil for reception. Images of 4 sagittal slices with a slice thickness of 0.6mm and field of view of 28x14mm^2 were acquired from each tumor using the following sequences: *Multi Echo Spin Echo*: TE=10.5ms, 32 echoes with 10.5ms echo spacing, TR=3s, 4 averages, matrix (MTX)=128x64; *Multi echo gradient echo*: TE=3.5ms, 30 echoes with echo spacing 3.5ms, flip angle=30°, 1 average, MTX=128x64; *RARE*: TE=13ms, TR=225/500/1500/3000/6000/12000ms, RARE factor=2, 1 average, MTX=64x32. Five mice were imaged on day 0 and 1 and the imaging protocols were repeated before and 15 minutes after i.v. injection of GEH121333 particles at a concentration of 3mg/ml in saline at a dose of 10mg/kg bodyweight. On day 0 right after the post contrast imaging three mice were treated i.p. with 5mg/kg Bevacizumab diluted in saline and two mice were given an i.p. injection of the according volume of saline (control). T1, T2 and T2* maps were computed voxelwise by monoexponentially fitting the signal intensity versus TR or TE, respectively. Voxels for which the fit did not converge were excluded. Pre contrast T2 and T2* maps were subtracted from the post-contrast maps to obtain ΔR2 and ΔR2* maps. Q values were calculated for each voxel[2] for both days and pre and post treatment Q values were compared. One bevacizumab treated mouse had unsuccessful T2 and T2* measurements on day 1. For two additional mice we performed a longitudinal investigation of the clearance of the GEH121333 particles. T1 maps were obtained using the RARE sequence before and 0.5, 24, 48, 72 and 96 hours (h) after a single injection of GEH121333. Median T1 values were calculated for each tumor. Image processing and analysis were performed using Matlab.

Results: Fig. A shows that Q is increasing significantly for the two control tumors (m1 and m2) from day 0 to day 1 while Q is rather stable for the treated mice (m3 and m4).The development in tumor T1 relaxation times the first 24h after injection of GEH121333 and treatment with bevacizumab indicates higher T1 relaxation times in treated tumors compared to controls 24h post injection (Fig. B). The longitudinal study of tumor T1 relaxation showed a recovery of the T1 values to 90% of the baseline values after 72h (Fig.B).

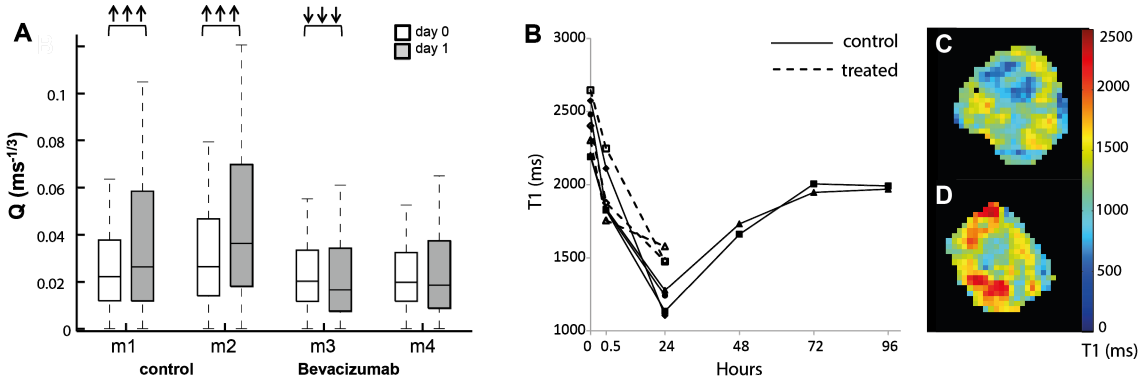

Fig. A) Box plots of Q for individual tumors for day 0 and day 1. ↑↑↑/↓↓↓ p<0.001 (increase/decrease from day 0 to day 1), two-tailed Mann-Whitney U test. B) Median T1 values for each tumor at different time points before and after the first contrast injection. 24h post contrast T1 maps obtained before the second injection from mouse m1 (control) and m4 (bevacizumab treated) are shown in C) and D) respectively.

Discussion: Bevacizumab is known to normalize the tumor vasculature already by 24h after administration by decreasing vessel density and by making it less leaky and more functional[3]. Compared to the control, the treated tumors have lower Q values on day 1, which indicates a lower vessel density one day after treatment. The particles remaining in the extracellular extravascular space (EES) 24h after injection (Fig. B) increase the pre contrast relaxation rates (R2 and R2*) on day 1. This might have caused the Q values to be higher than expected on day 1 for both control and treated tumors, which could possibly have been avoided if there were less particles remaining in the tissue from day 0. The longitudinal study of tumor T1 relaxation times indicates that a considerable amount of particles remains in the tissue 24h post injection and thus suggests that longitudinal studies of changes in the vascularity measured by Q-maps should be performed with > 72h (Fig.B) between injections to allow washout of particles from the EES. A decreased tissue T1after clearance of iron particles from the blood pool will give information about the tumor vessel permeability, since the measured T1 effect is due to iron particles which have leaked into the EES. The relaxation times after treatment are higher for the treated tumors, which suggests that the treated tumors show lower leakage of particles into the EES and therefore have less permeable vessels.

Conclusion: The results from this pilot study suggest that the novel contrast agent GEH121333 can be used to detect vascular changes after anti-angiogenic therapy. The magnetic properties of the particles allow evaluation of changes both in T1 and susceptibility imaging, attributable to underlying changes in permeability and blood vessel density, respectively.

References: 1) Shi et al. Contrast Media Mol. Imaging 2013; 8:281-288. 2) Jensen JH, Chandra R., Magn Reson Med 2000; 44:224-230. 3) Dickson VP et al. Clin Can Res 2007; 13 (13): 3942-50.

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